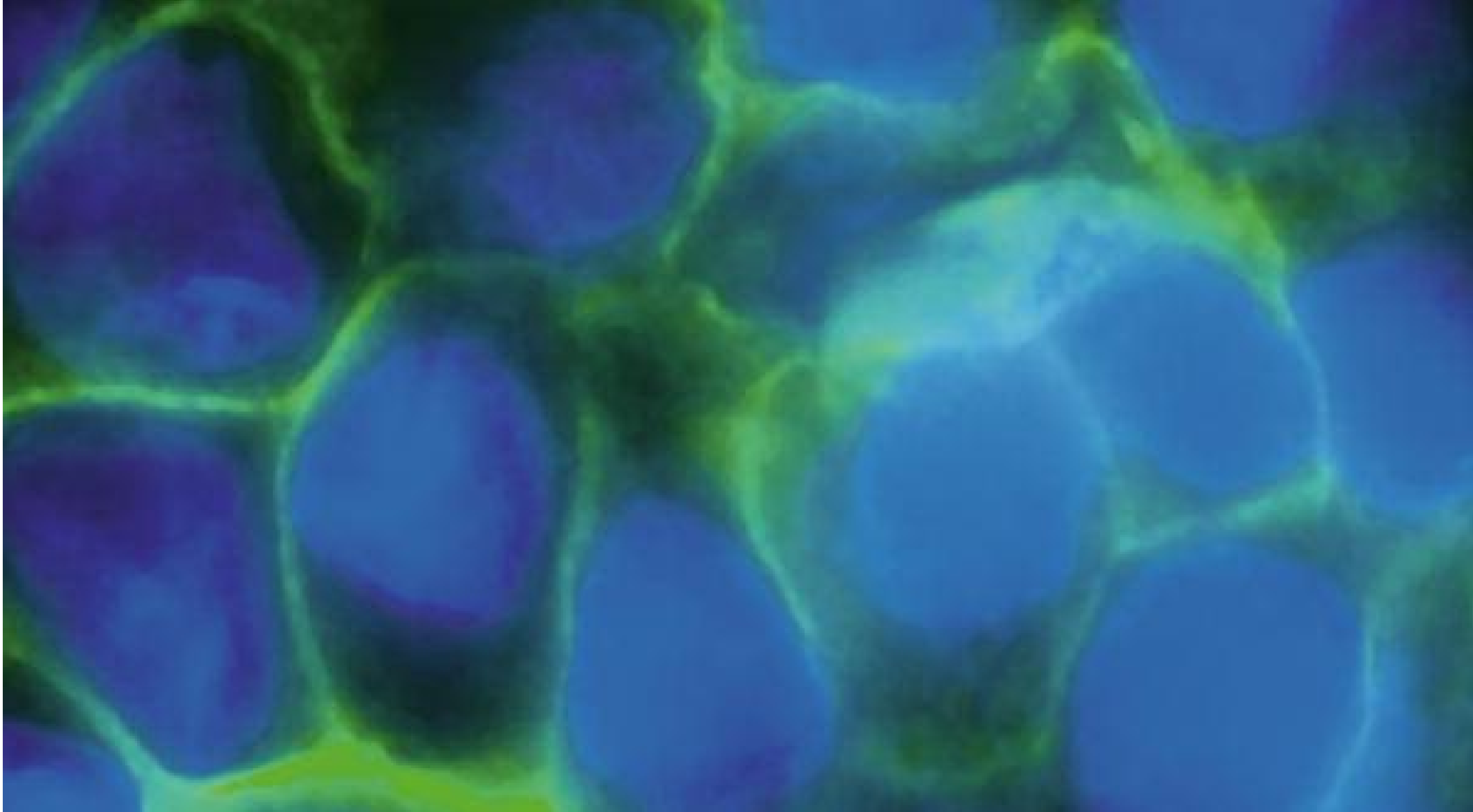

Annual Report

2007-2008

EMBL

Annual Report 2007-2008

European Molecular Biology Laboratory

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IN 2007 LUXEMBOURG BECAME EMBL'S TWENTIETH MEMBER STATE. *This event was significant not only because of the round number, but also because it means that all Western European countries are now members of the Laboratory. The pace of new member state accessions has recently picked up, with Luxembourg the fourth country to join since 2003. Interest from several of the more recent EU member countries in joining the Laboratory is also high. These developments underline the ever-increasing reputation and international role of EMBL and its usefulness to the scientists in its member states.*

Further afield, Australia became the Laboratory's first associate member state in 2008, a step that we hope will increase the interaction between Australian life scientists, EMBL and the wider European research community. EMBL Council has agreed to a number of programmes that distinguish associate membership and we are working with the very engaged Australian scientific community, as well as the research ministry, to develop joint activities.

In two of the outstations, the year was marked by milestones in building projects. The very attractive EMBL-EBI extension building was completed, relieving for the time being their pressure for space and providing a state-of-the-art training room that had been eagerly awaited by all those engaged in providing courses and workshops. Ian Pearson, the UK Minister of State for Science and Innovation, attended the event as did the EMBL Council chair and representatives of EMBL-EBI's major funders, the European Commission, The Wellcome Trust, and the UK MRC and BBSRC research councils. The celebration was rounded off by a series of scientific talks from EMBL-EBI staff and alumni.

In EMBL Hamburg, the PETRA III project construction got underway at DESY. This coincided with the move into high gear of the EMBL beamline design and construction team, who have a very busy few years ahead of them.

I am very happy to welcome Anne Ephrussi as the new Head of the Developmental Biology Unit in Heidelberg, succeeding Stephen Cohen who left for Singapore. Anne has played an enormously important role in the success and development of the EMBL International PhD programme, often working together with Matthias Hentze, for example in their planning of the reorganisation of our scientific training activities into the EMBL International Centre for Advanced Training (EICAT). Her typically energetic start to reshaping the Developmental Biology Unit bodes well for the future of this facet of EMBL's activities.

Finally, I trust you will find much to enjoy in the rest of this report. I hope it provides you with some insight into what makes leading EMBL such an exciting and rewarding task.

Iain W. Mattaj
Director General

State of the Laboratory

New member states

Two new states have joined EMBL. The first, Luxembourg, joined in 2007. Octavie Modert, Secretary of State for Culture, Higher Education and Research in Luxembourg, visited EMBL during the Winter Council meeting in November 2007. She presented Luxembourg's plans for expanding research and higher education initiatives in the Grand-Duchy to EMBL Council.

Iain Mattaj with Octavie Modert, Secretary of State for Culture, Higher Education and Research in Luxembourg (middle) and Josiane Entringer, the Council representative for Luxembourg.

In March 2008, Australia joined EMBL as the first associate member state. The membership will initially last seven years. Collaborations and exchange will take place between the five European sites of EMBL and leading Australian research institutions. Monash University, The University of Western Australia, The University of Queensland, The University of Sydney and the Commonwealth Scientific and Industrial Research Organisation (CSIRO) all worked actively with the Commonwealth Government in establishing Australia's associate membership, and have indicated their interest in engaging in collaborative activities with EMBL. Through its associate membership, Australia will contribute to the diverse activities at EMBL by sending early-career scientists to join EMBL through a faculty development programme, while EMBL will share its world-renowned expertise in researcher training and research infrastructure development with Australian institutions.

At a recent meeting with Iain Mattaj, the Australian research minister Senator Kim Carr confirmed the shared goal of linking European molecular biology research more closely with the Australian scientific community.

Research

The Annual Report showcases the best examples of research projects carried out at EMBL over the past year and I will only provide a few general comments on our research in my report.

Given EMBL's fixed-term contract policy, staff turnover is a fact of life, and one that is of major benefit to both the Laboratory and the member states. Two new Heads of Unit took up their posts in EMBL Heidelberg during the past twelve months. Christoph Müller was first, replacing Luis Serrano in Structural and Computational Biology (SCB). Anne Ephrussi then took over from Stephen Cohen in Developmental Biology (DB). Both are already making their presence felt, partly because of their involvement in this year's group leader recruitment (DB will, for example, have space to recruit either three or four new group leaders this year) and their roles in redefining the strategy to be followed by their units.

In the smaller outstations, the rate of turnover can sometimes lead to what resembles a mass exodus. EMBL Grenoble has just gone through a period in which three of its six faculty members had to be replaced. Stephen Cusack has used the opportunity to reshape the outstation's expertise toward the goal of tackling the structures of large multicomponent macromolecular complexes. EMBL Monterotondo is entering a similar phase. Having recruited one new group leader and one staff scientist last year, the outstation will need to replace three more of its six faculty members in the upcoming year. Nadia Rosenthal sought input on this recruitment from this year's Sci-

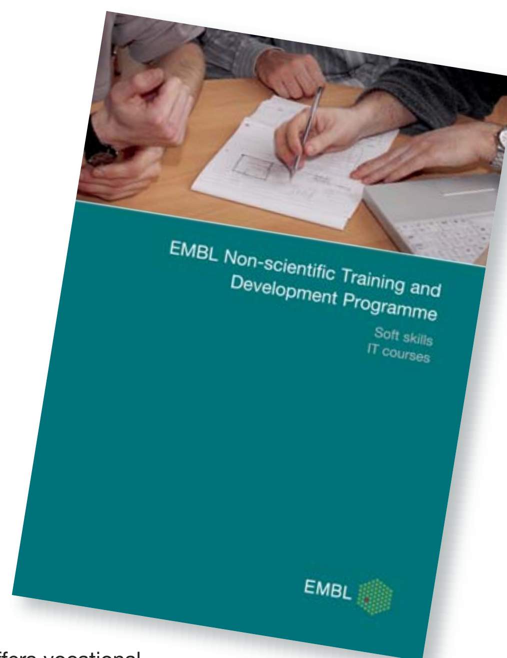
entific Advisory Committee (SAC) review panel, which evaluated EMBL Monterotondo in February.

One major reason for studying the mouse is because of the insight that can be gained into the basis of human disease by using mouse models. This year, Claus Nerlov's group used mice to demonstrate that a mutation found in many patients suffering from a form of leukaemia is indeed causative for the disease. They also found that the mechanism by which this occurs has had important consequences for the cancer stem-cell model. In Heidelberg, Matthias Hentze's group was involved in a collaboration that unexpectedly showed – using a mouse model – that a common genetic disease that leads to iron overload is due to a defect in the liver, rather than in the intestine as previously believed.

There is also an increasing trend toward direct studies of the causes of disease in humans, where knowledge gained from our basic research provides clues as to where to look for defects in patients. Epigenetics is an area of rapid progress and two of the groups working in this field found links between their areas of interest and human cancer this year. Asifa Akhtar's group works on dosage compensation in flies, one of the regulators of which is a histone acetylase called MOF. The human homologue of MOF turns out to be upregulated in at least two different forms of tumour: breast carcinoma and medullablastoma. Similarly, Frank Gannon's group demonstrated that estrogens, which play a critical role in some forms of breast cancer, set in motion a series of epigenetic changes in responsive calls, and that these changes are affected not only by estrogens but also by anticancer drugs. This suggests that these specific changes may be important in the development of the cancer phenotype. New DNA sequencing technologies will revolutionise our ability to carry out direct studies of the genetic basis of human disease, and the involvement of EMBL-EBI in the 1000 Genomes Project – a major pilot study to document human variation – is an important pointer to future research interests both within and outside EMBL.

EMBL Scientific Publications and Collaborations 2007

- Total number of peer-reviewed publications: **446**
- Internal collaborations: publications co-authored by more than one EMBL group leader: **45**
- External collaborations: **753** in total of which **99** resulted in a publication



EMBL offers vocational training for all staff members

Interdisciplinary research has long been a hallmark of EMBL, thanks to its collaborative culture. In recent years, we developed EMBL Centres as focal points for interchange of expertise in certain areas of common interest to many EMBL scientists (imaging, computation, high-throughput technology and disease mechanisms).

These centres have succeeded admirably in increasing communication and training in these areas. However, they also underlined the need for EMBL to hire more scientists with skills and training different than those of the 'standard' EMBL recruits in order to facilitate the pursuit of interdisciplinary scientific projects. These deliberations led to the decision to use some of the funding that was granted by Council to support a new postdoctoral scheme, the EMBL Interdisciplinary Postdoctoral Fellows (EIPODS). We advertised interdisciplinary projects that were aimed at recruiting postdocs who would work with (at least) two EMBL groups of different expertise. Most of these projects involved groups from more than one EMBL unit, and often from more than one EMBL site. The response exceeded expectation, both in terms of the quantity and quality of the applicants, and we hired 15 EIPODs in the first selection. Aside from the boost to interdisciplinary research, this programme will have a second advantageous side effect for EMBL. Almost all of the other 205 postdoctoral fellows who were at EMBL in 2007

View inside the 280 m long PETRA III experimental hall

applied to individual group leaders and came to EMBL with external funding. Since many of our young group leaders are in their first independent position, they are not very visible to potential postdocs and do not receive many applications. Because the EIPOD programme, following the tradition of our PhD programme, is designed to favour projects offered by more recently recruited group leaders, the scheme will help our young group leaders to recruit excellent people into their labs.

Services

The main areas of service provision are structural biology, bioinformatics and the core facilities. Many thousands of scientists use these facilities and services every year and they are an essential contribution to the European research infrastructure in molecular biology.

Roadmaps for research infrastructures have been developed in some European countries and in other parts of the world such as the USA and Japan. They help to identify projects that are essential to the scientific community and require researchers to join forces across borders and disciplines. The first European roadmap for research infrastructures was published by the European Strategy Forum on Research Infrastructures (ESFRI) in October 2006.

The preparatory phase of the 35 European research infrastructure projects has begun and EMBL is involved, to varying extents, in four of the six selected biomedical science projects. We believe that making the ESFRI process work is critical to the future success of European science, particularly in the case of life science research. Janet Thornton, Director of EMBL-EBI, is coordinator of one of the projects, ELIXIR, whose ambition is to prepare the future of European bioinformatics infrastructure. In an interview in this annual report, she provides some insight into this endeavour (see page 13). Like the other ESFRI projects, this will be a huge and complex undertaking, but one to which there is no reasonable alternative.

Structural biology services

Activities at EMBL Hamburg focus on structural biology methods using synchrotron radiation provided by the German Synchrotron Research Centre (DESY). DESY has started to convert the PETRA storage ring into a dedicated synchrotron radiation facility with world-class optical parameters, and the current facilities on DORIS III will be replaced by new beamlines built on PETRA III.

EMBL Hamburg is planning to build two beamlines for protein crystallography and one for small-angle X-ray scattering, complemented by an on-site sample preparation facility and an online data evaluation infrastructure. The EMBL@PETRA3 project team is headed by Thomas Schneider and includes Florent Cipriani from EMBL Grenoble. The project is now in its second year and is in the process of defining the design of the three beamlines in detail. A Scientific Advisory Board (SAB) to the project was formed in March 2008, chaired by Dr Liz Duke (Diamond Light Source, UK) and Professor Dino Moras (IGBMC, Strasbourg, France). The SAB consists of world-leading experts in the construction and operation of

beamlines for small angle X-ray scattering on biological samples (BioSAXS) and macromolecular X-ray crystallography.

During the construction phase of the PETRA III beamlines, EMBL will continue to operate four experimental stations in protein crystallography (beamlines X11, X12, X13) and small angle scattering of biological material. Two stations have become test facilities for EMBL's future PETRA III beamlines (beamlines BW7A, BW7B) and the remaining two EMBL beamlines at DORIS III (beamlines X31, EXAFS) will be closed.

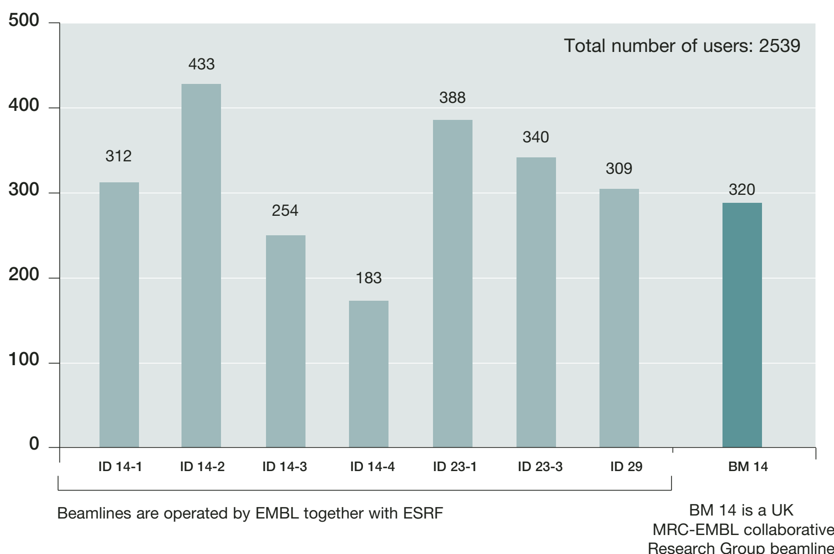
EMBL Grenoble: More than 2500 scientists have made use of synchrotron and neutron radiation for life sciences applications at beamlines that are operated in collaboration with the European Synchrotron Radiation Facility (ESRF) and the Institut Laue-Langevin (ILL). EMBL Grenoble has entered into negotiations to establish a formal collaboration between the ESRF, India and the International Centre for Genetic Engineering and Biotechnology (ICGEB) in Italy to provide access to users from their respective member states as well as emerging regions. Beamline BM14 has been jointly operated by the UK's Medical Research Council (MRC), ESRF and EMBL since 2002. The MRC will discontinue operation of the beamline after 2009 when an equivalent beamline will be available at the new UK synchrotron, Diamond. Depending on the formal agreement of all partners, the plan is to organise the transition gradually so that India joins the MRC-EMBL-ESRF collaboration in 2009 to train staff and then in 2010 the ESFR-India-EMBL-ICGEB consortium will take over from the MRC.

Bioinformatics services

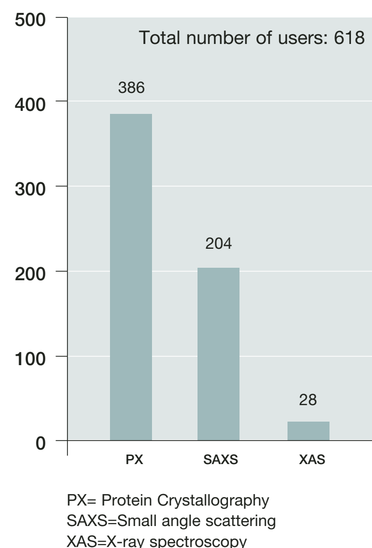
The European Bioinformatics Institute provides core biomolecular data resources to users in EMBL member states and beyond. These services continued to be well used in 2007: on average 339,629 unique hosts were served per month (293 000 in 2006). For the period from September 2006 to August 2007, there were on average 2,328,857 requests per day (1,481,000 in 2006) for the EMBL databases and 3,169,862 when Ensembl (a collaboration between EMBL and the Sanger Institute) was included. The main databases include EMBL-Bank for nucleotides, Ensembl for genomes, ArrayExpress for gene expression data, UniProt for proteins, MSD for macromolecular structures and InterPro for protein motifs. In the past year EMBL-EBI received and processed more than 38 million EMBL-Bank entries (compared with 18 million in 2006), including more than 450 new genomes (200 in 2006). This brings the total to 650 complete genomes, excluding viruses and organelles. In 2007, 10 new eukaryotic genomes were added to Ensembl (10 in 2006) and 1.1 million UniParc entries were processed, together with 28,000 microarray hybridisations and 9263 macromolecular structures. Data from environmental sequencing projects are also accumulating, and EMBL-EBI has recently agreed to take over the DNA Trace Archive, a database of the world's raw sequence trace data, from the Wellcome Trust Sanger Institute.

EMBL-EBI's DNA and protein sequence database teams have undergone a major reorganisation, reflecting the need to coordinate DNA and protein sequence data more closely and to integrate DNA data from an increasing

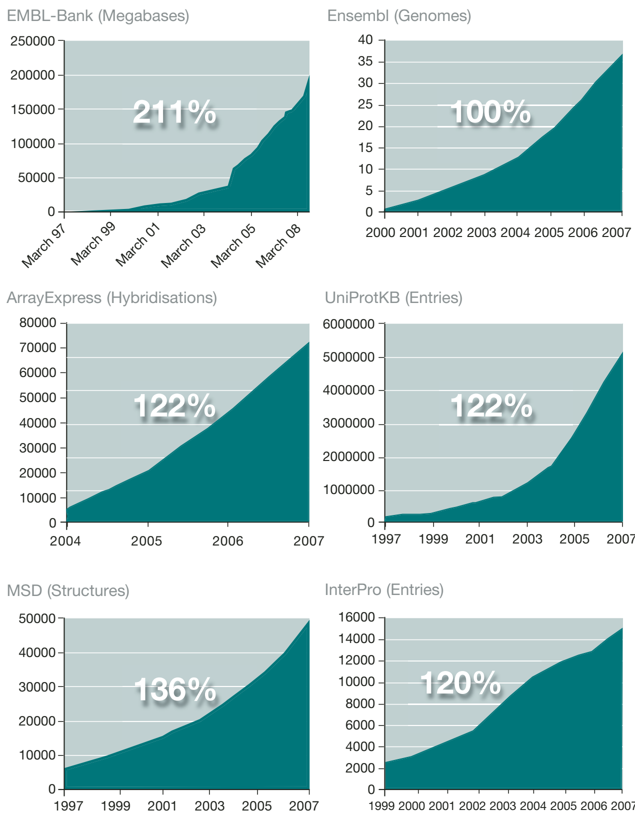
Beamline Users EMBL Grenoble 2007



Beamline Users EMBL Hamburg 2007



Growth of EMBL-EBI's core data resources in 2006/2007



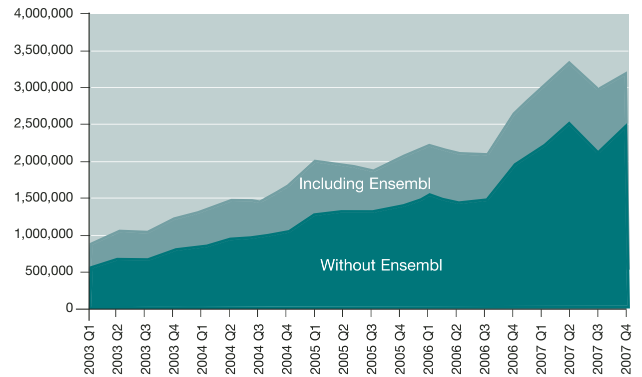
diversity of high-throughput methods. The new team, PANDA, is jointly led by Rolf Apweiler and Ewan Birney. Several team leaders have taken over responsibility for areas within PANDA: Sarah Hunter for InterPro, Henning Hermjakob for proteomics services, Christoph Steinbeck for cheminformatics, Paul Flicek for human genetic variation, and most recently Paul Kersey for bacterial genomes and PANDA-wide sequence data pipeline coordination.

EMBL core facilities

EMBL offers services to its staff and external academic users in nine core facilities for genomics, proteomics, imaging, and so forth. The services of the Advanced Light Microscopy Facility (ALMF), headed by Rainer Pepperkok, have recently been significantly extended to include image processing as well as a new imaging technology developed at the EMBL Centre for Molecular and Cellular Imaging. The new technology for high-throughput microscopy for systems biology was developed by Jan Ellenberg and Rainer Pepperkok to enable confocal microscope screening of large scale experiments using RNAi, chemical or other perturbations. The integration into the ALMF will enhance synergies between image acquisition, processing and high-throughput microscopy.

Usage of the EMBL-EBI website

Ensembl is a joint project with the Wellcome Trust Sanger Institute



It will add state-of-the-art technologies – which are in high demand – to the services offered by the ALMF. The joint EMBL-DKFZ Chemical Biology Core Facility headed by Joe Lewis has recruited a new partner: the University of Heidelberg officially joined the facility in 2007.

In early 2008, the Genomics Core Facility was equipped with a new high performance, next-generation sequencer which allows a new range of genomic analyses and will be used intensively for systems biology at EMBL.

The new EM tomographic microscope (FEI) was delivered by the end of 2007 to the Electron Microscopy Core Facility. This device is now fully operational and will help to solve many cell and developmental biology questions in the coming years.

Training

All scientific training activities at EMBL are organised under the umbrella of the EMBL International Centre for Advanced Training (EICAT).

The EMBL International PhD Programme (EIPP) was founded in 1983 and has always been run by faculty members who maintained their research groups while organising the programme. The EIPP has been extremely successful and has been copied by many other institutions, frequently with help and advice from EMBL. We have established collaboration agreements with 28 universities in 18 countries with whom EMBL awards joint degrees to the members of the EIPP in the students' home countries. Several hundred students have graduated since the beginning of the Programme. It has now grown to include more than 200 students, and has an annual intake of about 50. Last year, the EMBL Scientific Advisory Committee suggested that the time had come to hire a full-time head for the PhD programme and we have recently recruited Helke Hillebrand, herself a scientist and former lab head, as the

new Dean of Graduate studies. Lars Steinmetz will serve as EIPP Academic Mentor and will share with Helke the role of advising students and interacting with faculty members.

Lars Steinmetz was instrumental in reorganising the PhD core course that is taught to all new PhD Students every year (see the interview on page 23). Matthias Hentze and Anne Ephrussi are stepping down from their direct EICAT responsibilities but will remain closely linked as advisors: Anne as the EICAT Coordinator and Matthias as Associate Director.

The EMBL Postdoctoral Programme has started to organise training activities for postdoctoral fellows at EMBL. A popular course has been 'Preparing for the job market', which took place twice last year. Detlev Arendt is the Academic Mentor of the Postdoctoral Programme, and his role is to set up and revise the programme, in addition to being the contact person for the Postdoctoral Association. Postdoctoral fellows from different EMBL Units are now meeting regularly at an annual retreat and at other events organised by the Association.

The outreach and training activities at EMBL-EBI were consolidated at the end of 2006 to create a single team that is also responsible for EMBL-EBI's scientific training programme. Cath Brooksbank is the Head of Outreach and Training and Nick Goldman serves as the Research and Training Coordinator. The training programme is designed to equip users of EMBL-EBI's bioinformatics services in the EMBL member states and beyond with the knowledge they need to use the data resources provided by EMBL-EBI. Last year, the existing training activities were restructured and several new ones added: the bioinformatics roadshow, a travelling user-training programme tailored to the needs of users of Europe's core biomolecular databases; the hands-on training programme; a series of short courses held at the new IT training suite; and the EBI e-learning pilot project, a web-based portal that serves both end-users and trainers.

The construction of the Advanced Training Centre on the Heidelberg campus is proceeding according to plan. The 2007/2008 winter has been mild and work continued without interruption. The project is likely to stay within the planned time schedule. The additional €3 million approved by EMBL Council in Summer 2007 will cover the bulk of the additional cost; any further cost increases due to higher material cost or inflation, for example, will be covered by Klaus-Tschira-Stiftung.

EMBL is promoting women in science, and together with EMBO and CERN has launched the EU-funded project SET-Routes to encourage more young women to pursue careers in science. The project has three main activities. The first was a conference organised by EMBO in May 2007 that was attended by 270 participants and provided an excellent overview of measures that have been taken across the world to improve the career perspectives of women in science. The second initiative is the ambassador programme that sends female science ambassadors into schools and universities. More than 130 ambassadors have been recruited and SET-Routes is now organising visits in more than 13 countries. Finally, a series of Insight Lectures by female scientists to school classes will be recorded and streamed as webcasts for use by teachers and students throughout Europe.

The helical architecture of the Advanced Training Centre on the Heidelberg campus is already recognisable.

EMBL Alumni

The EMBL Alumni Association continues to grow and now has more than 1300 members. It also has six active local chapters, with those of Greece and Germany holding their first meetings this year in Dilofo and Heidelberg respectively. Spain and Portugal held their third local chapter meeting in Derio (Bilbao), and EMBL-EBI generously organised an EMBL alumni day for all EMBL alumni and EMBL-EBI staff on 24 October to coincide with the opening of their new extension building. The meetings were extremely successful, providing participants with the

opportunity to present the progress of their careers and science since leaving EMBL, and to network with EMBL. There was also ample opportunity for alumni and current staff to discuss plans for future meetings.

In October 2007, the EMBL Alumni Association Board announced the first winners of the John Kendrew Young Scientist Award. The extremely high standard of the applications made the selection very difficult and the Board decided to select joint winners: Antonio Giraldez for his major contributions to science in studying microRNAs, and Giovanni Frazzetto for his inspirational and creative achievements in incorporating societal needs into his research in neuroscience as a Society in Science Branco Weiss Fellow. Antonio and Giovanni were both former PhD students in the EMBL International PhD Programme and they received the award in a ceremony on EMBL Lab Day from EMBL/EMBO alumnus John Tooze.

Former EMBL group leaders Elena Conti and Elisa Izaurralde have been awarded the Gottfried Wilhelm Leibniz Prize 2007 for their work in the area of RNA export, processing and turnover. The two researchers, who left EMBL in 2007, combined their expertise in structural biology and biochemistry during their time at EMBL. The prize, awarded by the Deutsche Forschungsgemeinschaft (German Research Foundation) is the most prestigious German research prize and is awarded in recognition of excellent scientific work. Six former recipients of the Leibniz Prize have gone on to win the Nobel Prize, including EMBL alumna Christiane Nüsslein-Volhard. Elena Conti is now a director at the Max Planck Institute of Biochemistry in Munich and Elisa Izaurralde is a director at the MPI for Developmental Biology in Tübingen.

Outreach

Many scientists at all EMBL sites have been involved in outreach activities organised by the Office of Information and Public Affairs, the EMBL-EBI Outreach and Training Group, the Science and Society Programme, parts of EICAT and many others. The events fall into several categories. Those intended for the general public usually attract the largest number of participants, but smaller events that are organised, for example, to help recruit PhD students or to encourage girls to consider a career in science are also important to EMBL. Last year EMBL was present at several large scientific conferences to raise our profile and to provide first-hand information to young scientists and users of EMBL services. We are now making webcasts of EMBL's Science and Society Forum and Distinguished Visitor lectures available on the EMBL website for all to enjoy at their own convenience.

Highlights of the events that were organised in the past year include the Long Night of Sciences in Heidelberg, and Girls' Day (see the Calendar on page 120 for more events). From a wide range of events that took place last year, here are a few examples.

This year's Cambridge Science Festival saw more than 30 volunteers from EMBL-EBI and the Sanger Institute introducing visitors to 'The world of DNA'. More than 2500 people dropped in on the 'Biology Zone', part of the Festival's 'Science on Saturday' activities on 15 March. Fun activities included making colourful DNA sequence bracelets, DNA origami and edible double-helices that illustrated the concepts of complementary base pairing and DNA structure. In addition, filming teams interviewed more than 25 visitors about their thoughts on personal genomics, and the resulting 'vox pops' are available to view on the www.yourgenome.org website.

In March 2008 an 'open house' was organised at EMBL Grenoble which attracted around 30 families of staff. This is an event that occurs every couple of years on the shared EMBL/ESRF/ILL campus during beamline shutdown, to allow spouses and kids to visit the microscopes and storage ring and to learn about structural biology.

Administration

The successful implementation of SAP HR phase 1 was completed at the end of 2007 and the programme went live with the first payroll run in January 2008. The programme runs well and offers much better HR management tools than we had before, as well as a better link between financial and personnel management. We will now start phase 2, during which additional functionalities and workflows will be implemented.

We have initiated the vocational staff training programme over the past year by first surveying the training requirements, testing potential trainers and finally putting together a catalogue of training courses that is made available as a booklet and online to all staff members. Specific scientific training courses for staff are organised by EICAT; some of these are available online.

A full-time resource development manager, Jörg Fleckenstein, has been hired for the new EMBL Office of Resource Development. The office will focus on three main areas: a corporate sponsorship programme for Advanced Training Centre courses and conferences, focused capital campaigns around key priorities, and a major donor development programme.

Integration of European Research

EMBL Partnerships

EMBL partnerships are special collaborations between EMBL and research institutes in the EMBL member states. They are established following certain organisational and scientific principles and aim to transfer some aspects of EMBL to the national institutes. The exact implementation of these partnerships varies depending on the needs of EMBL and the local partner.

In the presence of the rectors of the three host universities Oslo, Umeå, and Helsinki, we signed an agreement to establish the Nordic EMBL Partnership for Molecular Medicine on 3 October 2007. The agreement will encourage scientific exchange and collaborations between the partners and will facilitate access to respective scientific infrastructures, facilities and services for an initial period of five years. The partnership between EMBL, the Institute for Molecular Medicine Finland (FIMM), the Centre for Molecular Medicine Norway (NCMM), and the Laboratory for Molecular Infection Medicine Sweden (MIMS) is dedicated to molecular medicine, a growing field in the life sciences that investigates the molecular basis of disease and explores molecularly and genetically based treatments.

The CRG/EMBL Partnership Unit for Systems Biology started in 2006 with funding for five research groups. Now all five group leader positions have been filled. A reflection of the high quality of the staff recruited is the fact that two of the EMBL-CRG group leaders, Ben Lehner and Mark Isalan, were awarded European Research Council young investigator grants in the first round of this new EU funding scheme for individual researchers.

Relations with the European Commission

The European Commission restarted the discussion about the future of the European Research Area by publishing a Green Paper in April 2007, entitled 'The European Research Area: New Perspectives'. The document identified a number of areas that need to be addressed to work towards a better integration of research activities in Europe: well-coordinated research programmes and priorities, world-class research infrastructures, research institutions, a single labour market for researchers, sharing knowledge, and international collaboration. Intergovernmental organisations are recognised as one of the major stakeholders in the European Research Area and the Commission asked a provocative question: should the EU be a member of the intergovernmental research organisations? In the course of the year, the Commission

Iain Mattaj and the rectors of the universities of Oslo, Umeå and Helsinki, Geir Ellingsrud, Göran Sandberg and Ilkka Niiniluoto.

carried out a broad consultation with stakeholders in Europe. EMBL prepared a formal response to the Green Paper with its EIROforum partner organisations. Our internal analysis came to the conclusion that the EU cannot, under current regulations, be a member of EMBL and the public consultation produced a similar result. Based on an administrative agreement that was signed between EMBL and the Commission in 1995, we will however invite an EU observer to the EMBL Council meetings. The relationship between EMBL and the EC should be built on mutual recognition and reciprocity. EMBL's main role in the European Research Area is as a provider of major infrastructure in the biomedical sciences. EMBL receives a significant part of its external funding from the EU Framework Programme (17.1 million Euro in 2007). In FP6, EMBL was responsible for the coordination of 18 projects and participated in more than 80. In FP7 EMBL is so far coordinating three projects and is participating in eight. For an overview of external funding please see the financial report on page xvi.

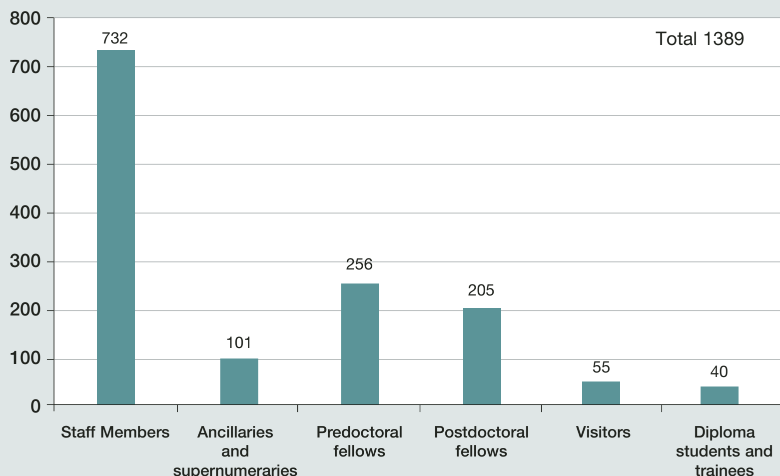
EIROforum

EIROforum is a partnership of the seven largest inter-governmental research organisations that provide infrastructure support to scientific communities in Europe: CERN, EFDA-JET, EMBL, ESA, ESO, ESRF and ILL. In September 2007, EIROforum submitted a formal response to the Commission's Green Paper. In this document, EIROforum emphasised the important role of the seven partner organisations in building the European Research Area, especially by providing some of the major research infrastructures and contributing to organising scientific communities. EIROforum endorsed the Commission's request for improved mobility of scientists, science education and coordinated international relations. It offered to continue to work together with the Commission towards solutions that will help to construct and upgrade major research infrastructures, improve career perspectives of researchers and make European science competitive on a global scale.

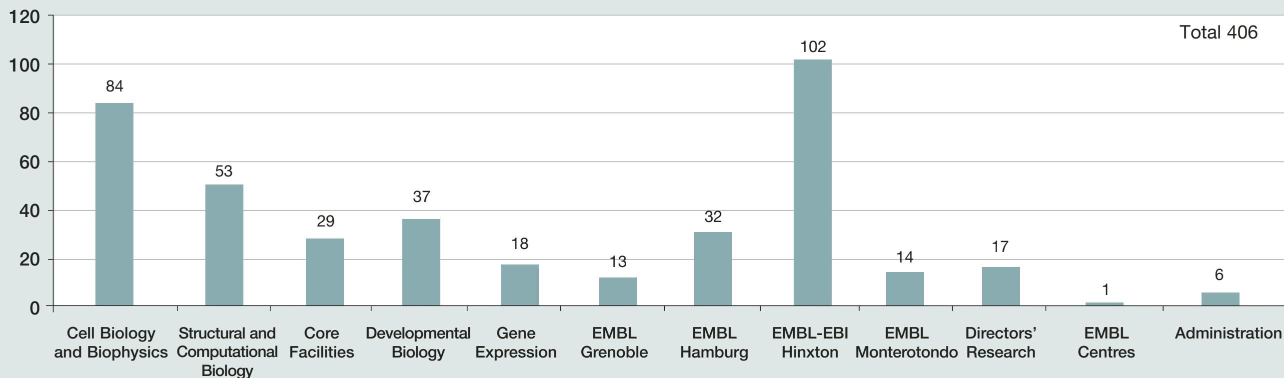
Personnel statistics

On 31 December 2007, 1389 people from more than 60 nations were employed by EMBL. 79% were from EMBL member states and 43% were female.

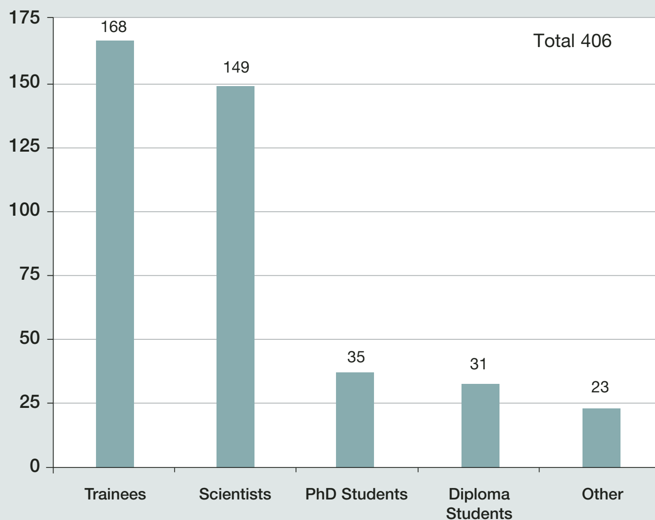
Personnel on 31 December 2007



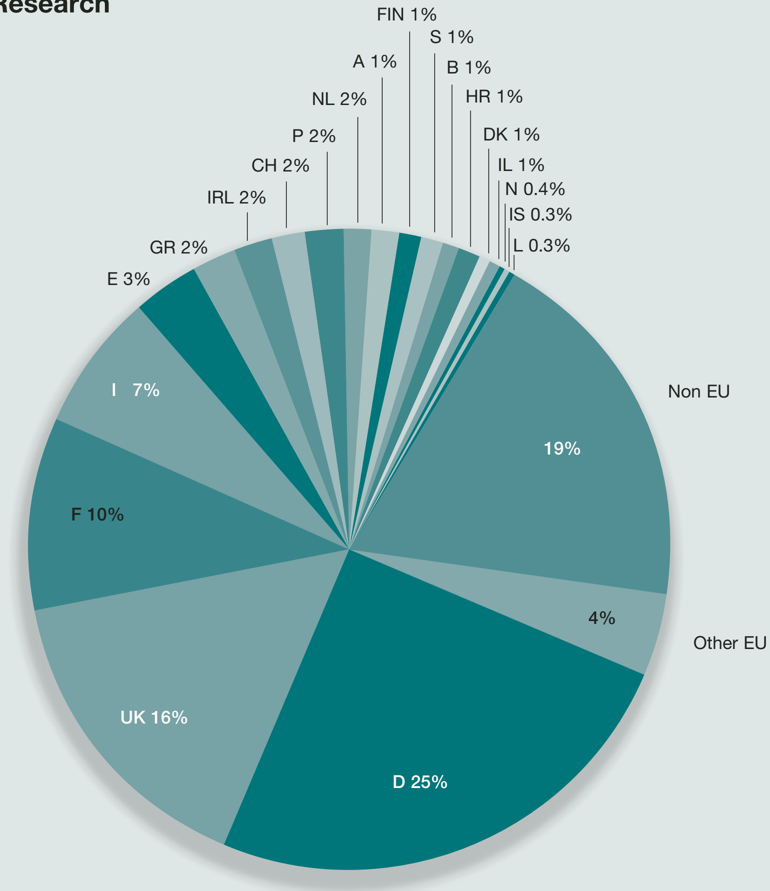
Visits to EMBL Units during 2007



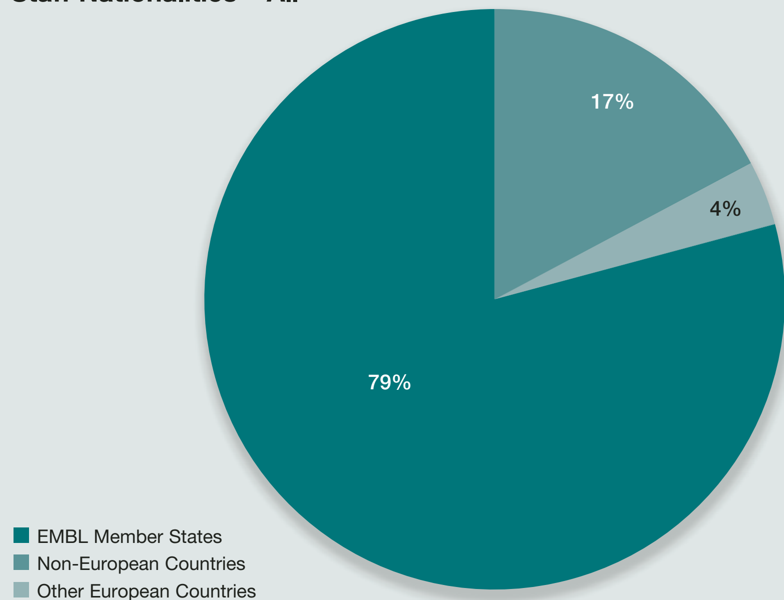
Visitor Types during 2007



Staff Nationalities – Research



Staff Nationalities – All



Financial report

External Funding (Grants)

	2007		2006	
	k Euro	*%*	*k Euro*	*%*
EU	17,124	47.5	15,648	50.5
NIH	5,908	16.4	2,981	9.6
BMBF	2,436	6.8	1,241	4.0
Wellcome Trust	2,038	5.7	2,717	8.8
BBSRC	1,244	3.4	1,210	3.9
DFG	1,090	3.0	1,619	5.2
Swissprot	957	2.7	1,343	4.3
HFSPO	583	1.6	406	1.3
VW Foundation	241	0.7	316	1.0
MRC	178	0.5	142	0.5
Others	4,265	11.8	3,383	10.9
TOTAL	**36,064**	**100**	**31,006**	**100**

Income/expenditure statement

INCOME	**2007**	**2006**
	k Euro	*k Euro*
Member states contributions	78,712	68,055
Internal Tax	18,139	16,972
External Funding	36,548	31,543
Other Receipts	13,786	10,861
Total Income Excluding pension contributions	**147,185**	**127,431**

EXPENDITURE	**2007**	**2006**
	k Euro	*k Euro*
Staff Costs	79,363	75,591
Operating Costs	40,107	36,320
Capital Expenditure	33,647	15,510
Total Costs	**153,117**	**12,421**
Surplus (deficit) transferred to reserves	(5,932)	10

Please refer to DVD for more information

Member states contributions

	Ordinary and one-off contributions				Pension contribution	
	2007		2006		2007	2006
	k Euro	*%*	*k Euro*	*%*	*k Euro*	*k Euro*
Austria	1,573	2.2	1,425	2.2	26	26
Belgium	1,927	2.7	1,746	2.7	31	32
Denmark	1,226	1.7	1,111	1.7	20	20
Finland	971	1.4	880	1.4	16	16
France	11,286	15.9	10,229	15.9	185	186
Germany	15,543	21.9	14,088	21.9	255	255
Greece	1,041	1.5	944	1.5	17	17
Israel	864	1.2	783	1.2	14	14
Italy	9,210	13.0	8,347	13.0	151	151
Netherlands	3,096	4.4	2,806	4.4	51	51
Norway	1,410	2.0	1,278	2.0	23	23
Portugal	822	1.2	745	1.2	13	14
Spain	4,945	7.0	4,482	7.0	81	81
Sweden	1,884	2.7	1,708	2.7	31	31
Switzerland	2,317	3.3	2,100	3.3	38	38
United Kingdom	12,731	18.0	11,539	18.0	209	209
SUB TOTAL	**70,846**	**100**	**64,211**	**100**	**1,161**	**1,164**
Ireland	730		463		12	8
Special contribution Ireland	–		146		–	–
Iceland	45		41		1	1
Special contribution Iceland	16		16		–	–
Croatia	67		62		1	1
Special contribution Croatia	23		23		–	–
Luxembourg	104		–		1	–
Special contribution Luxembourg	41		–		–	–
TOTAL CONTRIBUTIONS	**71,872**		**64,962**		**1,176**	**1,174**

Additional one-off contributions

	2007	2006
	k Euro	*k Euro*
Germany – for ATC construction	4,062	3,093
UK (BBSRC & MRC) – for EBI East Wing construction	2,778	–
TOTAL	6,840	3,093

2007/2008 Reviews of EMBL Scientific Units

EMBL Units are reviewed in depth every four years by expert international panels organised by the Scientific Advisory Committee. To ensure openness, the review reports are submitted in confidence to EMBL Council and the Director General. The formal responses of the Director General to the reports are made public, to communicate the adjustments made by the Laboratory in response to the reviews, when needed.

Director General's Response to the Developmental Biology Unit Review Report, Heidelberg, 9 and 10 May 2007

1. I thank the panel and its chair for their thorough evaluation of the Unit, and for their formal and informal input on the question of the future of the Unit after Stephen Cohen's imminent departure. The extensive experience and broad expertise of the panel together with the significant overlap in membership with the EMBL SAC meant that these discussions were particularly helpful.

2. In the view of the review panel, the research programmes of a considerable number of the groups in the Unit are outstanding. This reflects well on the individual group leaders as well as on the Unit Coordinator for his part in group leader selection and in providing an environment where the initial promise of young group leaders can be realised.

3. The review panel praised the contributions of several members of the Unit to the broader EMBL community. Specifically the commitment of time and effort to EMBL graduate and general training programmes (Anne Ephrussi) postdoctoral association (Pernille Rørth and recently Detlev Arendt) and the oversight of the Heidelberg Laboratory of Animal Resources (Mathias Treier) were all mentioned and commended.

4. Finally, I wish to join the panel in congratulating Stephen Cohen on his successful leadership of the Developmental Biology Unit over the last decade and to personally thank him for his many contributions to the Unit and to EMBL during his time here.

Iain W. Mattaj
Director General
16 May 2007

Director General's Response to the EMBL-EBI Services Review Report, Hinxton, 26 and 27 March 2007

1. This was the first review dedicated specifically to the EMBL-EBI service activities. These represent a complex array of diverse but interlocking activities that involve a diverse range of expertise. I thank the review panel for their detailed analysis of the service teams and leadership, and for several excellent suggestions for future planning.

2. The panel was unanimous in praising the enormous progress made in dealing with service-related issues raised at the last review four years ago. Positive mention was made in particular of the new web portal and search engine (EB-eye) and of progress towards a data integration strategy. I am pleased to acknowledge the effort put into these and other improvements by the leadership (Director, Associate Director) and staff of the EBI.

3. We acknowledge and agree with the panel's conclusion that it is important to maintain a strong integration of research and service activities at EMBL-EBI.

4. We note the panel's support for the plan to further increase our efforts in user training.

5. The panel recommends that advanced strategic planning is continuously required in order to deal with new challenges such as; the inevitable and imminent huge increase in DNA sequence data production; the need for an extension of the Ensembl model to the many poorly annotated genomes, both current and future; the need for changes in the structure of service teams to reflect their increasing size and complexity, as well as to prepare for inevitable future staff turnover at all levels. EMBL is constantly engaged in future planning and both I and the leadership of EMBL-EBI will act on this advice.

6. I acknowledge the support of the review panel for the view that EMBL-EBI needs to play a strong leadership role in planning for the future of bioinformatic data resource provision in Europe as part of the ongoing ESFRI process.

7. I appreciate the panel's advice that every effort needs to be made to ensure international (world-wide) integration of the core biomolecular data resources. As the panel points out, the EBI leadership must continue its energetic efforts in this direction for those data resources that currently see themselves in an intercontinentally competitive situation.

8. I will discuss with the EBI leadership the best way to implement the panel's excellent suggestion to establish a technical advisory board to provide state-of-the-art input to those engaged in provision of computing facilities or database administration.

9. Finally, we will implement the panel's suggestion to survey internal users of the computing facilities to asses their satisfaction levels during the preparation of the next such review.

Iain W. Mattaj
Director General
16 May 2007

Director General's Response to the EMBL Monterotondo Review Report, Monterotondo, 21 and 22 February 2008

1. I am very pleased by the conclusions of the review panel, who consider EMBL Monterotondo to be in a very healthy state and who provide a very positive evaluation of the output of all the research groups who have worked at EMBL Monterotondo over most or all of the review period (2004 – 2008). These conclusions validate the decisions of Council, following the last review, to recognise EMBL Monterotondo as an EMBL Outstation, rather than a programme, and to agree to modest growth of the Outstation in the 2007 – 2011 indicative scheme.

2. The panel greatly appreciated the excellent leadership of Nadia Rosenthal. They praised in particular her successful efforts in recruitment and in raising external funds, partly via participation in a number of important European initiatives in mouse biology, and her generosity to the other group leaders with respect to these funds. They note that participation in European projects contributes to raising the profile of the Outstation.

3. The panel notes that the Outstation is entering a period of considerable turnover. One established group left towards the end of the review period and three others will soon leave. Added to this will come the planned recruitment of at least one additional group. The panel points out that these changes will impose a stronger requirement than in the recent past for both leadership and mentorship from senior staff. I am exploring ways in which this need can be met without placing an undue load on Nadia Rosenthal.

4. The panel suggests that the profile of the Outstation may be further improved in the future by concentrating new recruitment around existing strengths, provided suitably qualified candidates can be found. We will follow this advice.

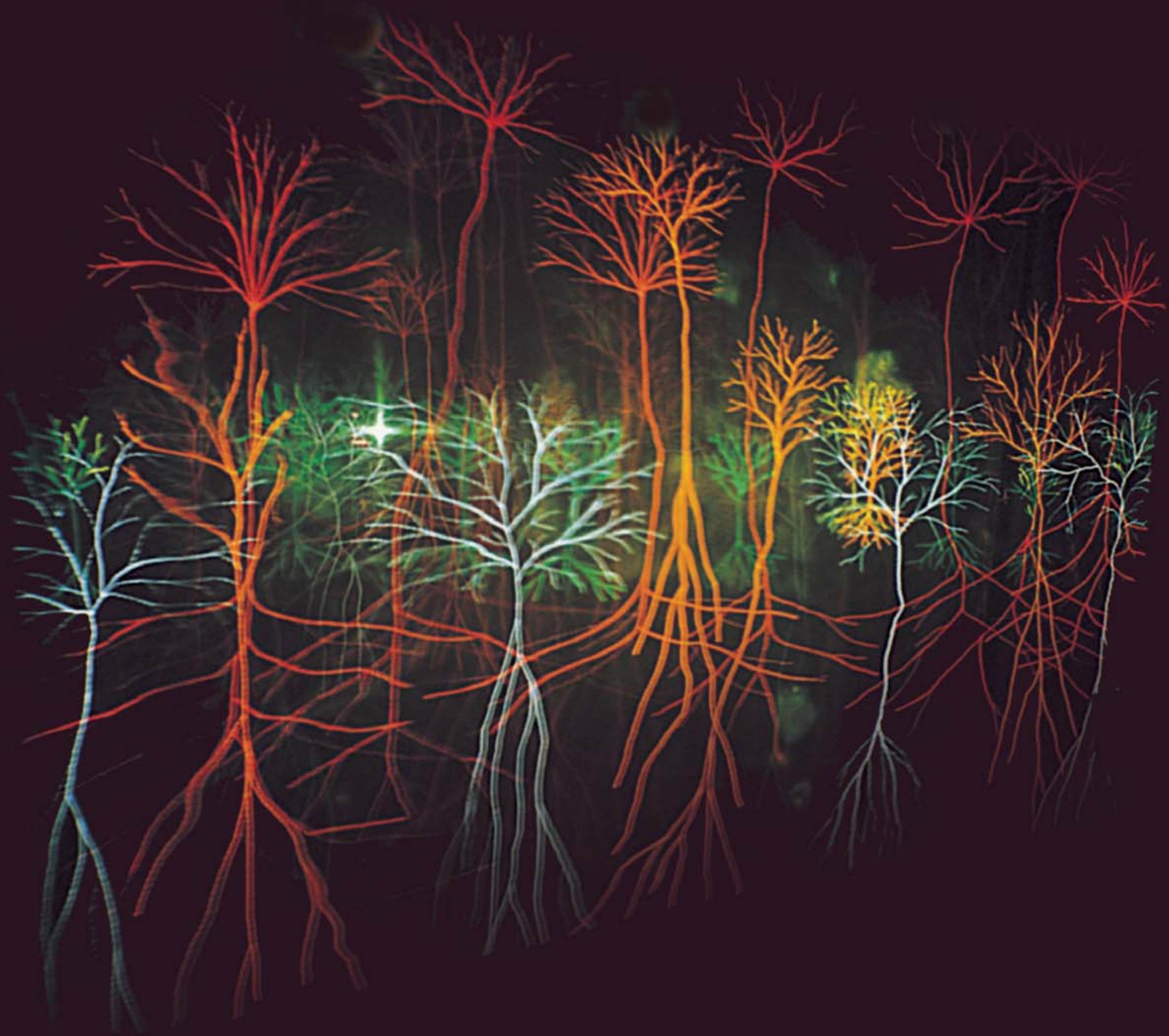
5. The panel notes the overall high quality of the facilities provided at EMBL Monterotondo but points out that success, and subsequent growth, of the Outstation has strained the capacity of a minority of the scientific support facilities, and provides good advice on where some investment may be required.

6. While being generally very positive about the productivity and scientific quality of the research groups, the panel provides constructive, detailed scientific advice to each of the group leaders particularly in regard to the focus of their future efforts. I am grateful to the reviewers for these comments, which are mainly aimed at encouraging the group leaders to sacrifice some breadth in their approaches, in favour of additional focus and depth.

7. Finally, I would like to thank the entire panel, and in particular the chair, Prof Roberto Di Lauro, for their concentrated effort in providing a detailed and extremely useful report on the Outstation in the rather limited time available for the review.

Iain W. Mattaj
Director General
31 March 2008

Scientific Report



The catalogue of life

MENTION DNA and most people immediately think of Watson and Crick's famous double helix. It has taken on a life of its own in the popular media as an eternal and unchanging icon, a Book of Life that contains precise instructions for making living things, a sort of biological reference text or encyclopaedia that keeps these instructions safe and constant.

But this image is deeply misleading. Real-life DNA is about as far from a lasting, immutable text as you can get. In the cell, it is constantly falling apart and coming under chemical attack – the DNA in your body suffers some 300 000 trillion damaging hits each day – and must regularly be repaired. What's more, DNA doesn't merely sit in the cell and issue instructions. It constantly responds to changes in its environment and alters its behaviour accordingly.

So DNA is less like a dusty tome and more like a vibrant wiki, a webpage that can be edited by its users and so keeps pace with an ever-changing world. One of the most famous wikis of course is Wikipedia, an online encyclopaedia whose vast content is shared and kept up-to-date by its diverse range of readers.

DNA and the work EMBL scientists perform on it have many parallels with Wikipedia. Perhaps the most obvious is the ENCyclopedia Of DNA Elements, or ENCODE, a huge international project that aims to catalogue all the functional elements in the human genome. Another concerns efforts to find and characterise all the genes in a given environmental sample – be it soil or the human gut – to create a 'metagenome' for that environment, an advance that will have a huge range of applications.

Of course, catalogues like these need to be shared effectively with users. Here, EMBL is leading the way by developing standardised methods that allow researchers around the world to share and manage data from a wide range of bioinformatics projects quickly and efficiently.

Such resources are smoothing the path of research into how DNA influences our lives, and that of living things in general. Researchers at EMBL are working on a number of projects, such as how our genetic make-up can affect our chances of becoming obese, or how environmental factors – such as disease pandemics or climate change – will affect life on Earth.

Always with an eye on the future, EMBL trains the next generation of biologists through its PhD programme. Now in its 25th year, the programme attracts students from diverse backgrounds. Their rich catalogue of life experiences will prove invaluable as they come to write the next chapters in DNA's dynamic inventory. ■

KAKSONEN
GÄRTNER
HAUSMEISTER
BRIGGS
CHRISTOPH MÜLLER
PROTEIN PURIFICATION
CARLOMAGNO
WILM
GAVIN
To the occupant
INTERNAL AUDITORS

Peer Bork, Joint Coordinator of the Structural and Computational Biology Unit in Heidelberg

A vast metabolism

'TO THE OCCUPANT'. A large brown envelope had just arrived with your household mail, and it doesn't look promising. Inside is a thick booklet full of questions to be answered and boxes to be ticked, together with a letter informing you that its completion is compulsory by law. A census questionnaire. Ugh. You hate filling out forms, especially ones from the government asking personal questions. You file it away in your 'to do' tray until you really can't put it off any longer.

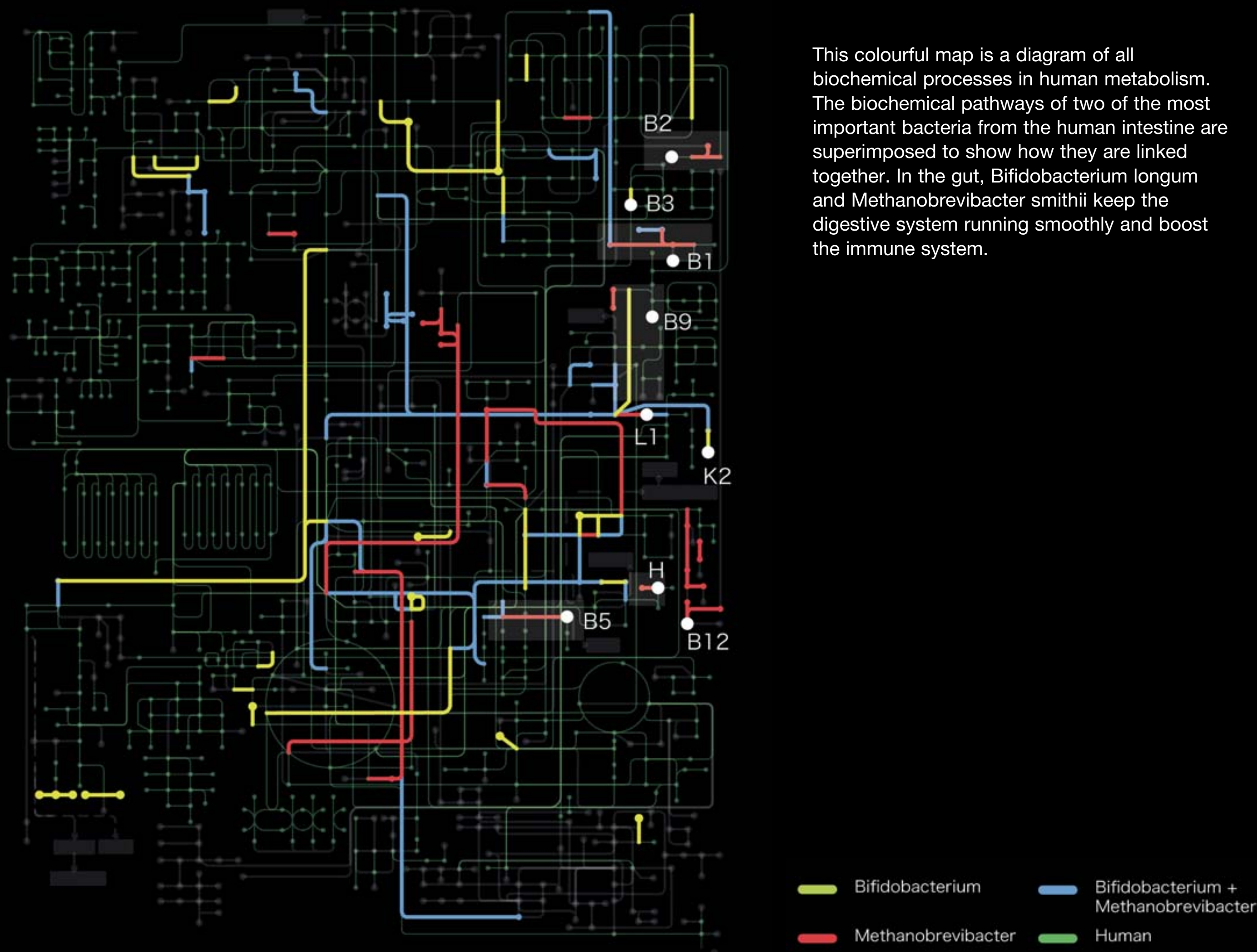
But if you think filling in the questionnaire is tedious, spare a thought for the government officials who then have to trudge from house to house, chasing up missing questionnaires and persuading grumpy residents to hand over information about what they do for a living and how many bathrooms they've got. Then imagine what this job would be like if you didn't have the threat of fines and penalties to extract your information, or if you had no idea who was living in your area and no way of seeing them or talking to them. That will give you some idea of the challenge faced by Peer Bork and his colleagues, who are involved in projects that aim to take a census of all the microbes living in a range of different environments.

Peer, Joint Unit Coordinator and Senior Scientist at EMBL Heidelberg, together with his team and other colleagues there and at EMBL-EBI, is blazing a trail in a new branch of genetics called metagenomics. Metagenomics involves sequencing DNA to find out what microbes are present in a sample, such as a fragment of soil. But unlike conventional genome sequencing, which relies on isolating an individual microbial species and sequencing its whole genome, metagenomics simply looks at all the genes present in the sample, regardless of where they came from. So rather than studying the genome of a single microbe, you are effectively studying the collective genome of an entire ecosystem: the metagenome.

There are several good reasons why scientists want to do this. The first is to overcome a problem that has dogged microbiology for decades: the fact that the vast majority of microbes can't be grown easily in the lab. This means they haven't been able to study such species or collect enough DNA from them for sequencing. As a result, biologists know only a fraction of the bacteria, fungi, viruses and other microbes that live in environments such as the sea, the soil, or even our own bodies.

As well as letting scientists get an idea of what is present in a sample, metagenomics also lets them see what these invisible residents do for a living. It's a bit like doing a census where, instead of talking to people, you wander around their houses or offices, looking for tools or equipment that tell you what their profession is. Metagenomics researchers sequence short fragments of the genes in a sample and then try to work out what metabolic processes the proteins they make are involved in. Many of these metabolic processes have evolved to let their owners survive in a particular niche. "We take this soup and look for metabolic adaptation to that habitat and to whatever environmental constraints exist," explains Peer.

What's more, advances in computing power and new bioinformatics techniques, including those developed by



This colourful map is a diagram of all biochemical processes in human metabolism. The biochemical pathways of two of the most important bacteria from the human intestine are superimposed to show how they are linked together. In the gut, Bifidobacterium longum and Methanobrevibacter smithii keep the digestive system running smoothly and boost the immune system.

EMBL scientists such as Peer, are letting researchers work out how these metabolic processes interact, giving them an insight into how the microbes are cooperating and competing with each other. "In a way, it's modern ecology," says Peer. "Only one level lower." So it's little wonder that the idea of metagenomics has really caught the imagination of biologists around the world. "Excitement really came with this topic," says Peer. "It's a new angle, with the hope for various applications such as new energy sources, better food and treatment for diseases."

At the moment, two fields are emerging. The first is the global approach, which aims to study the microbial metagenome of the whole world by looking at environmental samples. The second has a medical slant: performing a census of all the microbes that live in and on the human body. In 2006, for example, biologists published the first metagenomic analysis of the microbes in the human gut. "The potential is obvious," says Peer. "In the gut, for example, the microbial communities are known to influence things such as obesity, cancer and bowel disease." But the gut is only the start.

In the USA, the National Institutes of Health has launched a €115 million programme to establish metagenomics research to exploit microbial communities in and around the human body. In parallel, the European Union, China, Canada, Japan, India and Australia also have projects. Currently, funding bodies and researchers around the world are putting together an International Human Microbiome Project, which will examine every nook and cranny of the body, including the mouth, vagina and skin. Peer's team will analyse the vast amounts of data that will be generated by a large European gut project, and his group, together with Rolf Apweiler, Ewan Birney and their colleagues at EMBL-EBI are also spearheading the bioinformatics efforts to tackle the unprecedented amounts of information and analyses being generated by such projects worldwide.

Metagenomics really got going in 2004, with the publication of two influential papers. One, from a group of researchers at the University of California, Berkeley, looked for genes from the microbial denizens involved in producing acidic drainage water from a mine. A month

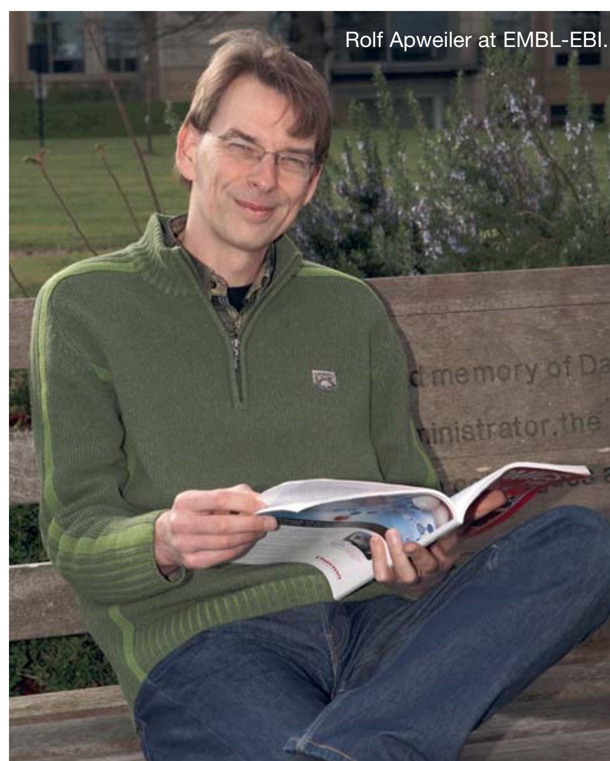
later, a team headed by the commercial genome sequencing pioneer Craig Venter published findings of a study on the microorganisms populating the sunlit surface waters of the Sargasso Sea. "These were two different habitats, but the concept was very similar," says Peer. "They just sequence whatever they find in their samples, to look for all the metabolic adaptation to that particular habitat."

It was around this time that Peer and his colleagues realised that there was a pressing need for new software, storage and bioinformatics techniques to manage and exploit all of the data. This foresight, together with its existing expertise in handling and annotating the sequencing data, has put EMBL ahead of the pack when it comes to metagenome bioinformatics. "We were early in the game," says Peer. "We have four years' experience in the field and I'm very proud of it. It gives us a jump start."

As a result, Peer is optimistic that he will be able to extract useful information from the bits and pieces of data coming from samples of many healthy and diseased people. This information could offer hints as to the causes of disease. "Two individuals are very different in their species composition," explains Peer. "We need a lot of sampling, not just from one human being but quite a few to understand the difference between species and between disease stages."

But Peer's sights are not confined to the human body. He and other EMBL scientists, including Eric Karsenti and Detlev Arendt, both at EMBL Heidelberg, are planning several environmental projects such as a two-year boat trip where samples are taken from all over the world. Bioinformatics teams in Heidelberg and at EMBL-EBI are helping to unite all the data from the various projects in a number of ways, such as storing the information in appropriate databases. One of these is a new extension to the International UniProt database called UniMES, short for the UniProt Metagenomic and Environmental Sequences database. UniMES is housed at EMBL-EBI, and was developed by Rolf's team there.

A key outcome of Peer's work is understanding how the metabolisms of all the different microbes in a sample work together as a network to let the community thrive. Although nearly all will have a core set of metabolic processes needed for life, many will also have specialist genes that allow them to flourish in their own particular niche. Going back to the census analogy, a microbial ecosystem is very much like the high street in a small town. Such a street might have a baker, a butcher, a fishmonger, a dentist and perhaps a plumber (with a rival plumber or two). No single person could hope to practice all these professions at once. But by sharing the effort between different specialists, the small town thrives as a community of interdependent individuals. "We're talking about huge complexity," says Peer.



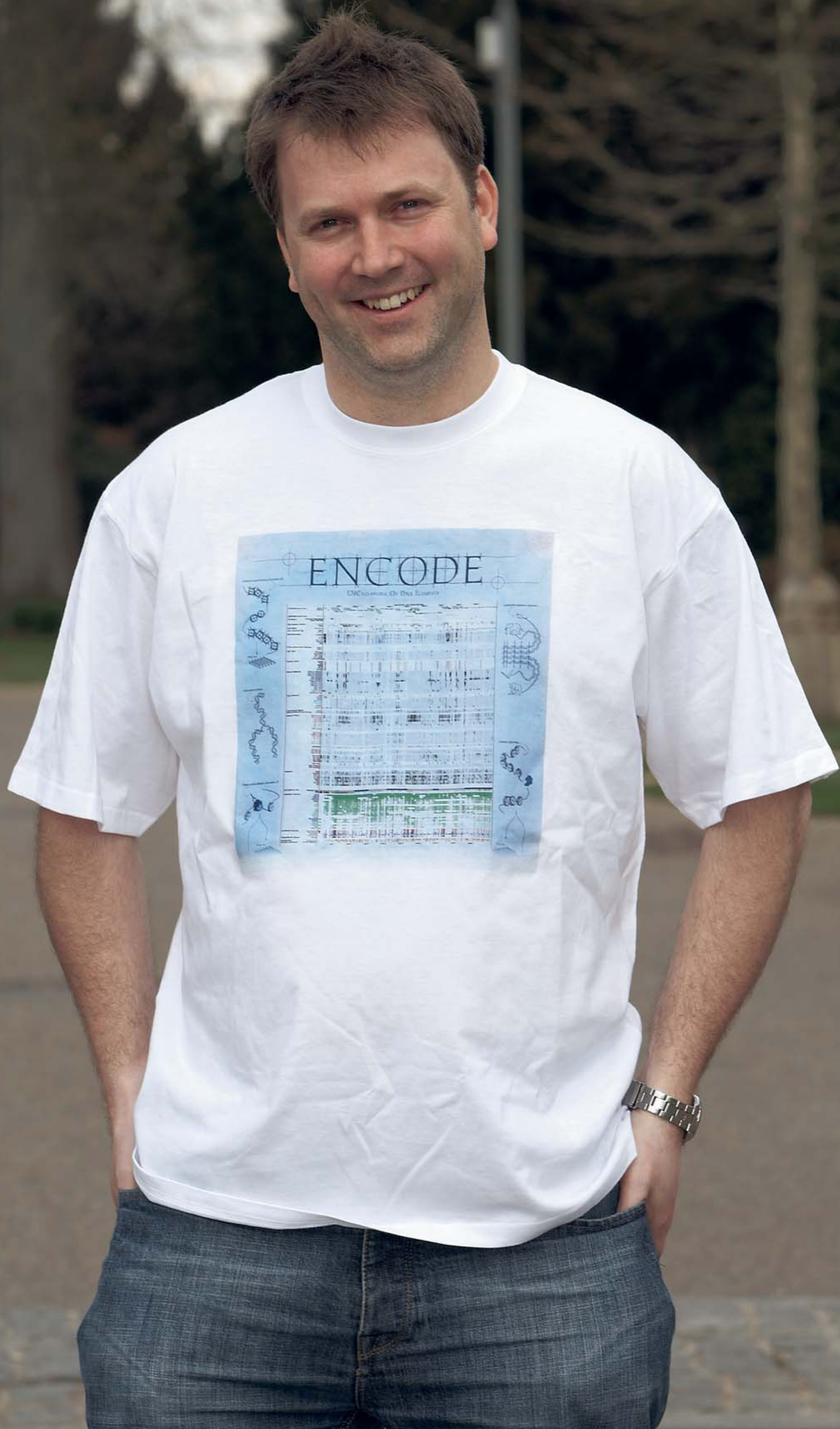
Rolf Apweiler at EMBL-EBI.

To help them get the most out of their analyses, Peer has teamed up with some Japanese colleagues to produce a kind of map through which one can easily navigate: a diagram of all the biochemical processes known to exist in nature (see page 6) and how they link together. They then see what processes are active in a given microbial ecosystem. This helps them identify new enzymes or processes that could be useful for cleaning up oil spills, for example. Or it could help identify bacteria that produce compounds that restrict the growth of harmful bacteria in the gut. Patients might then be able to eat foods or other harmless substances that favour the growth of these 'pest-control' bacteria – an option that avoids the side effects of antibiotics. "You could drive the community into more of a healthy state again," says Peer. "There are tonnes of other exciting outlooks."

Despite the considerable challenges facing the field, it seems that metagenomics is set to transform our understanding of the biological world – a vibrant and exciting prospect that is about as far from a boring census form as you could get. "This whole field is in an early phase," enthuses Peer. "We hope we can contribute to early discoveries."

Human Microbiome Project:
http://nihroadmap.nih.gov/hmp/

European MetaHIT Project:
http://locus.jouy.inra.fr/metahit/index.php ■


ENCODE

Ewan Birney led the data integration and analysis work in the ENCODE project

A new world order

WHAT IS A GENE? It seems like a simple enough question. After all, it is more than half a century since molecular biology pinned the mechanism of inheritance on DNA, and figured out its structure. Thanks to these discoveries and others like them, biologists thought they had a nice pithy definition of exactly what a gene is. It now seems they were wrong.

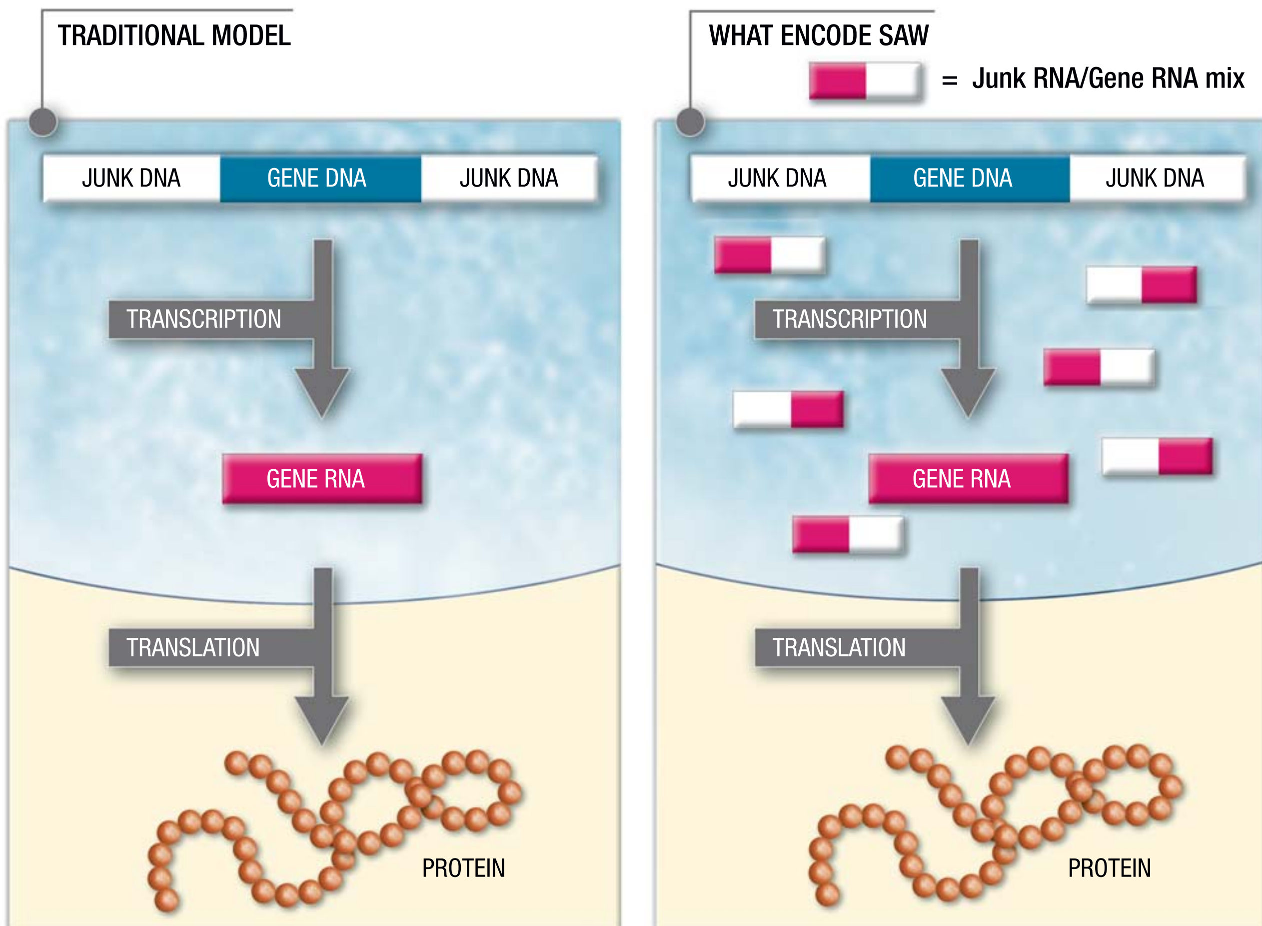
Over the past few years, there have been whisperings in the scientific literature that not all genes in the genome fit the tidy description detailed in molecular biology textbooks. But last year, EMBL-EBI researchers, together with their colleagues around the world, unveiled findings that are forcing a major re-think of these long-cherished ideas. Genes are not, as was once thought, discrete stretches of DNA, arranged like stately mansions fenced in on neat private estates. Instead, they are far more like the camping site at the Glastonbury rock festival, packed with pot-brained hippies blundering across each others' tents and spouting gibberish. For many biologists, the idea has taken some getting used to, according to Ewan Birney, head of genome annotation at EMBL-EBI.

The source of this consternation is a massive international research effort called the Encyclopedia Of DNA Elements, or ENCODE. Ultimately, ENCODE aims to discover and catalogue every molecular transaction that takes place across our genetic blueprint, or genome. It's building on the Human Genome Project, which discovered the order of DNA bases – chemical letters – that make up the genome, but didn't reveal what most of these letter sequences meant. "There are lots of unknown things in the genome," says Birney, who led ENCODE's data integration and analysis. "We want a much richer view that tells us which bits do what, where, why and how."

But scrutinising the entire 3 billion letters of the human genome is a truly Herculean task. Rather than jumping in headfirst, researchers decided to begin with a pilot phase, studying a mere 1 per cent of the sequence. "That's actually quite a lot of human genome!" says Ewan. "You can draw a lot of inferences about what is going on in the rest from that 1 per cent."

The US National Institutes of Health (NIH) funded 20 groups around the world to conduct the pilot phase and one of these was Ewan's group, which, together with Nick Goldman's group at EMBL-EBI, headed the analysis of the data produced by other members of the ENCODE consortium. When the findings were published in the journal *Nature* last year, it listed a total of 308 authors, which gives you some idea of the Gordian challenge facing the four-year project. "A lot of what ENCODE told us is that the genome is quite a complicated place," says Ewan, "which shouldn't surprise anyone who thinks about how the genome encodes something as complex as us."

Although ENCODE filled in a lot of the finer details about our genome's biology, the biggest surprise came when the team looked at a process called transcription. Genes are DNA sequences that spell out instructions for making proteins and other molecules needed for the cell to function. Transcription is one of the first steps in the process of producing such molecules from these instructions.



It begins when the cell selects a stretch of DNA and starts making a copy of its sequence using a chemical relative of DNA called RNA. The cell then edits this transcript and, if it is made from a gene that encodes a protein, sends it off to be used as a template in the protein-making process. For a long time, biologists thought that all genes encoded only proteins. It is now clear, however, that many do not. Instead, they contain instructions for making RNA molecules that perform particular jobs within the cell.

The discovery that genes could encode a vast range of RNA molecules as well as proteins had already shaken ideas about what a gene really is. But the ENCODE findings revealed that the cell is transcribing far more RNA than biologists ever imagined. Even more surprising, the transcription was often running through the supposed boundary between one gene and the next. There were ten times more 'start' signals for transcription than protein coding genes. Bizarrely, many of these start sites cropped up in unexpected places, such as in between or even within protein-coding genes. "Probably the thing that's freaked some people out the most is the amount of transcription we saw," says Ewan, "and that transcription didn't really fit into nice gene boundary-style packages. The transcripts just seemed to be refusing to behave in that way." Exactly what these 'wacky' transcripts are doing remains an open question.

For Ewan, however, the biggest revelation was the scant similarity between the functional elements of different mammals. The pressures of natural selection mean that evolution tends to keep important processes, such as how the transcription of a particular gene is controlled, broadly the same through successive generations. So biologists expected to find more similarities than differences between different mammalian species. "Instead we saw this very big non-overlap," says Ewan. On average, about 50 per cent of the non-protein-based functional elements were not conserved across mammals. "That's not what we expected when we went into this."

Part of the explanation lies in the fact that some of these differences are what makes each species unique. But Ewan and his colleagues suggest that a lot of these elements are 'neutral', created by chance, but not selected. "Evolution doesn't really care whether it's there or not there," says Ewan. "I think it's quite a challenging concept for a lot of molecular biologists to embrace."

This has important consequences for researchers. At the moment, says Ewan, when biologists discover a biochemical process happening in the human genome, they assume that it has an important function that has been favoured by natural selection. What ENCODE reveals, however, is that they should instead assume the opposite:

that it is not significant. They should then perform further studies – such as looking to see whether other mammals share a similar biochemical process – before passing judgement.

Ewan's group is one of ten that have now been funded by the NIH to scale up ENCODE to cover the whole genome over the next three years. In the meantime, the pilot phase has given biologists plenty to chew on. What are all these extra transcripts doing, for example? Ewan isn't convinced that they have to be doing anything special. They could, he suggests, merely be the by-product of the cell's day-to-day metabolism, or simply a way of stabilising a section of DNA while it is being transcribed.

Although this is possible, a number of scientists argue that many of these RNAs could indeed have a function, albeit one that remains to be discovered. They point to the wealth of studies demonstrating the wide range of important jobs that RNA molecules perform in the cell. The answer could involve all of the above, thinks Ewan. "That's where the drama in the field is," he says. "How much of it is RNA doing stuff, how much is it the action of transcription but not the RNA itself, and how much of it is just neutral?"

This idea of neutral, or unimportant, molecules could also apply to another puzzling ENCODE discovery, made with the help of the BioSapiens consortium. Once transcripts are produced, the cell edits them before using them to make proteins. But a lot of this editing results in transcripts that don't make sense: any proteins produced from them would be misshapen and non-functional. Why the cell should allow this to happen is a question structural biologists are already pondering.

So where does all this leave the gene? While ENCODE questions the simple molecular description founded in the late twentieth century, biologists can still fall back to the 1860s, when the father of modern genetics, Gregor Mendel, published his laws of heredity. He described 'particles' of inheritance that transmitted traits down generations of pea plants, ideas that were built on by later scientists to define the gene by what it does. So although the molecular details may need reworking, the essential concept still stands. "Does this change the definition of the gene?" asks Ewan. "Personally, I think it's fine. What ENCODE tells us is that transcription is much more complicated than we thought before."

The ENCODE Project Consortium (2007) Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. *Nature* **447**: 799–816

Tress ML, et al (2007) The implications of alternative splicing in the ENCODE protein complement. *Proc Natl Acad Sci* USA **104**: 5495–500 ■

The new East Wing of EMBL-EBI offers modern office space and a new training suite

Janet Thornton, Director of EMBL-EBI

Roadmap to the future

BIOLOGY IS GETTING BIGGER. Disciplines such as genomics are generating vast amounts of data that need to be managed effectively, while others, such as structural biology, also need access to large or expensive resources. They are not alone: sciences such as physics and astronomy need costly instruments and huge facilities, such as particle colliders and telescopes. To tackle the massive costs and logistics of doing this kind of science, countries need to cooperate, share materials and put infrastructures in place to help them meet these new challenges.

In 2002, the European Strategy Forum on Research Infrastructures, or ESFRI, was set up to develop a more coordinated approach for policy making in the field of research infrastructures. In 2005, it published the first European roadmap for research infrastructures in which it identified 35 large-scale projects that need to be put into place. The Commission issued a call for a preparatory phase, with a view to getting each of the 35 projects to draw up plans that would allow them to secure long-term funding.

EMBL is involved in four of the six biomedical projects. EMBL-EBI is leading a proposal called the European Life Sciences Infrastructure for Biological Information, or ELIXIR for short. Its mission is to make a plan for the construction and operation of a sustainable infrastructure for biological information to support life science research in Europe and its translation to medicine and the environment, the bioindustries and society. All EMBL structural biology units are involved in another proposal, INSTRUCT, which is geared towards structural biology infrastructures. Below, EMBL-EBI Director Janet Thornton, who is heading the ELIXIR proposal, talks about its goals and plans for the future.

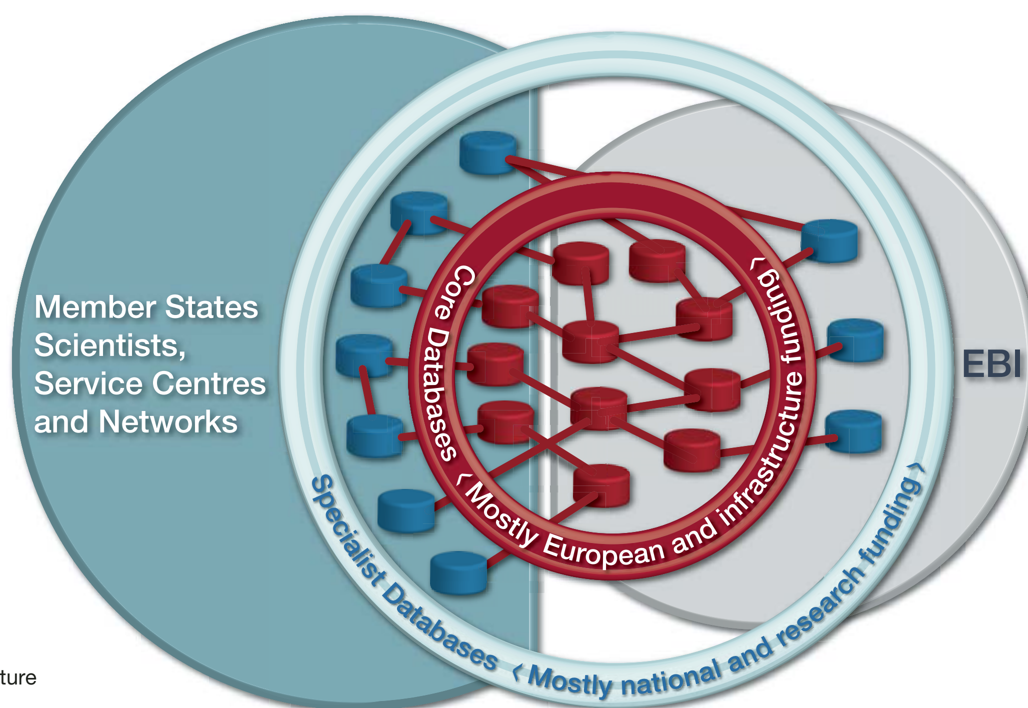
How does the ELIXIR proposal fit in to the ESFRI scheme?

Bioinformatics was seen as a very good biomedical infrastructure: biological data is really what drives biological research, pharmaceutical research and the biotech industry. The provision of this data, its curation and looking after it is critical. When research councils fund experiments, they want to see the output, and one concrete way to do this is to get it stored in a data resource of some description that anybody can access – so that it's not lost.

EMBL-EBI is now in the process of coordinating the definition of the scope of ELIXIR, by consultation with our users, the depositors of data, the industry partners and with all the different countries. We are also trying to work out a mechanism that would fund it.

EMBL-EBI already provides excellent data resources and management. Why is there a need for a pan-European infrastructure?

In Europe, we are fortunate because we do already have EMBL-EBI. Our resources are part of a global network of data repositories, with whom we exchange data on a regular basis. These 'core' resources are really the fundamental reference databases. But there is also a number – about 700 – of specialised molecular data resources that are dis-



A possible structure for ELIXIR

tributed in different people's laboratories around the world. They don't tend to be international collaborations, and are often only in one lab, made by people doing their own research and are not accessible from a centralised portal.

Obviously, we need to integrate all of this data where appropriate and make sure it remains accessible and available long-term. It's very timely to exploit the new developments in e-science, such as the Data GRID, to really establish a robust infrastructure across Europe for our data. As well as benefiting academic research, it's clearly of great relevance for medicine, agriculture, health, forestry, fishing, biotechnology – for many disciplines.

What elements would be included in the new infrastructure?

We would interlink the core and the specialised resources with each other and with the literature. We would also be responsible in some way for developing the standards for new data, such as image data, and models for how it should be stored, used, exchanged or compared.

We would also develop new resources often generated by new technological advances, as appropriate. We need to integrate and interoperate between the data, some of which will be here at EMBL-EBI, some of which will be distributed. And we obviously need to build access through user-friendly portals: for example, we're working with our industry partners to develop a druggability portal that would allow users to ask questions about which protein in the human genome are druggable.

Why does it need new money? Isn't this already covered by government and institutional funding?

The problem is that, because of the worldwide web, bioinformatics is international. Everybody uses the databases, but it's not clear who should pay for what. Because of this, national governments are often reluctant to give funding.

Currently at the EBI, we get 50 per cent of our funding from EMBL, representing the investment of member states, which will rise to 60 per cent by 2011.The remaining funding comes from grant applications from many diverse sources, including the EU, the US NIH and the Wellcome Trust, but the non-rolling nature of these grants makes it hard to plan and build a sustainable resource. Even to keep pace with the current data flow will require additional investment. We are already facing the challenge of a second explosion of data from many new technologies, for example the new sequencing methods. A new physical infrastructure will be needed at EMBL-EBI to store and provide all the data. In addition a coordinated European response to new challenges requires additional investment to build a trans-national network of resources, plus the infrastructure to provide the compute power needed as more complex computational methods and approaches, which exploit huge data sets, are developed and used.

Instructive Experience

Bioinformatics is not the only arena in which EMBL is playing a key role. Structural biologists at the Grenoble and Hamburg outstations as well as in Heidelberg are involved in an infrastructure proposal known as the Integrated Structural Biology Infrastructure, or INSTRUCT.

Coordinated by David Stuart at the University of Oxford, INSTRUCT's goal is to build a network of centres of excellence, or core centres, where state-of-the-art facilities for techniques such as electron microscopy, nuclear magnetic resonance, X-ray crystallography, advance light microscopy and protein production will be developed in an integrated environment and made available to the European community.

As well as spreading the considerable cost of these technologies between nation states, INSTRUCT aims to promote the integration of structural biology with molecular and cell biology and so help researchers understand how molecules work together as a system to create the cell as a whole. "You have to be connected to biology," explains Stephen Cusack, Head of the EMBL Grenoble outstation and EMBL's representative in INSTRUCT. "But to do this, structural biologists need access to a broad spectrum of different techniques."

The infrastructure will also help drive the development of new research technologies – an area where EMBL structural biologists already have an impressive track record. A key feature of INSTRUCT will be to aid cooperation between the core centres and with secondary centres in many countries and increase the accessibility of their facilities to researchers. Here, EMBL Grenoble can point to the success of the Partnership for Structural Biology, a five-year-old collaboration between the outstation, the European Synchrotron Radiation Facility, the Institut Laue Langevin and the Institut de Biologie Structurale, as a model for coordinating different technological platforms.

INSTRUCT's preparatory phase began in April 2008 and is expected to take two years, and involves a total of seven core centres (including EMBL) across Europe in France, Germany, Italy, Israel and the UK, together with their governmental agencies. "With €4.5 million of funding, the aim of this phase is to turn the initial forward-looking vision into a practical plan that will be supported by the different centres and their governments," says Matthias Wilmanns, Head of EMBL Hamburg.

What stage has ELIXIR reached so far?

We're just starting. We've got €4.5 million for three years from the EU to deliver an agreement to allow us to construct and operate the infrastructure. To me, the challenge is to create something that is sustainable, fundable and sensible and that will be long-lasting; a framework for addressing these issues over the next 20 years. We have to get the stakeholders on board, and that includes the funders, the people who provide the databases – both the core and the specialists – the experimentalists who provide the data that go on these data resources, the people who provide the tools and all the people who use the data. This is a huge constituency.

There are many ways in which this initiative will contribute to European science. It will optimise access and exploitation, it will make sure that the data are not lost, so it protects previous investment and increases the competence and the size of the user community by strengthening the national efforts. Hopefully we will be more effective because of this infrastructure in research and in industry in Europe. It would also give Europe one voice in the global community on these aspects.

www.elixir-europe.org ■

Mathias Treier and Henriette Uhlenhaut:
What is the secret of staying slim?

The years of plenty might be numbered

IT IS SO UNFAIR! Some people – not even great athletes or sports fanatics – can eat as much as they like without gaining a single pound. You, on the other hand, only look at a piece of chocolate cake and you can already feel it on your hips.

Inspired by the question of why some people are more susceptible to obesity than others, Mathias Treier and his group at EMBL Heidelberg set out to uncover the genetic and molecular mechanisms that control body weight. The same mechanisms are key to understanding the processes that lead to obesity. "Maintaining a healthy, stable weight is not easy, neither in everyday life nor with respect to the biology involved," says Mathias. "The control of body weight depends on many different mechanisms and factors."

> *"Maintaining a healthy, stable weight is not easy, neither in everyday life nor with respect to the biology involved."*

Broadly speaking, body weight is a balance between food intake and physical activity, ignoring the energy spent for basal metabolic activities such as digestion. So simply put, your weight is determined by how much you eat versus how much you move. It sounds straightforward, but this equation is not quite as simple as it seems, because only a fraction of our physical activity is voluntary motion like walking, running or playing sports. The other substantial part is so-called spontaneous physical activity, subconscious movements such as fidgeting while working on the computer or the nervous tapping of fingers on the table without realising it.

Even though we are not consciously aware of these movements, our brain triggers and controls them. More precisely the hypothalamus, a structure deep inside the brain that manages fundamental and evolutionarily determined behaviours such as eating, drinking, sleeping, reproduction and spontaneous physical activity. As fundamental as these processes are, little is known about the neural circuits that govern them. In particular, the biology underpinning the control of subconscious movements is far from being understood. Scientists are sure that there is a genetic basis to involuntary motion, but the genes and molecules involved have not yet been identified.

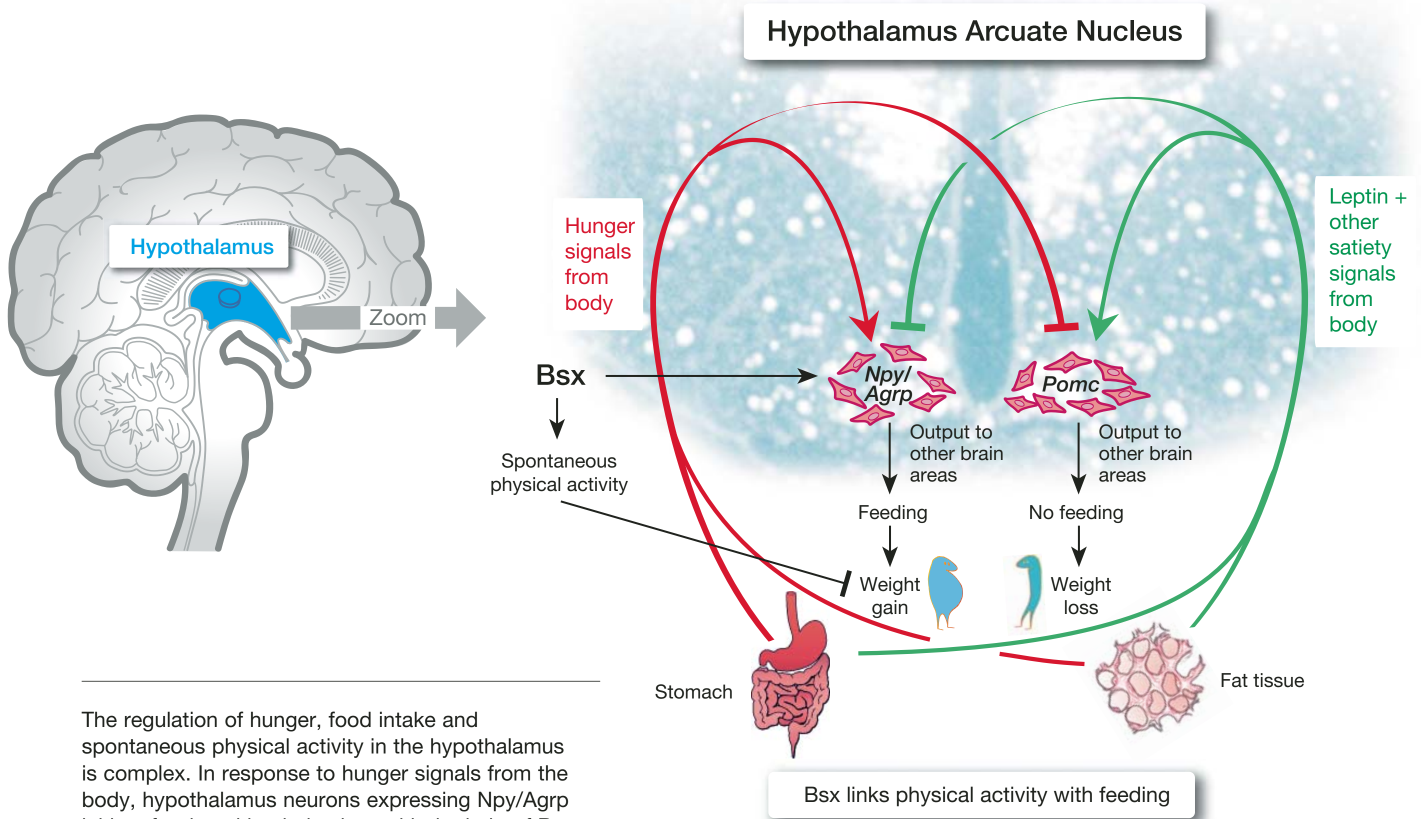
"We know that spontaneous physical activity is directly linked to eating," says Mathias. "When we are full we are lazy and only move little, but when we get hungry we start to be more agitated. This connection is rooted in our past, when humans still needed to go hunting and foraging. The body prepares itself for food seeking by becoming more active."

Together with PhD student Maria Sakkou, Mathias went looking for the molecular link that connects feeding behaviour with spontaneous physical activity in mice.

Using knockout techniques (see page 92), they genetically engineered mice that lack Bsx, a molecule thought to be crucially involved in feeding behaviour. Observing the resulting behaviour of their mice, the scientists knew they had hit gold when they realised that the mice were much lazier than their normal counterparts. Locomotor activity, the mouse equivalent of spontaneous physical activity in humans, was reduced by half and the mice only rarely went looking for food even after extended periods of starvation. Paradoxically, they still had an increased fat mass. Maria discovered the reason for this lack of food-seeking behaviour using sophisticated molecular techniques: without Bsx the mice had a much lower concentration of feeding hormones in their hypothalamus. These hormones, called NPY and AgRP, are normally produced in the hypothalamus in response to the hunger signals released into the bloodstream by stomach, intestine and fat tissue and transported to the brain. Neurons in the hypothalamus detect these signals and respond by releasing NPY and AgRP, which signal to higher brain centres that then induce feeding behaviour.

Bsx acts as a transcription factor, a regulatory protein that binds to DNA and influences the expression of other proteins including NPY and AgRP. Without Bsx, hunger signals from the body no longer trigger the release of these hormones, which results in the mice feeling no hunger. With no feelings of hunger, there is also no point for the mice to become agitated and increase their locomotor activity in the search for food, which explains why they are so lazy. Influencing both food intake and spontaneous physical activity, Bsx acts as a molecular link that connects the two behaviours at their control centre in the hypothalamus.

One paradox remains, however. Why are mice that lack Bsx fatter than their normal fellows if they never feel hungry? This is due to the artificial conditions that life in the lab imposes on the mice. “They do not have to go foraging, because they are fed. This means the mice eat even though they do not feel hungry. And since the lack of Bsx reduces their spontaneous physical activity, they burn less fat than normal mice and turn out a bit chubbier. This is essentially the same dilemma that we are facing in wealthy



The regulation of hunger, food intake and spontaneous physical activity in the hypothalamus is complex. In response to hunger signals from the body, hypothalamus neurons expressing Npy/Agrp initiate food-seeking behaviour with the help of Bsx. Satiety signals inhibit these neurons and instead activate neurons expressing pomc, which induces weight loss.

societies where food is always available. As we see, the consequence in many cases is obesity," Mathias explains.

Does that mean Bsx might be the key to obesity? The fact is, there are variations in the genetic predisposition towards fidgeting and other spontaneous movements and the mouse study suggests that people who fidget might have an advantage in staying slim. But before you start trying to fidget yourself thin, wait for the patient studies that are necessary to show if the findings in mice are also true for humans. "The gene that codes for Bsx is widely conserved across species. That makes it likely to fulfil a similar function in humans," says Maria. "Differences in Bsx activity between individuals could help to explain why the same diet makes one person fat while leaving another one unaffected. But the only way to find out is to look at patients with mutations in the Bsx gene." This is what the group is planning for the future.

Patient studies will also help to shed light on a second interesting link between the Bsx molecule and obesity. The researchers discovered that the effects of knocking out Bsx counteracted another genetic defect in mice, known as leptin deficiency. Leptin is a hormone produced by stomach and fat tissue when satiety has been reached. Humans and animals that cannot produce leptin suffer from a constant hunger sensation that can never be satisfied. The result is obesity, which often leads to serious diseases including cardiovascular disorders, diabetes and ultimately death at a very early age.

Surprisingly, mice that were engineered to lack both leptin and Bsx were much closer to normal body weight and no longer suffered from the complications of obesity. "This suggests that Bsx and leptin may work as antagonists to generate the appropriate response to peripheral satiety signals in the hypothalamus," Mathias interprets. "These results confirm the idea about Bsx as a crucial switch between peripheral signals about the body's energy balance and the guiding of behaviour by the central nervous system."

This finding is particularly interesting in the light of rising leptin resistance, the foremost reason for obesity in western societies. Through permanent overeating on a high-fat diet and years of actively ignoring satiety signals, the body can develop a resistance to leptin and no longer respond to it even though the hormone is present in high quantities in the blood. Since Bsx appears to be one of, if not the, crucial switch at the central control centre that couples hunger to feeding and is involved in the processing of satiety signals, drugs that target the molecule are likely to be highly effective in treating leptin resistance and pathological obesity.

Yet many more years of research will be necessary to find ways to successfully manipulate Bsx to counteract obesity and to develop drugs that can be applied to humans. In the meantime, Mathias remains optimistic. "Bsx is currently one of our best bets in the fight against obesity," he concludes. So, it seems the years of plenty might indeed be numbered.

Sakkou M, Wiedmer P, Anlag K, Hamm A, Seuntjens E, Ettwiller L, Tschop MH & Treier M (2007) A role for brain-specific homeobox factor bsx in the control of hyperphagia and locomotory behavior. *Cell Metabolism* **5**: 450-63 ■

Henning Hermjakob

Raising the standard

PROTEOMICS RESEARCH around the world is set to become more efficient and cost-effective thanks to two new initiatives created by scientists at EMBL-EBI. These initiatives, headed by bioinformatician Henning Hermjakob, will allow researchers to share and compare their data much more easily. They work by making sure that when it comes to submitting information to databases, everyone is singing from the same hymn sheet.

The hymn sheets in this case are not the verses of sacred music, but instead guidelines that detail the information that scientists need to include with their experimental data to ensure that the databases can record, retrieve and integrate them accurately. One set of guidelines – the minimum information about a proteomics experiment, or MIAPE – deals with submitting proteomics data in general, while the other – the minimum information for reporting a molecular interaction experiment, or MIMIx – explains how scientists should describe data on how molecules, such as cellular proteins and drugs, react with one another.

Until now, the lack of guidelines has meant that proteomics database curators have struggled to extract and integrate vast amounts of fragmented data from research papers, explains Henning. Such papers often do not contain enough information about the methods or reagents they describe: for example, they may fail to say what species of animal a protein comes from, or exactly what fragment of a protein was used. Another problem is that different researchers use different terms to describe the same thing: one common experimental method has 20 different names in the literature. "For a scientist, all of this is clear, but for a computer, it isn't," says Henning.

EMBL-EBI was ideally placed to take the lead in addressing these issues: as well as being Europe's centre for bioinformatics, it can boast of a previous success in drawing up a set of now widely used guidelines called MIAME, for a kind of RNA experiment called a microarray. Together with other members of the Proteomics Standards Initiative, a working group drawn from the Human Proteome Organization, HUPO, Henning invited representatives from academia, commercial organisations and research institutions to provide their input on developing the new standards. The result was MIAPE and MIMIx, published last year.

Both were soon put to the test by researchers at EMBL spin-off company Cellzome, when they reported the results of a screen for drug molecules that interact with a particular subset of cellular proteins. Although the dataset was dauntingly large and complex, the fact that it complied with the new standards made it easy to enter into the PRIDE, IntAct and ChEBI databases hosted at EMBL-EBI, says Henning.

A number of major journals are now pushing researchers to submit MIAPE- and MIMIx-compliant data, and researchers themselves are realising the advantages of doing so. "It is a benefit for the community and also researchers themselves, as it will increase their citations," says Henning. "It's really something that flows back into the community."

Bantscheff M, et al (2007) Quantitative chemical proteomics reveals mechanisms of action of clinical ABL kinase inhibitors. *Nat Biotechnol* **25**: 1035–44

Orchard S, et al (2007) The minimum information required for reporting a molecular interaction experiment (MIMIx). *Nat Biotechnol* **25**: 894–8 ■

Context is everything

SPACE AND TIME ARE IMPORTANT. That is what Peer Bork, Joint Coordinator of the Structural and Computational Biology Unit at EMBL Heidelberg, and his colleague Lars Jensen say as they disentangle the vast networks of protein interactions that keep cells and organisms ticking over.

DNA sequencing has provided biologists with an overwhelming wealth of information, but understanding that information is not easy. So, hot on the heels of genome sequencing technology, computational approaches mine those sequences for information about cellular processes.

Peer develops computer algorithms that decode sequence information to predict what proteins look like, how they function and what their targets are. Conventional bioinformatics approaches look at the sequence and shape of proteins to predict what they might interact with. But to make their predictions more powerful, Peer and his colleagues had the idea of adding 'contextual' information into their algorithms. Working with former PhD student Rune Linding, and colleagues Tony Pawson at Mount Sinai Hospital in Toronto and Michael Yaffe at the Massachusetts Institute of Technology, Peer reasoned that proteins produced in similar tissues will be more likely to interact with one another than those expressed in different tissues, and a wealth of such contextual information has been accumulating in published papers and databases over the years.

So does this contextual information improve the reconstruction of protein networks? Peer and his co-workers tested this by applying the idea to a particularly important set of protein interactions called the phosphorylation–kinase network. The behaviours and functions of proteins are dramatically altered by adding or removing chemical tags. 'Phosphorylation', the addition of a phosphate chemical group to a protein, is a very common type of protein modification, and is involved in crucially important cellular processes such as cell division and DNA repair.

The enzymes that carry out phosphorylation are called protein kinases. Animals and plants possess hundreds of different types of protein kinases that modify thousands of different types of proteins. For a few kinases, the target substrates are known, but Peer and his colleagues want to systematically build a comprehensive picture of which kinases modify which proteins. This will make it easier for researchers to pinpoint why some phosphorylation-controlled processes go wrong. "Thousands of proteins are changed through phosphorylation, but until now, it has not been possible to know which kinase targets which protein," he says.

It turns out that adding contextual information to their algorithm, which the researchers have dubbed NetworKIN, more than doubles the success of correctly linking protein kinases with their phosphorylation targets. The researchers have already used NetworKIN to build up a picture of the kinase networks in humans. For example, they used this information to examine the networks of kinases involved in DNA-damage repair – crucial for understanding the processes that lead to cancer. Peer says, "With our method, we not only correctly predicted many of the known protein targets of these kinases, but also identified several proteins not previously known to be targets of DNA repair kinases."

Their results suggest that this approach could help to predict targets that are therapeutically relevant. Many important diseases, particularly cancer, involve mistakes in the phosphorylation process; identifying those mistakes, and understanding the consequences, could make it possible to intervene when things go wrong. Peer says, "In the future, the treatment of complex human diseases will be treated by targeting multiple genes. By getting a network-wide view, we will more easily identify the targets of kinase-controlled processes."

Linding R, et al (2007) Systematic discovery of *in vivo* phosphorylation networks. *Cell* **129**: 1415–1426 ■

Lars Steinmetz interviewed by Vienna Leigh

A solid basis for a future in science

AS RESEARCH AT EMBL evolves to embrace new scientific knowledge, technologies and techniques, so too must the training programmes which support it. Nowhere is this more true than with the Core Course of the EMBL International PhD Programme (EIPP), which every year welcomes and initiates around 50 students embarking on the first important leg of their scientific career. Its mission is to teach interdisciplinary research in molecular biology to first-year PhD students and to familiarise them with the distinctive EMBL culture.

Last year, the programme's newly appointed Academic Mentor, EMBL Heidelberg faculty member Lars Steinmetz, took on the task of revamping the course to reflect the needs of today's PhD students – and if the feedback is anything to go by, he did a great job.

"The number of PhD students at EMBL is increasing – to date we have a total of 183 students currently enrolled, from 32 countries," explains Lars. "It was becoming clear that the structure of the course would need to be different to accommodate the numbers." This increase is set to continue, so much so that the EIPP, for the first time in its history, now has a full-time Dean, Helke Hillebrand. But the more pressing need for change has more to do with the type of students who are being accepted. "We're getting more and more students with different backgrounds. The focus of the course was originally on experimental biology, and it needed to be adapted for other students. The response from the evaluation, which we carry out with questionnaires following every course, also suggested that the outstation students felt the course was very Heidelberg-centric."

Anne Ephrussi, former Dean of the EIPP, agrees. "The course needed to be overhauled mainly because of the growth of EMBL-EBI. We were taking on more and more computational students with no biology, and they just couldn't follow the science. We tried to find ways to improve that, and students from previous years came forward to offer help with tutorials for the bioinformaticians, but it was clear that a major revamp was necessary."

Lars and the working group, which comprised scientists at all stages of their careers – Alice Young, Carl Neumann, Heidi Dvinge, Klaus Scheffzek, Nils Gehlenborg, Marcel Souren, Wolfgang Huber and Vladimír Benes – examined the results of 2006's course evaluation and identified several aspects that needed updating. The new course is now arranged into themed modules instead of by unit, and nearly every research group is involved.

"The research within an EMBL unit can be very diverse. That means there's a lot of overlap between different units, and the students were being told about some topics twice," says Lars. "By focusing on areas by biological subjects, we guarantee that students hear about something only once, and also that subjects are taught in an interdisciplinary way, because group leaders from different units have different approaches to a particular biological question."

The scientific content of the course reflects the research activities of the different EMBL sites. The seven modules

are Genomics; Evolution; Proteomics and Structural Biology; Developmental Biology and Behaviour; Cell Biology; Chromatin and Translation; and Disease Mechanisms and Pathogens. Each module lasts for about a week and contains lectures and practical lessons, in which students are exposed to the diversity of scientific ideas that are defining new fields of integrative research. Lectures are taught by group leaders from several units and outstations.

With interactive seminars and rotations through multiple laboratories at EMBL, the course, which runs from mid-October to mid-December at EMBL Heidelberg and includes a 4-5 day outstation visit, encourages students to approach problems collaboratively, to complement each other's expertise, to learn how to communicate with specialists in different scientific disciplines – and to begin to experience EMBL's unique culture.

Also new to the course are the journal clubs: students read one or two papers which are the basis of the module, sometimes classical biology papers which made a big impact decades ago, and discuss them in a group. In addition, the presentations and course materials are now made available on the web, a resource that has been very popular.

The results of the evaluation last year show that the new recruits rated the majority of the modules 'good' or 'very good', and felt that the course as a whole was useful preparation for helping them start their PhD work. Importantly, 32 of the 48 respondents could see connections between the individual modules and felt that the interdisciplinarity of the field as a whole was well represented. They responded favourably to the non-scientific parts – which cover good scientific practice, patenting and effective scientific communication, among other topics – feeling that they contributed to the learning experience. More than 80 per cent of the respondents – a huge improvement – felt that the course was appropriate to their background. 'Fun' and 'informative' were the descriptions that most students opted for in their overall conclusions.

The course has been a crucial part of the EIPP since the beginning, but it has taken time to achieve the right balance and effective structure. The first students enrolled in 1984, and for the next few years the programme remained small, accepting up to just eight students per year working alongside others who were not part of the programme. In 1987, Dean Wieland Huttner took steps to formalise graduate work at the Lab, requiring that all students be recruited via the EIPP. Director General Lennart Philipson provided internal funding, allowing the number of new students joining EMBL each year to reach 20.

EMBL Hamburg group leader Manfred Weiss teaches predocs about structural biology.

Establishing the programme in this way reflected the awareness that there was more to having a student in the lab than just an extra pair of hands. In return EMBL owed the students a formal training structure, which would offer a solid basis for their career in science. The PhD Core Course was introduced, but was very different to what we have now. Teaching activities were spread throughout the year, and each unit would organise a week-long set of practical exercises. Things weren't as organised as they are now and sometimes the timing wasn't very convenient for the participants.

In 1993, Director General Fotis Kafatos, EIPP Dean Frank Gannon and the Graduate Committee reorganised the course from separate activities into one integrated programme. The outstations were involved, and non-scientific activities such as safety, bioethics and science communication were included in the curriculum. "It's important to remember that our scientists come from different countries and academic settings and have differing abilities. Many benefit from learning how to write more effectively or give a good seminar," says Anne. "Since the basis of any kind of interaction and collaboration is efficient communication, the course provides the language and communication skills required to address various audiences, from experts to lay people." Similarly, social, ethical and legal aspects of science in general, and molecular biology in particular, are discussed to encourage critical thinking among the students and to prepare them for their future.

"It's really important to have a broad overview of the research that's going on, to know about the additional services and training opportunities that are available here, and to have the opportunity to forge strong scientific collaborations and friendships."

Though the structured academic programme and non-scientific modules are now honed almost to perfection, there's a hugely important by-product of the two-month course which nobody predicted. "It wasn't until a year into the course that we realised one of its major effects, which was to create a network for the students," says Anne. "That wasn't premeditated, but turned out to be one of the most important aspects. The course helps the students form links with one another and to get to know all of the group leaders – they know exactly who is doing what within the laboratory."

Based on the feedback, the course will undergo a little more tweaking before the next intake of new students, but the basic premise is now established in the new structure. "We hope that the incoming PhD students will find the course both useful and enjoyable," says Lars. "It's really important to have a broad overview of the research that's going on at EMBL, to know about the additional services and training opportunities that are available here, and – perhaps most importantly – to have the opportunity to forge strong scientific collaborations and friendships." ■

At the core...

The Hentze lab and the Molecular Medicine Partnership Unit

Together with Matthias Hentze and Martina Muckenthaler of EMBL's Molecular Medicine Partnership Unit (MMPU) with the University of Heidelberg, GeneCore has developed technology which allows the profiling of microRNAs that are active in different species. MicroRNAs are small molecules with powerful regulatory functions. They play crucial roles in many cellular processes such as haematopoiesis, the production of blood, and diseases affecting this process. To be able to compare the microRNAs that are active in humans with those found in the mouse – the most important model organism used in MMPU research – GeneCore helped to develop the so called miChip. The miChip is based on the same principles as a microarray and enables profiling of mature microRNAs from human, mouse and other organisms.

The Gannon lab

Together with George Reid from Frank Gannon's lab, GeneCore is working on a whole human genome scale analysis of estrogen binding sites. In response to the hormone estrogen, transcription factors bind to specific regions on the DNA to activate or inactivate genes. These processes are important especially in embryonic development and the development of certain types of cancer.

The Akhtar lab

Asifa Akhtar's group studies how male flies produce the same amount of proteins from their single X chromosomes as females generate from two X chromosome copies. This is achieved with the help of a protein complex that binds to the male X chromosome and increases its transcription. Asifa discovered where exactly this complex binds on the X chromosome with the help of a localisation analysis carried out by GeneCore.

The Cohen lab

An expression analysis of a microRNA called *bantam*, which GeneCore carried out for the Cohen lab, shed light on the important Hippo signalling pathway which controls the life and death of cells.

The Genomics Core Facility

Affectionately referred to simply as GeneCore, Vladimír Benes' Genomics Core Facility is not only one of the first EMBL core facilities to be founded – in 2001 – but also one of the most frequented. "It is probably easier to mention the groups that we don't work with than to list all our customers at EMBL," says Vladimír. In 2006 alone, GeneCore processed 44 000 sequencing reactions and several hundred microarrays on samples from almost all wet lab groups at EMBL. These numbers show the great importance of functional genomics, the processing and assigning of functions to sequence data, to modern molecular biology.

Thanks to close collaboration with the scientific instrumentation industry, GeneCore offers state-of-the-art technology and the expertise of nine highly qualified staff to help tackle a wide range of problems in genomics. The repertoire of the

The Arendt lab

With the help of the miChip, which allows the comparison of microRNAs active in different species, Detlev Arendt and his group are looking for similarities in the microRNA contents of the evolutionarily old ragworm *Platynereis dumerilii* and more recent organisms. Insights gained will likely shed light on the origin and evolution of all bilaterian species.

Since 2001, EMBL has offered its research groups and the scientific communities of its member states essential scientific infrastructure, services and specialist expertise through centralised units. These eight so-called core facilities are coordinated by Christian Boulin and are engaged in a variety of research efforts inside and outside EMBL. GeneCore has contributed to countless scientific and technical projects and, collaborating with research groups from all five EMBL sites, constitutes a central node in EMBL's research network.

The Rosenthal lab

Using the microarray facilities of GeneCore, Nadia Rosenthal and her group investigated how gene expression differs between healthy heart cells and cells that had undergone an artificially induced heart attack.

facility comprises DNA sequencing, microarrays, a technology to assess which genes are active in a given tissue under specific conditions, and liquid handling robotics for large-scale projects involving huge pipetting efforts. To spread their knowledge, Vladimír and his staff organise many practical courses on the operation of technology and data analysis.

The latest addition to GeneCore is a cutting-edge Illumina Genome Analyzer sequencing infrastructure, which will enhance sequencing efforts and at the same time improve their quality and extend their coverage to entire genomes. "We have all our tools in place now. The future challenge will be to better integrate different techniques to gain a more complete picture of gene expression and regulation," says Vladimír, depicting the facility's future.

Protein Expression and Purification Core Facility

Hüseyin Besir and the Protein Expression and Purification Core Facility regularly work with GeneCore to verify their starting material. GeneCore sequences the clone from which a protein should be expressed, to make sure the protein of interest is actually present and has the correct properties.

EMBO Young Investigator Programme

To support the Young Investigator Programme of EMBL's sister organisation, EMBO, GeneCore grants members of the programme access to its resources.

The Furlong lab

GeneCore assisted Eileen Furlong's work on the transcription factor network that controls muscle development in flies by producing the first tiling arrays of the Drosophila genome. Unlike a microarray, which is used for transcription profiling, a tiling array contains sequences that cover the whole genome – including not only genes but also regions with unknown functions. The tiling arrays helped to identify the sequences of the genome to which transcription factors bind.

Chemical Biology Core Facility

To elucidate the mode of action of small molecules identified in the Chemical Biology Core Facility, Joe Lewis and his team often collaborate with GeneCore. If a small molecule disrupts the cell cycle, for example, a microarray-based comparison between the genes expressed in a normal and a treated cell can shed light on how the molecule achieves its effect.

The High-throughput Functional Genomics Centre

Close collaboration between GeneCore and the Functional Genomics Centre, coordinated jointly by Eileen Furlong and Lars Steinmetz, establishes a complete pipeline for genomics research at EMBL. The core facility provides the hardware and technology; the centre adds expertise in software solutions and support with data processing and analysis. Together they try to promote a stronger culture of functional genomics at EMBL. ■

EMBO EMBL

The future of our species

Evolution, disease and sustainable development

On the 8th EMBL/EMBO Science and Society Conference 2007

The future of our species

BIOLOGICAL SCIENCE is a powerful engine for change, and its scope extends well beyond the laboratory. To consider the role of contemporary biology within the context of wider society, EMBL, together with its sister organisation EMBO, hosts an annual 'Science and Society' conference. Over two crisp autumn days in Heidelberg, delegates from around the world gathered for the 2007 conference – entitled 'The Future of our Species' – to consider the next century of human society, and how disease, climate change and evolution might remodel that future.

Reflecting the complex network of professions that evaluate, disseminate and use biological knowledge, the conference drew on a broad range of specialities, from research scientists and ethicists, to lawyers and philosophers. "The conference aims to promote dialogue and understanding between various sectors of society," said EMBO's Andrew Moore, one of the conference organisers.

The medical fates of humans and other animals are inextricably linked. In the first session, Albert Osterhaus, a vet turned virologist at the Erasmus Medical Centre in Rotterdam, the Netherlands, explained that wild and domesticated animals are the source of almost all new human infectious diseases. He stressed the vital importance of sharing information on diseases in wild and agricultural animal populations on a global scale. Sharing information is not always easy in today's political climate, but diseases often transcend political and geographical boundaries – just as disease monitoring and medicine should, said Dr Osterhaus. Scientific research groups, which have their share of political complexities, must also cooperate in their responses to such diseases, he said.

Dr Osterhaus cited the response to severe acute respiratory syndrome (SARS) as a model for how responses to new diseases might unfold in the future. He described how, during the global SARS outbreak, affected countries tended to monitor the disease closely and that many introduced quarantine measures. During this time, there was also unprecedented cooperation between scientific research groups to identify and characterise the virus. SARS demonstrates that such cooperation can happen, but, he cautioned, whether the same level of cooperation can be expected in response to a virulent strain of flu remains to be seen.

If medicine has changed society over the last century, climate change is likely to be the most important influence in the century to come. The second session of the conference focused on the interaction between human society and the natural world. How will global ecosystems change in the future? How will such change affect biodiversity and human society? According to conservation biologist Chris Thomas of the University of York, UK, the plant and animal species at greatest risk are those that live in rare climates. These are species that are only able to live in narrow climate ranges, and are likely to be pushed out by rising temperatures.

Dr Thomas also pointed out that changes in ecosystems are predicted to be most severe in those countries with the highest biodiversity levels. "These are also often the poor-

Albert Osterhaus

A question from the audience

Sarah Chan

est countries, where humans rely most directly on natural ecosystems for their livelihoods," he said. "Finding ways to direct resources to help such countries adapt to climate change is a daunting task." He and the participants of a panel discussion that followed agreed that a key challenge for sustainable development is to unhook economic growth from competition for resources. And the only way to do that, says Chris, "is for the true environmental and other costs of unsustainable exploitation to be reflected in the prices we pay for commodities".

Technological solutions to climate change may be on the horizon, but looking back, biomedical innovations have produced some of the most significant advances in human history. However, new medical treatments often require delicate ethical negotiation. The third session considered ways in which society evaluates new biotechnologies, including stem cell therapies, regenerative medicine and genetic manipulation.

A strong focus of this session was why treatments with a genetic dimension tend to attract so much scepticism. Fulvio Mavilio, a molecular biologist at the University of Modena in Italy and one of the founders of the gene-therapy company MolMed, suggested that misgivings over genetic modification often obscure the rational evaluation of gene therapies. Dr Mavilio uses genetically modified stem cells in novel treatments for diseases such as severe combined immune deficiency (SCID) and epidermolysis bullosa (a skin adhesion defect), but is concerned that stem cell therapies are not evaluated fairly alongside more conventional treatments. All therapies have side effects. "Decades of practice has meant that the medical community has learned how to deal with them," he said. "The

The interested audience in the Operon of EMBL Heidelberg.

risk–benefit ratio should be the only ethical criterion underlying the decision to apply an experimental therapy." He explained that genetic manipulation should not be considered an exception.

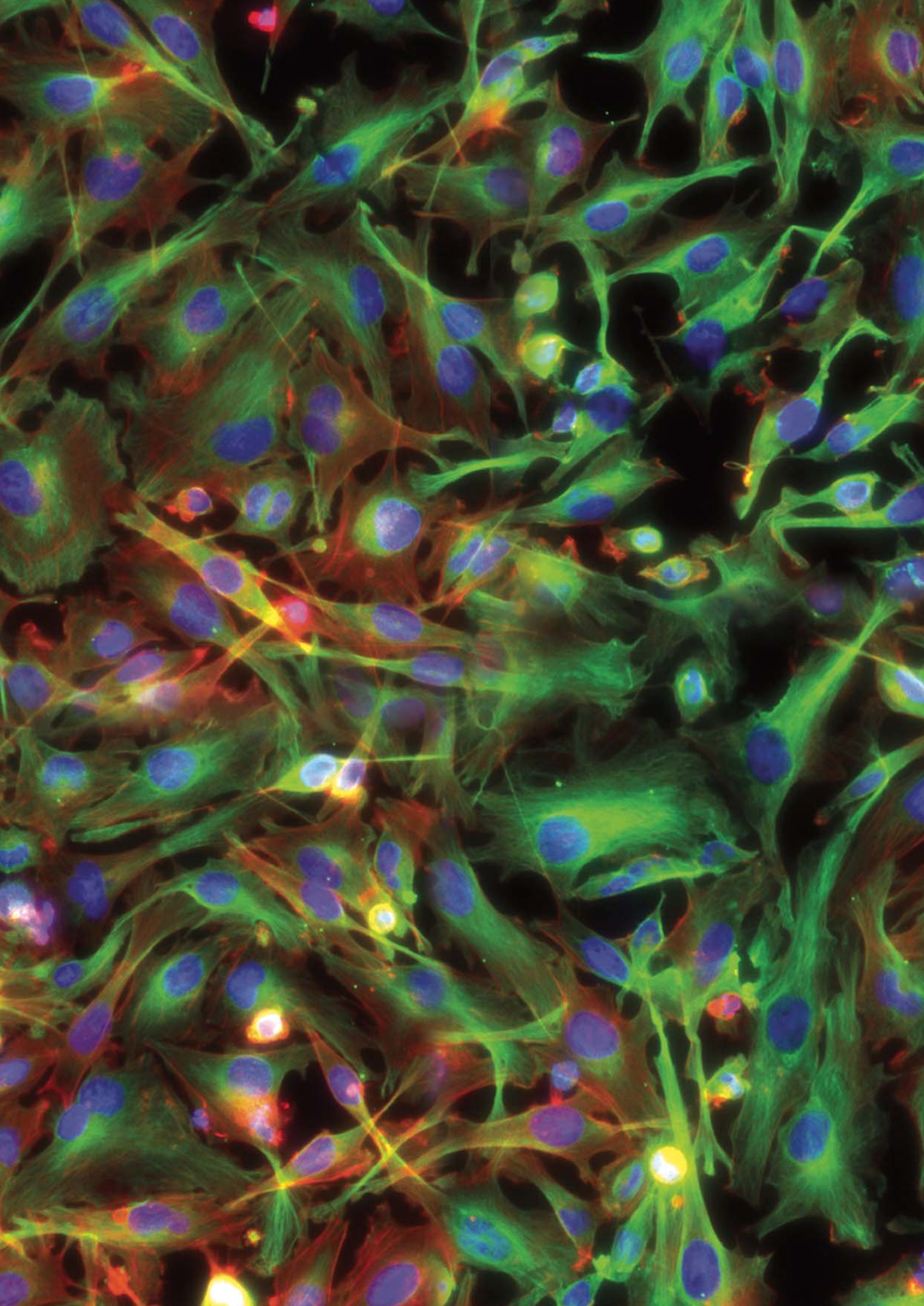
The boundaries of technological acceptability were also the main focus of philosopher Sarah Chan from the University of Manchester, UK. She discussed biological technologies in relation to both medical treatments and to 'enhancements', which improve normal function rather than treat dysfunction. At its most benign, an enhancement might be a pair of spectacles or a cup of coffee. But when enhancements take the form of performance-enhancing drugs, or the potential manipulation of a person's genetic code, then many feel suspicious.

Dr Chan asked whether such suspicions are justified. As a philosopher, she says that the line between treatment and enhancement is remarkably thin. She argued that technologies could and should also allow us to enhance our normal function, and calls for a thorough and rational debate on these matters. "We are dealing with a typical example of the 'dual use' problem of scientific research and its applications," said Dr Chan.

Like other technologies, enhancements could be seen as a step in the process of human evolution. How will such technology affect the future course of humanity? The last session of the conference considered what evolutionary biology has told us about our own history, and what it might have to say about our future. Mark Stoneking, who works at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, explained how molecular biology – specifically, understanding the patterns of DNA sequence variation – has uncovered the past migrations of our species. Mark described the role of molecular genetics in answering questions that have traditionally been defined within the realm of anthropology. "Genetics can help to answer questions about the origins, migrations and relationships of human populations, and what kinds of selection pressures have shaped *Homo sapiens*," he said.

Another talk in the session considered whether the human cultural environment has influenced biological adaptation. Jay Stock of the University of Cambridge, UK, studies the way in which human behaviours have influenced skeletal anatomy. He explained that culture has had an important influence on biological evolution, and in particular, has had a strong impact after the origins of agriculture. Other presentations in the session considered the boundaries between biological evolution and cultural evolution. Ian Pearson, a futurologist for British Telecom, walked on the wild side and considered how technology might physically intersect with human biology. Anthropologist Jerome Barkow from Dalhousie University, Canada, said that biological and cultural evolution are as intertwined as physics and chemistry. He explained that culture and society are the means by which humans express their biology and should be considered together. Dr Barkow also discussed the problems we have in coping with our increasingly larger societies, given that we evolved as small-band social primates, and expressed concern that humans are emotionally ill-equipped to adapt to this expansion.

Appropriately perhaps for a conference considering the future of mankind, the audience included several high-school children, which tempered the presentation style of the talks and debates. Delegate Dr Ali Saib, coordinator of Les Apprentis Chercheurs, a French organisation that forges collaboration between high schools, universities and research institutes, said that, as scientists, "Communicating life sciences to the public and especially to youngsters should be part of our daily job." Many at the meeting agreed that this is important – both to give the new generation the minimal scientific tools for understanding our world and the future, and to recreate a link between scientists and society. ■



Molecular managers

"THERE MAY BE NO 'I' IN 'TEAM', but there's a 'me' if you look hard enough." David Brent, the lead character in the British sitcom *The Office*, was the epitome of a ghastly manager – so much so that he and his catchphrases inspired similar TV series and spinoffs around the world. Audiences in France and Germany, for example, were treated to the toe-curling antics of Brent's equivalents, Gilles Triquet in *Le Bureau* and Bernd Stromberg in *Stromberg*.

But you don't have to see any of these sitcoms to recognise the problem. Brent's weaknesses – his self-importance, lack of empathy and remarkable ability to alienate his team – show how vital a good manager is to the survival of any organisation. The same is true of cells. Many of the processes that drive their biology depend on the efficiency of just one particular molecule, and if this goes wrong, the cell – and even the whole organism – suffers.

Several teams at EMBL are studying these essential cellular managers. One, for example, is looking at the large proteins that link cells together and transmit information about the environment. Others are looking at the molecules that relay signals inside a cell in a precise and controlled manner – a process that often goes wrong in diseases such as cancer. Yet others are unpicking the intricate systems which control the molecules that build a cell's internal skeleton, a vital structure that performs a myriad of functions from dictating cell shape to transporting crucial components from one part of the cell to another.

Although these molecules are very different, they share one thing in common: to do their jobs properly, they have to work as a team with many other molecules in the cell. Working out how molecules interact and what relationships they have with one another is key to understanding how life works. Like human resources consultants building teams in the workplace, biologists have to study how their molecules behave in different situations to really get a grip on what is going on.

Performing this kind of analysis on one or two molecules is hard enough. But cells are exquisitely complex things, and EMBL researchers are finding new ways of piecing together the myriad interactions that keep them running smoothly. These include working out how enzymes make subtle chemical alterations to DNA and chromosomes, for example, so that the cell can alter gene activity in response to a changing environment, or how proteins coordinate the electrical connections that let our nerves communicate. Thanks to molecular managers like these, cells can stick together, grow and move – without having to suffer the David Brents of this world. ■

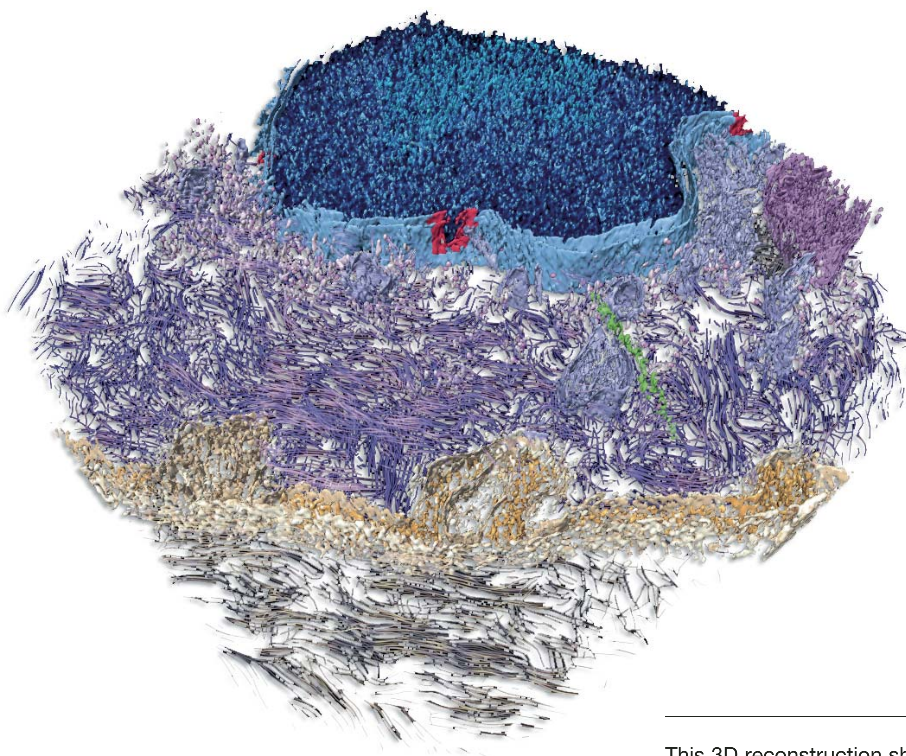
A safari through the cell

Achilleas Frangakis,
Matthew Betts and
Ashraf Al-Amoudi

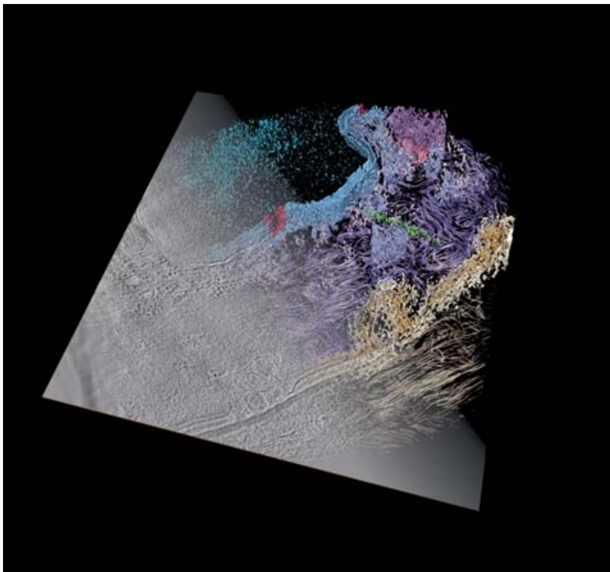
CLOSE YOUR EYES and let your mind travel! Straight ahead you see a huge, blue mountain looming in the distance. When you get closer, you realise that it is surrounded by a thick wall with small, red gates that lead inside. From this perspective the cell nucleus appears like a fortress, well-protected by its membrane and only to be entered through safely guarded pores. When you turn around to your left and right you recognise other cell organelles: a mitochondrion, ribosomes and parts of the endoplasmic reticulum. Far away, through a dense mesh of thin, purple filaments, you can even spot the cell membrane on the horizon. You are looking at the marvellous landscape of a cell – a human skin cell, to be precise.

What sounds like the beginning of a second-rate science fiction novel comes close to capturing the impression you get when Achilleas Frangakis talks you through the electron tomogram of a skin cell. The three-dimensional (3D) reconstructions that he and his group produce with the help of an electron microscope (EM) are not just any kind of model – they are the most accurate representations of cells. "This is the closest we can currently get to knowing what the inside of a cell looks like," says Achilleas while zooming in and out of the model on his computer screen. "All features, from large organelles to complexes of molecules, are shown in their natural positions and interactions." He makes an apt comparison: "Cryo-electron tomography takes you on a safari whereas other imaging techniques take you to the zoo."

What he means is that cryo-electron tomography is currently the only EM technique that studies biological samples preserved in something very close to their natural state. Unlike other EM approaches the sample is not dehydrated, treated with chemicals and dyes and coated in metal, a procedure necessary to protect it from the destructive force of the microscope's powerful electron beam. In the 1980s, Jacques Dubochet and his group at EMBL discovered that freezing a cell and covering it in a thin layer of ice was enough to make it withstand the electron beam and preserved the native state of the cell – capturing all dynamic processes in ice. "In conventional EM you only ever see imprints of the real thing. It is like studying a fossil of a fish conserved in a stone. With the freezing technique you can look at the real fish in the water – or better in ice," Achilleas says.



This 3D reconstruction shows the marvelous landscape of a human skin cell. The image was produced by electron tomography and shows organelles in different colours: regions of cell-cell contact (sandy brown), nucleus and nuclear envelope (blue) with pores (red), microtubules (green), mitochondria (purple) and endoplasmic reticulum (steel blue).



3D electron tomograms are generated by combining many electron microscope images taken at different angles of thin cell slices. The montage represents how the colourful 3D representation gradually emerges from 2D, black and white electron microscope images.

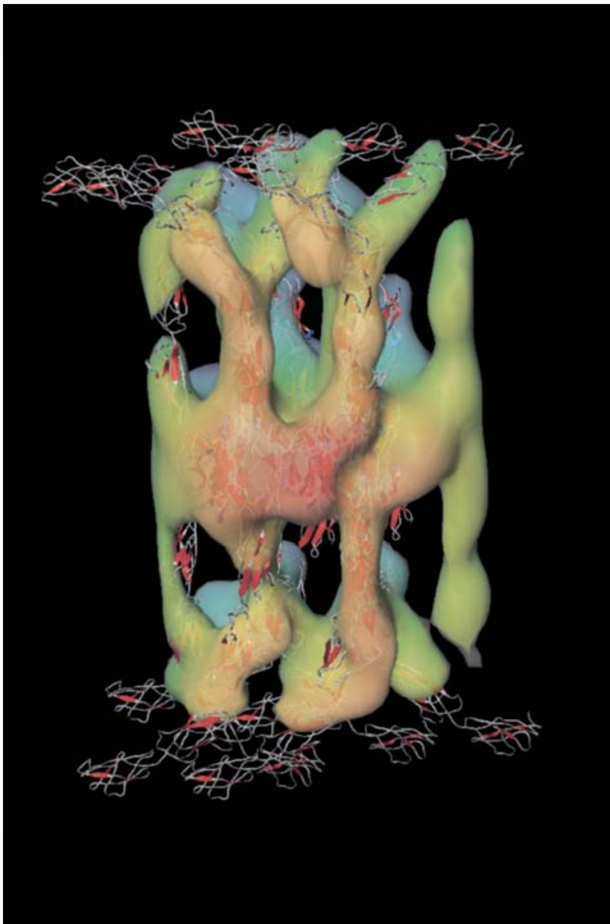
A two-dimensional (2D) image, however, does not say much about how a fish interacts with its environment. The same is true for the molecular organisation and interactions of a cell. This is why electron tomography works in three dimensions. The specimen is rotated in the electron beam of the microscope and images are taken from all possible angles. These images are then assembled into a 3D representation by a computer. Such 3D reconstructions allow the exploration of the whole inner space of small prokaryotic cells. For eukaryotic cells it is a bit more difficult. Since an EM can only work with subjects up to 500 nanometres in size – 100 times thinner than a human hair – eukaryotic cells need to be cut into sections. An EM can achieve a resolution of 4 nanometres. In their latest study, the members of Achilleas' team were the first to see an individual protein in its natural state, but the ultimate goal of electron tomography – a cellular cartography that shows all molecules and their interaction in a single snapshot of the cell – still remains a challenge.

Despite its eye for detail, electron tomography never loses sight of the cellular context. It works at the highest resolution possible to see molecular details, but the coarsest resolution necessary to still see the cell, or large portions of it, as a whole. This unique arrangement allows the mapping of molecules in the cell to find out their precise locations in all three dimensions. "EM tomography could act as the interface that bridges the gap between high-resolution structural approaches like X-ray crystallography or nuclear magnetic resonance and light microscopy. It localises atomic structures of molecules inside the cell," Achilleas explains.

With this thought in mind, the group used cryo-electron tomography to address a longstanding and as yet unsolved problem in biology. How do two cells make contact and adhere to each other? This cell–cell contact is very important for the integrity of tissues, especially skin, heart and other tissues that are exposed to a lot of physical stress. Specialised structures, called desmosomes, connect two cells establishing contact between their membranes. One key component of a desmosome are proteins called cadherins. They are anchored in the cell membrane and interact with each other to bring cells close together and interlink them tightly. How cadherins bind each other and why this binding is strong enough to hold two cells together has remained unclear even in the face of solved cadherin crystal structures and various microscopy studies of desmosomal areas.

To tackle the question, postdoc Ashraf Al-Amoudi produced an electron tomogram of human skin cell sections. "Thanks to Ashraf's meticulous work, his biological expertise and great experience with the tissue, he managed to generate very fine sections of perfectly preserved skin cells," Achilleas says. The sections, with a width of only 60 nanometres, helped to obtain images at very good resolution. But extracting minute details like the molecular organisation of a desmosome from the tomogram required powerful image processing.

"This is where the other, computer-oriented half of the lab came in. We are a truly interdisciplinary group," says Achilleas. Daniel Castaño Diez develops software that maximises the information that can be extracted from a tomogram. This software allowed the researchers to zoom in on the molecular details of the desmosomal area, where they discovered minute, W-shaped bridges between two juxtaposed membranes. To identify the nature of these bridges, the team superimposed the existing crystal structure of cadherin onto their tomogram and got a perfect match. Two interacting cadherin molecules were enough



3D visualisation of interacting cadherin molecules in their native arrangement. Known molecular structures of cadherins (grey and red ribbons) are fitted onto the electron tomogram (multicoloured) of the complex.

Four fingers are enough to generate a simple model of the interaction between cadherin molecules on two skin cells.

to reconstruct the W-shape between the membranes. But the regular arrays of cadherin bridges, located at a distance of 70 Ångstroms along the whole length of the desmosome, are not the full story. A second crucial interaction takes place between the molecules located on the same membrane, so that rigid cadherin grids are formed.

The insights into the twofold binding of cadherins proposed a new model of cell adhesion based on a principle that is reminiscent of Velcro. A few interactions between the cadherin grids of two juxtaposed membranes bring the cells close together, thereby promoting binding between more molecules. The more cadherins that bind, the stronger the connection between the two cells.

Apart from shedding light on tissue integrity, the new insights into cell–cell contact formation might have implications for scientists' understanding of other processes based on cell adhesion, including embryonic development, cell proliferation, membrane fusion and wound healing. The work is also an important landmark from a technical point of view. "This is the first time ever that an individual protein was observed in its native environment inside a cell," says Achilleas enthusiastically. "The technical advances achieved in cryo-electron tomography open up new possibilities to explore the molecular organisation of cells. It will allow us to investigate many other molecules in their natural context and to address new biological questions."

In Achilleas' case, answering the next question is already in progress. Together with Jan Ellenberg, Coordinator of the Gene Expression Unit, his group investigates how nuclear pores, the protein complexes that grant entry into the nucleus, are assembled. The future he sees for electron tomography is bright and full of potential. As the resolution of microscopes improves further, investigations might no longer be limited to protein complexes or bigger single proteins, but might address smaller molecules, for example the structure of DNA in the nucleus. Achilleas and his group are working hard to advance the technology and might soon be able to take us on safaris into a realm of even smaller molecular details.

Al-Amoudi A, Diez DC, Betts MJ & Frangakis AS (2007) The molecular architecture of cadherins in native epidermal desmosomes. *Nature* **450**: 832-7

Finding the X-factor

Asifa Akhtar

HOW'S THIS FOR A STRANGE THOUGHT: sex discrimination can be good for you. Not the sort that wrongly bars women from the boardroom, but the kind that cells use to compensate for the fact that males and females are endowed with a different set of chromosomes. Now, EMBL researchers have uncovered the intricate and finely balanced mechanism that cells use to do this.

Cells regulate the activity of genes on the differing chromosomes to ensure that both sexes end up with the right levels of the proteins and other molecules encoded by these genes. Disrupting this process can prove fatal. "It's a matter of life and death," says Asifa Akhtar, a group leader in the Gene Expression Unit at EMBL Heidelberg.

Asifa and her colleagues have been studying fruit flies to discover how cells discriminate between genes on ordinary chromosomes and those on sex chromosomes – the famous X and Y that are responsible for determining sex in many organisms. Females have two copies of the X chromosome, which balance the two copies they have of each of their ordinary chromosomes. Males, however only have one X, meaning that the genes on it are present in only half the numbers they should be to equal those on the ordinary chromosomes. So cells have to find ways of making sure that X ends up giving the cell its fair dose of gene activity.

Fruit flies are useful to study because, as well as having X and Y chromosomes, they are easy to manipulate genetically. They compensate for the sex chromosome difference by doubling the activity of genes on the X chromosome in males. Biologists already knew that a molecular machine called the MSL complex was responsible for ensuring that only X chromosome genes get ratcheted up in this way, but they weren't sure how the complex identified the correct genes.

Asifa's team knew that it had to be something about the genes themselves, because male fruit fly cells are still able to find X chromosome genes if biologists transplant them on to other chromosomes. "It's like finding a needle in a haystack," says Asifa, "but the complex is able to find it." If the team blocked the ability of cells to read, or transcribe, two X chromosome genes called *mof* and *CG3016*, the MSL complex could no longer be visualised on them, suggesting that transcription is needed for discrimination. But it couldn't be the whole story: after all, many other genes in the genome get transcribed, but don't have their activity doubled like genes on X.

Further work revealed that X chromosome genes also contain mysterious labels towards their ends, such as short DNA sequences, that help the MSL complex to recognise them. These labels are too subtle to be seen if the gene is not already active. They only become 'visible' to the cell when transcription is underway. "You need two signals," says Asifa. "The complexity is exquisite."

Although the biology of the fruit fly's sex chromosomes is different to ours – human females shut down one of their two Xs – the work offers new insights into how cells can achieve this kind of sensitive and highly specific control over gene activity. Asifa's next challenge is understanding exactly how this cellular labelling works. "The system is complex," she says. "It will keep us busy for a while."

Kind J, Akhtar A (2007) Cotranscriptional recruitment of the dosage compensation complex to X-linked target genes. *Genes Dev* **21**: 2030–40

Thomas Surrey, Peter Bieling
and Damian Brunner

The seeds of change

"NO. THAT'S NOT QUITE RIGHT." Damian Brunner is contemplating the view from his office window, wearing a concentrated frown. "Scaffolding is the wrong word," he explains. "This scaffold is never static. The building builds itself and constantly changes shape."

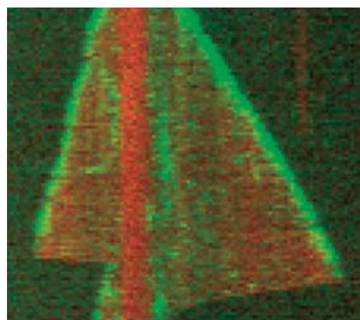
Damian and his colleague Thomas Surrey, both group leaders at EMBL Heidelberg, are trying to think of user-friendly analogies for microtubules – the tiny, dynamic cellular structures that they study. Microtubules are, as their name suggests, tiny tube-shaped structures that perform many different functions in the cell. Because they play an important role in controlling a cell's shape, they are often likened to a kind of cellular scaffolding. But, as Damian points out, 'scaffolding' is too poor a word to convey the ever-changing, organic nature of microtubules.

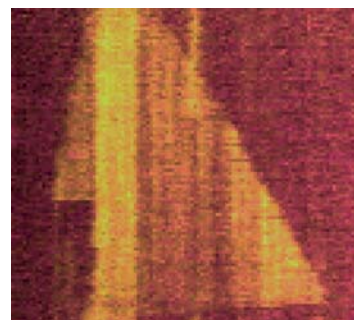
This evanescence is what makes microtubules so hard to describe. Never exactly the same from one moment to the next, microtubules respond rapidly to changes in their environment. Each tubule is made of protein building blocks called tubulin, which are constantly being added or removed from the tubule. If the balance between these two processes is exact, the tubule remains a constant size. Tip the balance either way, and the tubule will either grow or shrink.

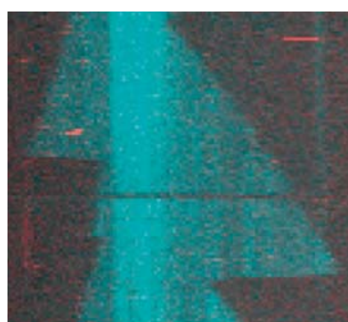
What's more, microtubules perform many different functions within a cell. Try picturing them in one of their roles during cell division, as pulley ropes that yank the copied chromosomes into the nascent daughter cells. Or you could visualise them as they act their part as molecular highways, tubulin-cobbled routes tramped by haulier proteins delivering cargoes to different destinations within the cell. And that is before you get to their role in building the dynamic internal skeleton that can rapidly construct or demolish structures such as cilia, tiny antenna-like organs that help cells sense their environment.

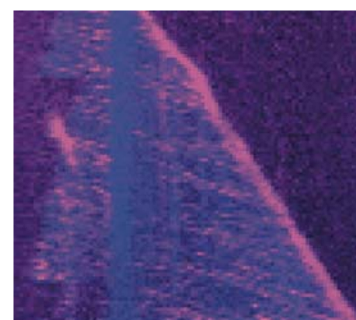
Put that all together and you'll get an inkling of what microtubules are. You'll also realise why they are so fiendishly difficult to study. But now, thanks to the efforts of Thomas and Damian, biologists have an insight into how cells control the behaviour of these dynamic structures.

Key to their success was sharing their expertise from different fields. "The first thing we did was get the wall between our labs removed," says Thomas. By combining Damian's cell biology background with Thomas's biochemical *savoir faire*, the researchers have pinned down a mechanism that directs proteins to the growing end of a microtubule – a mechanism many biologists had previously assumed would be too difficult to unravel. "At the time, it was thought to be very complex," says Thomas.

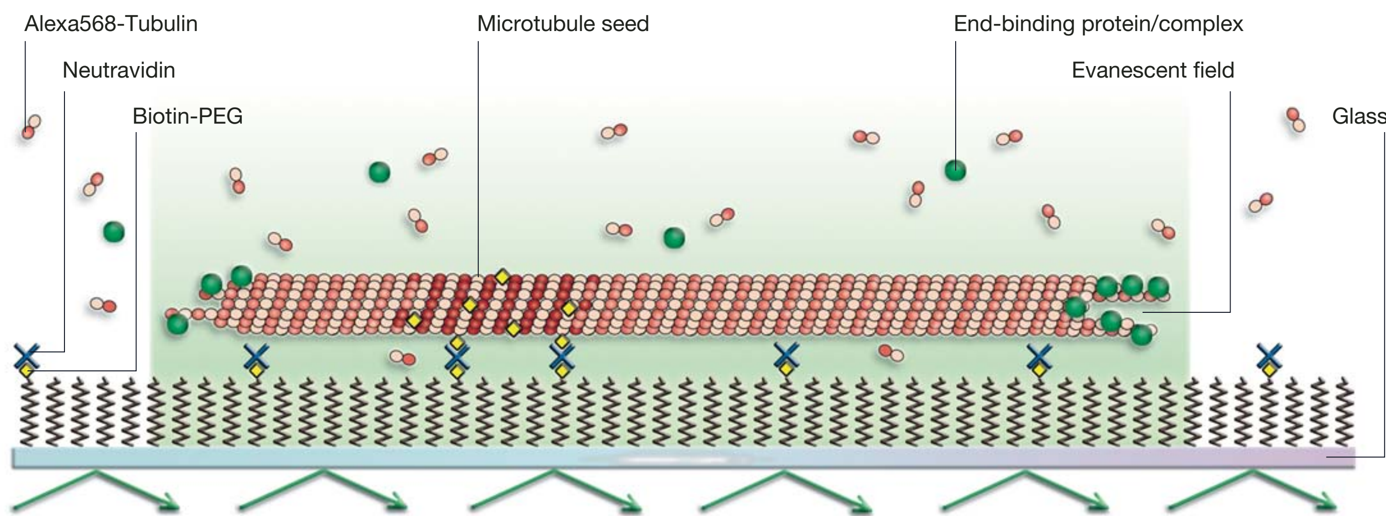








Damian, who works on yeast, first became interested in the topic through his curiosity about how cells control and change their shape. Microtubules were already known to be central to this process, and Damian wanted to find out more about how cells alter the behaviour of their microtubules. One way they can do this is via proteins that follow, or track, the growing, or 'plus end' of a microtubule. These proteins, called +TIPs, act as sensors at the tip of the tubule that monitor conditions within the cell and help the microtubule respond accordingly. This selective end tracking is quite a remarkable property that has fascinated scientists since it was discovered. So far, it could only be observed inside living cells. The mechanism of plus-end tracking was a mystery.



The diagram shows the setup of the experiment. Microtubules were grown in the presence of free tubulin (red) and fluorescently labelled +TIPs (green) on a glass slide and observed with microscopy in the evanescent field close to the glass surface.

Trying to study the molecular mechanism underlying such a dynamic system within cells is nigh impossible. This is where the expertise of Thomas and his team came in. Thomas works on the biochemistry of how microtubules assemble and of how motor proteins move along these tracks. "Our long-term goal is to understand the principles underlying large scale organisation within the cell," says Thomas. "Microtubules and motors make intracellular organisation different from what purely chemical systems can do."

Peter Bieling, a PhD student in Thomas's lab, has developed a unique system for growing microtubules in a lab dish. This involves sticking a short piece of microtubule, or 'seed', on to a dish treated with a special cocktail of chemicals. Only the seed sticks, so Peter can add any other components he wishes and see how the tubule grows. The ends of the tubules are free to move, as they would in a living cell. As well as being able to manipulate the conditions within the system, Peter is able to make precise measurements and so quantify the behaviour of the system's components.

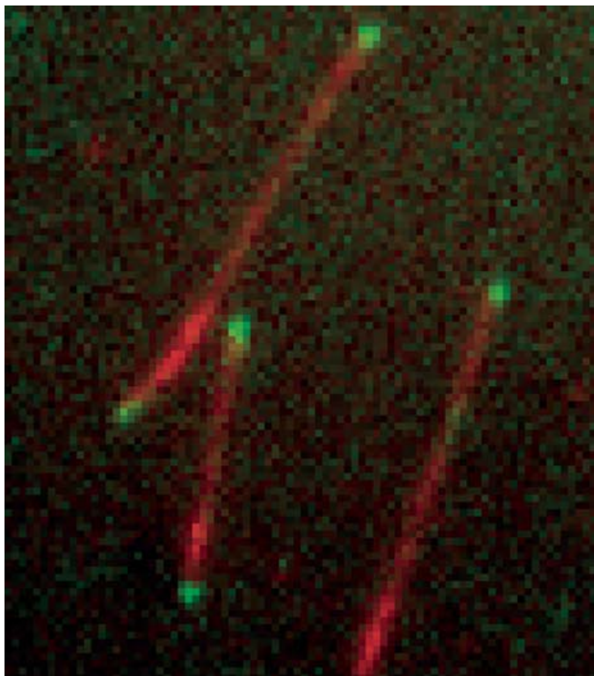
The two approaches complement each other very well. Damian likens it to studying how a car works. "I pull things out to see what goes wrong," he explains, "and Thomas and his colleagues build it bottom-up from components." Damian looked for mutations that affected microtubules in yeast. Having identified these, he gave Thomas a shortlist of potentially interesting components to test in his lab-dish system. "It's the difference between dissection and engineering," says Thomas.

The first challenge was to produce all proteins needed and treat them so that they could be seen using a special kind of fluorescence microscopy established in Thomas's lab. Once all proteins were happy and visible, Peter was able to focus in on a system that biologists had previously avoided on the grounds that it was too complex to study. "This is why many don't even dare to start trying," Peter says. "They thought there were too many parts."

Previous cell biology work on yeast had shown that many aspects of a cell's internal structure depended on how its microtubules were distributed. What's more, this distribution depended on the activity of three +TIPs called Mal3, Tip1 and Tea2. By watching living yeast cells under the microscope, researchers saw that Tip1 and Tea2 both tracked the growing plus ends of microtubules. Tea2 is a motor protein, and Damian and his team saw it move Tip1 along microtubules in cell. They had also found that Tip1 and Tea2 tracking depended on the presence of Mal3, which follows the plus ends without needing the other two. How was Mal3 doing this?

One possibility was that Mal3 was hitching a ride on the individual tubulin building blocks as they were added to the growing plus end. Alternatively, it might depend on one or more of the thousands of proteins present in a yeast cell – although biologists had so far found no hint of this. Could it be that Mal3 alone is able to recognise the growing plus end and latch on to it independently of tubulin and other proteins?

To find out, Peter added Mal3 and tubulin to his lab-dish model, and was excited to discover that Mal3 does indeed



The fluorescence microscope image shows microtubules in red, and plus-end tracking proteins that label growing microtubule ends in green.

act alone. What researchers had feared was a very complex and hard to study process turned out to be governed by a single, small protein. To unravel the mechanism of its action, the ability to manipulate the experimental conditions was key. By carefully looking at how individual molecules of Mal3 bound to the microtubule end, Peter was able to show that Mal3 was not hitching a ride, but that it must instead be recognising and sticking to a subtle structural feature of the growing plus end.

The researchers then turned their attention to Tea2, the motor protein that clambers up microtubules, delivering its cargo, Tip1. They wanted to know whether Mal3 was the only additional protein that Tea2 and Tip1 needed for their microtubule end tracking and if so, how it worked. Liedewij Laan, a PhD student in the laboratory of Marileen Dogterom, a collaborator based in the FOM Institute for Atomic and Molecular Physics in Amsterdam, visited Thomas's lab to work on this problem.

Liedewij found that, again, the situation was simpler than other cell biologists had expected. By using a mixture of Mal3, Tea2 and Tip1, she was able to reconstitute plus-end tracking of Tea2 and Tip1. Looking at the jiggling of single molecules again, Peter then worked out how all three plus-end trackers function together. Mal3 is not only an autonomous end tracking protein, but by binding to Tea2 and Tip1, it helps Tea2 to start stepping along the microtubule. Meanwhile, Mal3 unbinds from the two after a short time, letting Tea2 and Tip1 wander off towards the microtubule plus end.

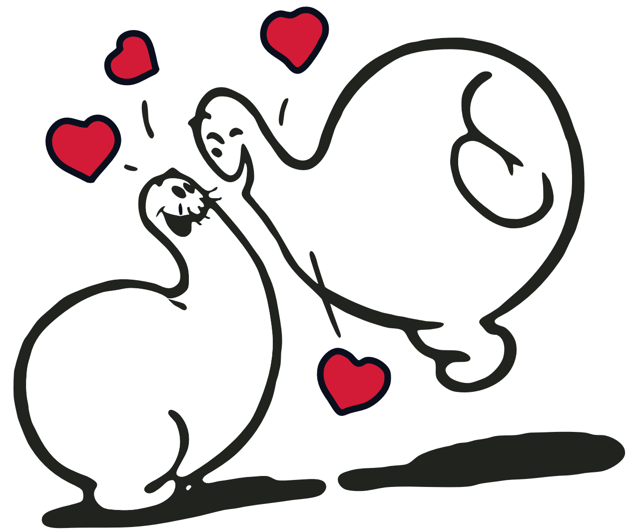
Thomas and Damian think that in this collaborative enterprise they have identified a defined protein interaction module, comprising Mal3, Tea2 and Tip1, that tracks microtubule plus ends. "Already, being able to define a module is amazing," says Damian. He and Thomas think that Mal3 acts as an overall organiser of plus-end behaviour, whereas Tea2 and Tip1 have more specialised functions.

The next challenge will be to look at how this newly found module interacts with other TIPs. "This is a very dynamic thing," says Thomas. For example, it is not clear whether the module is completely independent of other TIPs, or whether it interacts with them in some way. But now that scientists have a way of both dissecting and engineering these mechanisms, they have a very good chance of finally being able to pin down these elusive structures once and for all. "The potential of this *in vitro* system is great," says Thomas.

Bieling P, Laan L, Shek H, Muntenau EL, Sandblad L, Dogterom M, Brunner D, Surrey T (2007) Reconstitution of a microtubule plus-end tracking system in vitro. *Nature* **450**: 1100–05 ■

Probably the world's biggest model of a microtubule is the chimney at EMBL Heidelberg.

How does a shmoo schmooze?



A NEW MICROSCOPY TECHNIQUE that lets scientists count individual protein molecules in living cells has revealed remarkable new detail about how cells communicate. It will enable scientists to study such communication with far more precision than before and perhaps eventually to predict how it will react to changes in a cell's environment. This would be invaluable for developing new drugs to treat diseases such as cancer, says Michael Knop, who heads the team that made the findings. "You can only do this if you really understand your system at the necessary level of detail," he says.

Michael, a group leader at EMBL Heidelberg, and his team have pioneered the use of a technique known as fluorescence cross-correlation spectroscopy, or FCCS. Although the technology has been around for many years, the team is the first to apply it to look at molecules in living yeast cells. "The difficult part is analysing that data," explains Michael. The researchers, together with former EMBL group leader Philippe Bastiaens and his colleagues at the Max Planck Institute of Molecular Physiology in Dortmund, Germany, have overcome the technical obstacles that have prevented cell biologists from using FCCS in the past.

Armed with the new technique, the team set about studying a signal reception system employed by yeast cells as part of their mating behaviour. The system, called the mitogen activated protein (MAP) kinase cascade, relays signals triggered by hormones arriving at the surface of a yeast cell to the rest of the cell. In response, the cell extends a protrusion known as a shmoo, which unites with its partner's shmoo during mating. MAP kinase cascades are a common feature of cell signalling in many species, including humans, and influence a huge range of cell behaviours. They can also go awry in a number of diseases, notably cancer.

Yeast cells are easy to manipulate genetically, which allowed the team to add fluorescent protein labels to key components of the MAP kinase system. These components come together into complexes. By making precise measurements of their diffusion within living cells, the team was able to deduce that changes in the composition of the complexes are not involved in relaying the hormone signal through the cytoplasm. Rather, a tiny fraction of the complexes is drawn to the cell membrane. Once there, the complexes activate one of their components, a protein called Fus3. This messenger then transmits the signal across the cell interior.

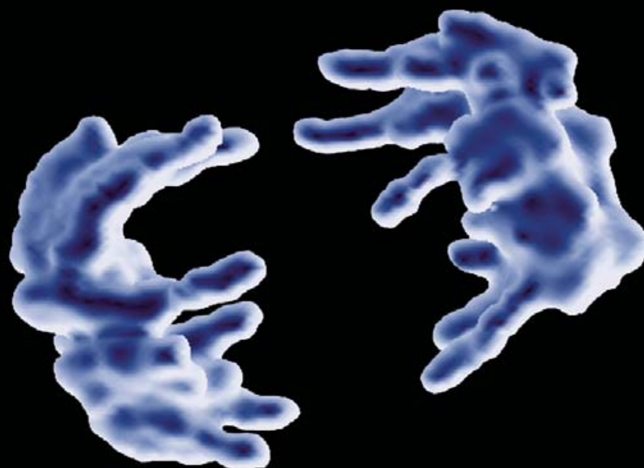
Fus3 makes its way to the cell's nucleus, where it switches on genes needed for mating. Using a different microscopy technique, Michael's team discovered that as Fus3 travels, its activity is reduced by other proteins in the cell, which results in a gradient of Fus3 activity. Fus3 activity is highest near the membrane, and lowest at the distal end of the cell, allowing just enough Fus3 activity inside the nucleus. This points to Fus3 relaying the hormone signal to other parts of the cell, and having different effects depending on its local level of activity, Michael thinks.

The great strength of FCCS is its ability to give precise and reliable measurements of proteins in living systems. It's not a simple system to use, however, so Michael and his colleagues have arranged an EMBO training course to bring the technology to the wider cell biology community. Ultimately, Michael hopes scientists will be able to count proteins within a single cell, using the techniques his team has established. "That's definitely a step further towards this goal," he says.

Maeder CI, Hink MA, Kinkhabwala A, Mayr R, Bastiaens PI, Knop M (2007) Spatial regulation of Fus3 MAP kinase activity through a reaction-diffusion mechanism in yeast pheromone signaling. *Nat Cell Biol* **9**:1319–26 ■

Push me, pull you

Jan Ellenberg, Coordinator of the Gene Expression Unit at EMBL Heidelberg, Sebastien Huet, Marina Freudzon, Melina Schuh, Péter Lénárt, Tomoya Kitajima



What looks like two fighting spiders is actually the 3D reconstruction of microscope images of a living mammalian cell showing the chromosomes just after separation.

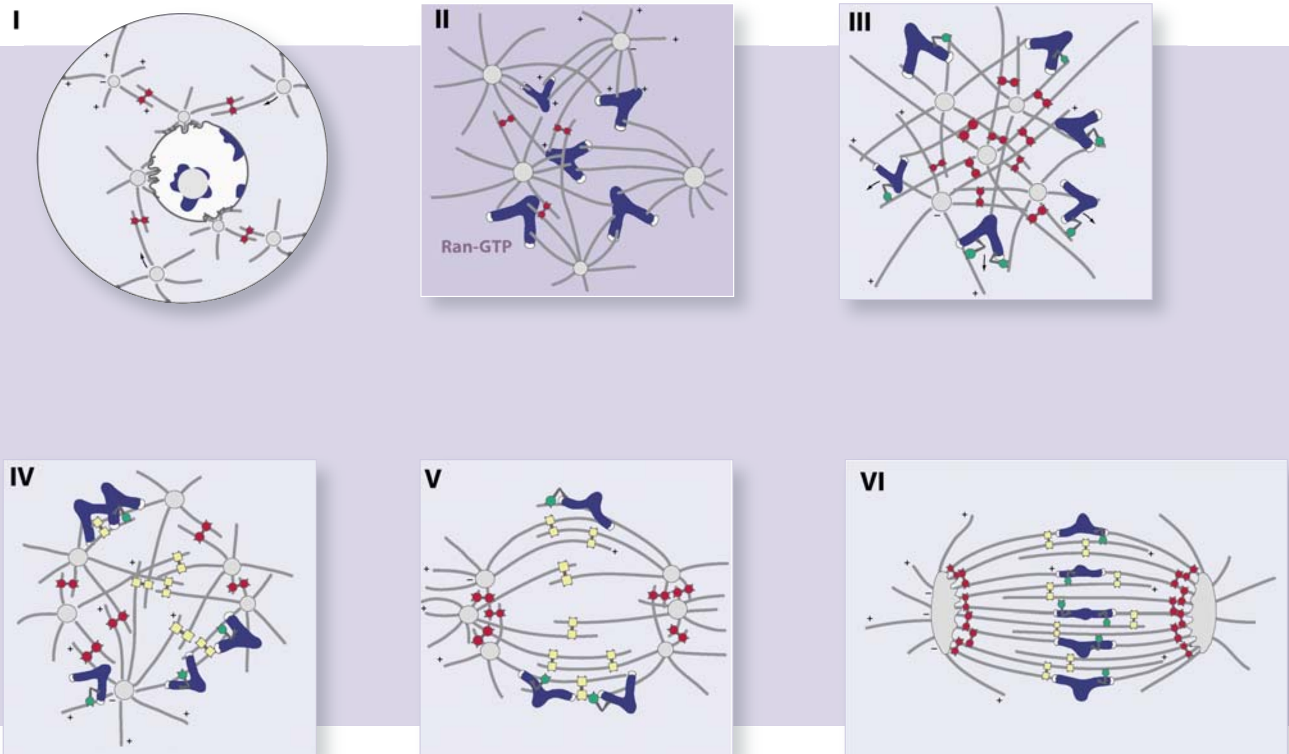
JAN ELLENBERG gently eases a drop of seawater with a starfish oocyte onto a microscope slide. The nearby computer screen – attached to a laser scanning microscope – fills with coloured wavy lines. He adds a small drop of hormone, and waits. Within minutes, the egg starts to work its magic. The colours dance on the monitor, and tiny strands wrestle with the cell's DNA to transform an oocyte into an egg that can be fertilised.

Jan is able to peer more deeply into the intricacies of cell division than most people. As Coordinator of EMBL's Gene Expression Unit in Heidelberg, he is interested in how cell division happens when eggs form from their precursor cells (called oocytes), a process known as meiosis. During meiosis, the maternal and paternal copies of each chromosome line up for a contest. Only one copy of each pair can remain in the maturing egg; the other has to be ejected from the cell to make space for the copy that the sperm brings in during fertilisation. To do this, the oocyte divides, but does so unevenly – all the nutrients and one set of chromosomes are kept in the egg for the growing embryo, while the unwanted second set of chromosomes is extruded in a miniature cell, called a polar body. If this process goes awry there can be problems in fertility or later in development. "The most important thing during cell division is the safe handling of chromosomes, which carry all of the cell's genetic material," Jan explains.

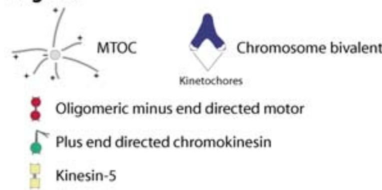
"It is curious that the process in mammals is so different to other types of cell division. But although this remarkable system of self-assembly looks complicated, it is clearly a reliable way of doing things."

Jan specialises in developing high-resolution microscopy to track what is going on inside living cells in real time. This technique involves three important steps. First, the researchers tag proteins inside the cell with fluorescent labels. Second, they use powerful laser scanning microscopes to take time-lapse photographs of the living, dividing cells. Third, and perhaps most importantly, the researchers develop computer software to quantify the processes they're watching. The researchers have automated many of these steps, which is crucial to the success of this technique. These powerful methods offer researchers a glimpse into cellular events that have never been seen before – and some of these new observations are shifting the classical textbook view of cell division.

Starfish eggs are perfect for watching cell division, because they are large, transparent, easy to manipulate and feel at home in just a small drop of seawater. But perhaps the most important thing is timing. When Jan adds a hormone to a clump of developing egg cells, they start to divide "with the precision of a Swiss clock", he says. Jan and his colleagues have mapped out how the chromosomes move during egg formation in exquisite detail. At the beginning of meiosis, the cell's internal skeleton – called the cytoskeleton – is reorganised. One part of the cytoskeleton, called actin, makes long flexible cables that move the chromosomes just underneath the surface of the cell. There, the chromo-



The schematic drawing shows the acentrosomal spindle assembly in mouse oocytes. Many independent microtubule organising centres (MTOCs) arrange themselves by pulling and pushing to form a bipolar spindle.



somes are taken over by a second part of the cytoskeleton called microtubules. Microtubules are long, relatively stiff, rods that emanate from so-called centrosomes, which are anchored just underneath the surface of the cell. "Then centrosomes send out a star-shaped array of microtubules to capture the chromosomes delivered by actin," explains Jan. These microtubule asters then form a 'spindle' – a bipolar structure along which chromosome pairs can be moved and one set extruded out of the cell.

But starfish are a long way from humans. And Jan wanted to know whether similar actin cable machinery was responsible for chromosome movement in mammalian eggs. "More than five percent of human eggs have the wrong number of chromosomes," says Jan. "This can sometimes lead to severe genetic disorders like Down Syndrome, and also to infertility or miscarriage."

So the researchers embarked on a similar study of mouse egg formation. This was much trickier, and they took advantage of EMBL's Laboratory of Animal Resources which provided mouse eggs at just the right stage of growth. But as they began the project, Jan was shocked to learn that no one knew exactly where and how the microtubule spindles form. "Biologists knew that mammalian meiosis does not involve centrosomes," he says. "But no one had managed to study meiosis at high enough resolution in a live cell to find out what went on instead." This led him to ask a basic question: How do you make a spindle without centrosomes?

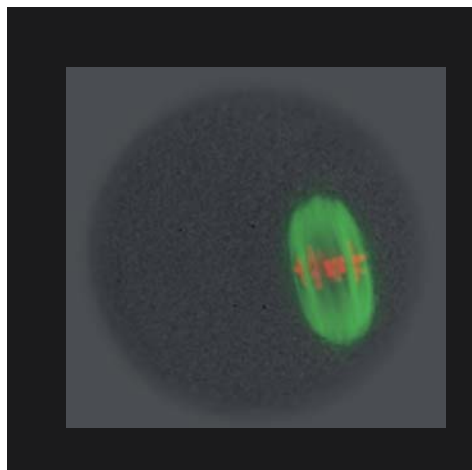
So Jan, and PhD student Melina Schuh, had to start at the beginning. And they were astonished by what they found. Labelling microtubules with fluorescent tags revealed that spindle assembly in a mouse oocyte involved more than 80 microtubule asters rather than only two centrosomes. They saw that at the start of meiosis these asters fill the cell with an amorphous web of microtubules. "Then by engaging with each other via their microtubule rods, the asters have a sort of fight, physically pushing and pulling on one another," says Melina.

In this molecular struggle, the asters first pull on and gradually merge with each other. They then start to push each other apart and through these simple attraction and repulsion forces, the system resolves into just two large group of asters: the spindle poles. "The multipolar system collapses into a bipolar system, which is the only stable structure able to balance the opposing forces," says Jan.

After that, the entire spindle, including the chromosomes, is moved to the surface of the cell to prepare for the

uneven cell division. The next question that Melina and Jan will tackle is whether this movement of chromosomes to the cell surface is orchestrated by actin, as it is in the starfish. "It is curious that the process in mammals is so different to other types of cell division," notes Jan. "But although this remarkable system of self-assembly looks complicated, it is clearly a reliable way of doing things."

Dividing a cell into two is always perilous, and problems with chromosome movement can also happen in other types of cell division. In normal mitosis, which is the cell division that happens in the body when cells other than eggs or sperm divide, chromosomes need to be replicated and divided equally between two daughter cells of similar size. And unless each new cell has a full set of chromosomes, cells are in danger of becoming cancerous. Jan became interested in a phenomenon known as 'chromosome compaction', which has an important role in chromosome segregation. After chromosomes have replicated but before they are captured by the microtubules, they condense to form neat, tight rods that line up along the equator of the cell. "This compaction process prevents the chromosomes from becoming tangled up during segregation."



The spindle apparatus of a mouse oocyte as seen through the microscope. Microtubules are labelled in green and chromosomes in red.

To find out how this compaction process was controlled, Jan enlisted the help of his PhD student Felipe Mora-Bermudez. "To do this, we first needed a precise idea of what the compaction process looks like," Felipe explains. Once again, they illuminated the chromosomes using fluorescent tags, and crucially, they tracked the chromosomes in three dimensions and developed computer software to calculate the volume of the condensing chromosomes.

"What we saw was totally unexpected," says Jan. Textbook wisdom says that chromosomes condense only until they are captured by the microtubule spindles. But instead, the researchers saw the chromosomes go on condensing even after they had been captured and segregated. At first they thought they must have made a mistake, so they repeated the experiment. But time and time again, they kept on getting the same result. "It was there in front of our eyes," says Felipe. "Chromosome compaction keeps right on going until after the chromosomes have been moved apart to the poles of the cell."

Certainly to prevent chromosomal threads from becoming tangled, chromosomes need to be compact. "But once segregation has happened," Jan asks, "why does a chromosome need to become any smaller?" The only way to get some clues was to somehow disrupt the compaction process and see what happened.

And the researchers found two new answers to the question of what chromosome compaction is good for. Mammals often have very long chromosomes. So when the mitotic spindle has done all the pulling it can, and the spindle poles are pushed right up against the edge of the cell, mammalian chromosomes are left dangling dangerously close to the middle of the cell where cleavage happens. When the researchers inhibited a protein called aurora kinase, the chromosomes didn't compact properly. "We saw that if the chromosomes don't shrink properly, the ends of the chromosomes often became trapped in the cleavage zone and prevented the cell from splitting in two," says Felipe.

The second crisis point comes right after the cell has divided. "It is very important that the chromosomes are rewrapped back into a nucleus as soon as possible," says Jan. If the chromosomes are not fully compacted, then this wrapping process becomes confused, and the daughter cells end up with lots of little nuclei. When the cell lacked aurora kinase, this is exactly what they saw. "Such micronucleation is something that we often see in cancerous cells," Jan says. "And it's very dangerous for the next cell division, because chromosomes will get lost."

This study illustrates just how powerful live cell imaging technology can be. "The chromosome-associated proteins are difficult to analyse biochemically, because they are not soluble, and at the same time difficult to analyse genetically, because they are so important to the life of the animal." Imaging is therefore one of the few ways to study them. "We're driven by the biological questions," says Jan. "But we're always pushing imaging technology to be able to look into new parts of the cellular universe." It seems that we really are living in an image-conscious age.

Mora-Bermúdez F, Gerlich D, Ellenberg J (2007) Maximal chromosome compaction occurs by axial shortening in anaphase and depends on Aurora kinase. *Nat Cell Biol* **9**: 822–31

Schuh M, Ellenberg J (2007) Self-organization of MTOCs replaces centrosome function during acentrosomal spindle assembly in live mouse oocytes. *Cell* **130**: 484–98 ■

Judging a gene by its cover

As much as we would like to convince ourselves that it is all about what's inside, sometimes outward appearance does count – at least in biology. The building plans for proteins are encoded as a string of four letters (A, T, C and G) on long DNA molecules. However, which proteins are made at any time is determined not only by this intrinsic letter code but also by the way DNA is packaged inside a cell's nucleus. With the help of proteins, DNA is condensed into a compact structure called chromatin. The more tightly the chromatin is packaged, the less accessible it becomes for the molecular machinery that transcribes the DNA to initiate the production of proteins. Changes to chromatin structure, so-called epigenetic changes, thus influence which genes are expressed in a cell.

Epigenetic regulation is a key player in embryonic development. A fertilised egg can still access all its genes, but in the course of development epigenetic silencing shuts down more and more areas of the genome. This allows cells to specialise and acquire specific functions. Epigenetic control is also crucially involved in the formation of cancer, making epigenetics a hot research topic for molecular biologists all around the world.

Scientists in the lab of former EMBO Executive Director Frank Gannon, who is now executive director of Science Foundation Ireland, are focusing on one particular epigenetic mechanism called DNA methylation. Adding or removing small chemical residues, called methyl groups, to or from key parts of the DNA alters the structure of chromatin.

Traditionally, DNA methylation has been described as long-term regulation of gene expression, because the methylation marks on DNA are maintained throughout rounds of cell division and passed on to the next generation of cells. But staff scientist George Reid and his colleagues recently made a discovery that suggests something very different. Applying the hormone estrogen or other small molecules to breast cancer cells to synchronise the expression of genes, they observed rapid changes in their methylation patterns. Estrogen and the established anticancer drug doxorubicin cause methyl groups to be removed from regulatory regions of specific genes within a few minutes. Such treatment then sets off a whole cycle of events: initial demethylation renders silent genes active and subsequent remethylation shuts them down again. This cycle repeats itself every 1.5 hours.

"We described changes in methylation of DNA acting on a very short timescale. These observations challenge our understanding of epigenetics as a means to regulate gene expression permanently. It also impacts upon our knowledge of how cells interpret their DNA," says Sara Kangaspeska from the Gannon lab.

The new insights into the cyclical nature of methylation might shed light on the molecular bases of cancer and inform the search for new, effective treatments. In particular, breast cancer is affected by estrogen signalling and changes in epigenetic control. "Our next step will be to find small molecules that target the cyclical methylation processes to elucidate their precise role and to identify possible ways to exploit them in the fight against breast cancer," George concludes.

Kangaspeska S, Stride B, Metivier R, Polycarpou-Schwarz M, Ibberson D, Carmouche RP, Benes V, Gannon F, Reid G (2008) Transient cyclical methylation of promoter DNA. *Nature* **452**: 112-15 ■

Sara Kangaspeska, George Reid and Maria Polycarpou-Schwarz

Tale of the unexpected

Life is like a box of chocolates –
Florence Besse and Anne Ephrussi,
Coordinator of the Developmental
Biology Unit at EMBL Heidelberg

"LIFE IS LIKE A BOX OF CHOCOLATES. You never know what you're gonna get." This line from the 1994 hit movie *Forrest Gump* was a philosophical take on the vagaries of fortune, but could just as easily apply to all life and the scientists who study it. The unpredictable nature of the world is a challenge faced by all living things, which must be flexible and adaptable to survive. And every once in a while, biological research turns up something unexpected which demands a nimble intellectual and practical response from scientists to turn it into a new and valuable discovery.

Last year, biology threw one of these surprises at Anne Ephrussi, Coordinator of the EMBL Heidelberg Developmental Biology Unit, and her postdoc Florence Besse. The pair had set out to find out more about how protein production within nerve cells might be targeted to specific areas of such cells in fruit fly larvae. Instead, they ended up discovering a protein that helps to build the connections, or synapses, between a larva's nerves and its muscles and to adapt these synapses in response to the environment. A similar protein exists in mammals and humans, and understanding more about it could shed new light on how our brain cells develop the remarkable flexibility that allows us to learn and remember new information.

The story began when Anne's group decided to expand the scope of their research into neurobiology. The group's focus is on understanding how cells deploy a particular kind of molecule, called messenger RNA or mRNA, which plays a central role in the process of turning the instructions encoded in genes into proteins.

In the first step of this process, the cell selects the stretch of DNA that contains the gene and makes a copy of it, using RNA, a chemical relative of DNA. This RNA copy then gets turned into messenger RNA, a form that can be read by ribosomes, the cell's protein-making robots. Although many of these RNAs are read in a centralised protein factory, others are sent to specific locations within the cell and read there instead. One reason for this is that cells use localised proteins as grid references that allow other molecules to determine their position within the cell. This is especially obvious in the fruit fly egg, the system Anne has studied for many years, where localised proteins and RNAs help define the head, tail and trunk of the future embryo.

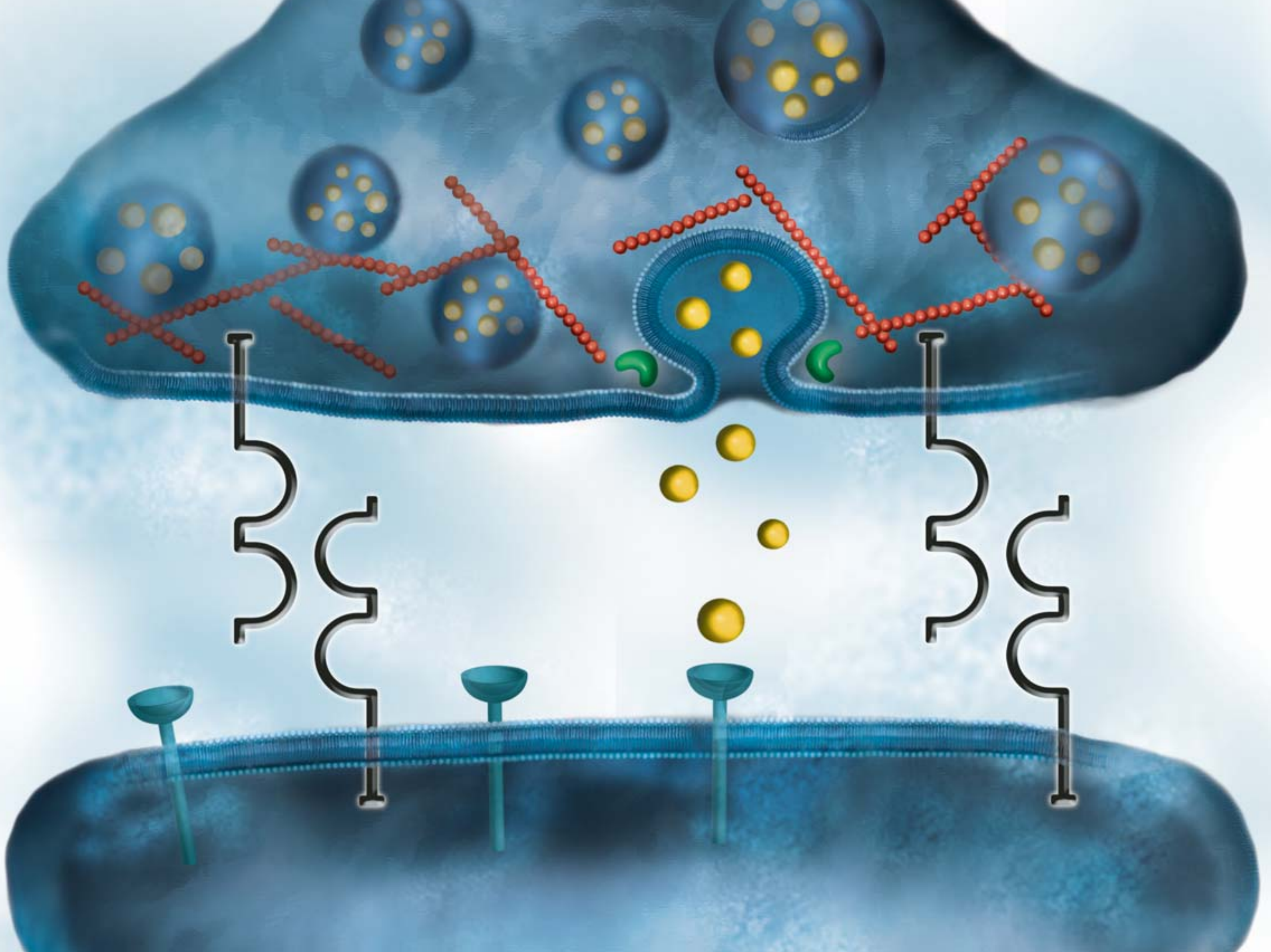
Another reason for localising RNAs is that it allows cells to respond very quickly to changes in their environment. If, for example, a cell receives a signal instructing it to make a protein that acts in a particular part of the cell, it can do so without having to waste time making an RNA copy and transporting the resulting protein to the correct location. This is particularly pertinent for nerve cells, where the region next to the synapse is usually a long way away from the nucleus, the main home of the cell's DNA.

Until now, however, most of the research into RNA localisation in nerve cells had been done *in vitro* – in the test tube – or on models that are not suitable for performing extensive genetic studies to search for new genes that might be involved in RNA localisation. Fruit flies, however, are ideal for this, so Anne decided to make the leap into neurobiology.

She and Florence teamed up with Stephan Sigrist, a fruit fly neurobiologist at the European Neuroscience Institute in Göttingen, Germany, who trained them in the key experimental techniques. Stephan had previously published a study on RNA localisation in a part of the fly nervous system called the neuromuscular junction, where a nerve cell forms synapses with a muscle cell. Given that Stephan had blazed the trail for this system, Anne and Florence decided to use it as model to screen for the genes involved.

Synapses are a specialised form of connection where the membranes of cells meet, leaving a tiny gap across which signals can be sent. The nerve cell, or neuron, sending the signal is known as the presynaptic cell, and the one receiving it – either another neuron or a muscle cell – is known as the postsynaptic cell. Synapses are usually located at the ends of the long, arm-like extensions or axons that extend

Florence at the Cell Sorter in the Developmental Biology Unit in Heidelberg.



A synapse – the connection between two nerves cells or nerve and muscle – consists of the presynapse (dark blue, top), the synaptic cleft and the postsynapse (dark blue, bottom). The protein basigin (black) spans the membranes of pre- and postsynapses and brings them into the correct proximity. It also controls how neurotransmitters (yellow) are released into the cleft, organising vesicles at the correct areas in the presynapse through interaction with the cytoskeleton (red).

from neurons and split into many branches. In the presynaptic cells, the ends of these branches terminate in swellings known as boutons.

When a nerve impulse travelling down an axon branch reaches the bouton, it triggers the release of chemical signals, called neurotransmitters, stored inside it into the tiny cleft between the cells. Receptors in the postsynaptic cell membrane detect these signals and trigger a response, such as relaying the impulse to another neuron, or making a muscle contract.

This sounds simple enough on paper, but the reality is far more complex. "A neuron can make upwards of 10 000 synaptic connections with other cells," explains Anne. What's more, the number of boutons is far from constant, and changes in response to the amount of activity within the nervous system. The number of boutons in a fruit fly larva, for example, depends upon how active its muscles are. So in addition to transmitting signals, neurons must also be able to monitor and respond quickly to their environment and alter their synapses accordingly. As well as helping an animal adapt to its environment, having flexibility, or plasticity, built in to synapses is thought to be one of the key factors underlying learning and memory.

But this kind of flexibility demands quick and accurately placed responses on the part of the neuron – the perfect job for locally stored RNAs. "To do things fast and very specifically and to build in memory, localised translation would be a very smart thing to do," says Anne. There were hints, but no firm proof, that something of that sort might be happening in mammals.

Florence began by screening for proteins that were present in the boutons. To do this, she had to work with tens of thousands of flies and screen thousands of larvae. "The day Florence started at EMBL we started the screen," says Anne. "I'm not sure when she first had the time to go to the grocery store!" A year of hard work paid off, however,

and Florence had a collection of proteins that looked promising. But it was here that she hit a wall. Try as she might, she was unable to identify one that looked like it was the product of localised translation.

While they were wrestling with this problem, Anne and Florence discovered that one of their promising proteins was the fly equivalent of Basigin, a mammalian protein known to play a role in invasive cancers. What's more, mice lacking Basigin have problems with learning and sensing, hinting at an important role in the nervous system. "It was clear that it could also have a developmental function," says Florence. "But nothing was really known about the function of the protein."

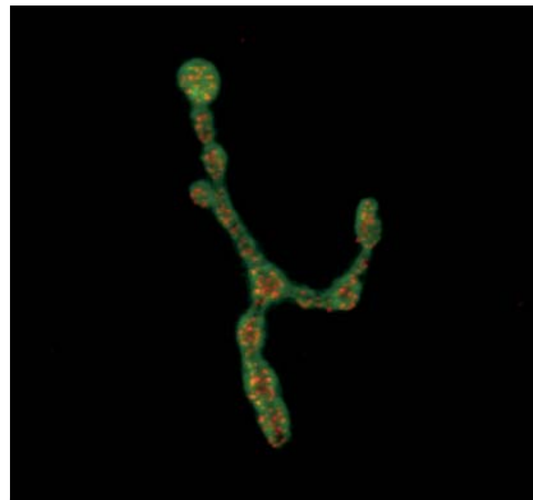
Anne and Florence combined lots of different experimental techniques to find out more about the fly Basigin, including electrophysiology experiments, which were performed by members of Stephan's group. What they uncovered was a picture of a multi-talented and versatile protein. Basigin spans the cell membrane of both the neuron and the muscle cell at the synapse. It makes sure that these membranes keep just the right distance from each other as the synapse develops: not so close that the synapse's function is restricted and not so far that synaptic signalling fails.

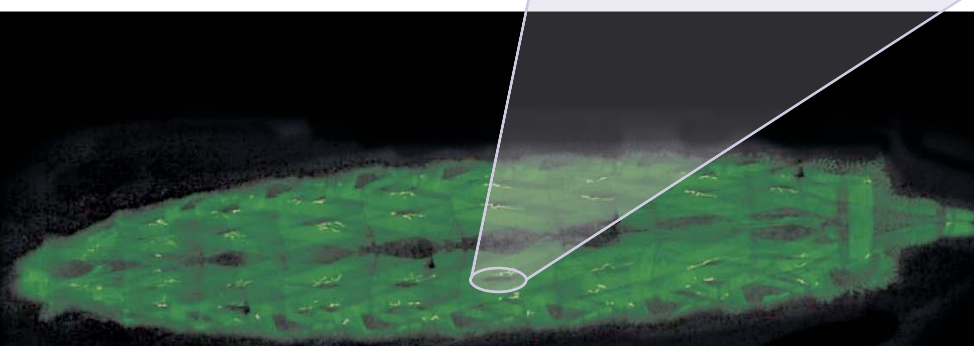
Intriguingly, Basigin also plays a vital role in managing the ever-changing molecular structure of the synapse. In the presynaptic cell, neurotransmitters are kept in little parcels called vesicles, which are stored right next to the cell membrane. The way they are deployed and released through the cell membrane changes depending on the feedback and signals being received by the cell. Basigin helps to ensure that the vesicles are stored and organised properly in the right areas of the neuron, and thus helps to control how and when neurotransmitters are released into the synapse. "This is a most original protein found to link the neuron's membrane at the synapse and the cellular components stored just inside it," says Florence.

All these processes are thought to underlie long-term increases in the strength of signalling across a synapse that are associated with learning and memory. Basigin may carry out some of its functions by interacting with part of the cell's internal scaffolding, its actin cytoskeleton. The section of the protein that might do this is similar across a range of different species, and would be a good starting point for future studies, say Anne and Florence.

For the time being, however, their focus is firmly back on RNA localisation in neurons, although there could be more surprises in store. "You don't necessarily come out with what you were looking for," says Florence.

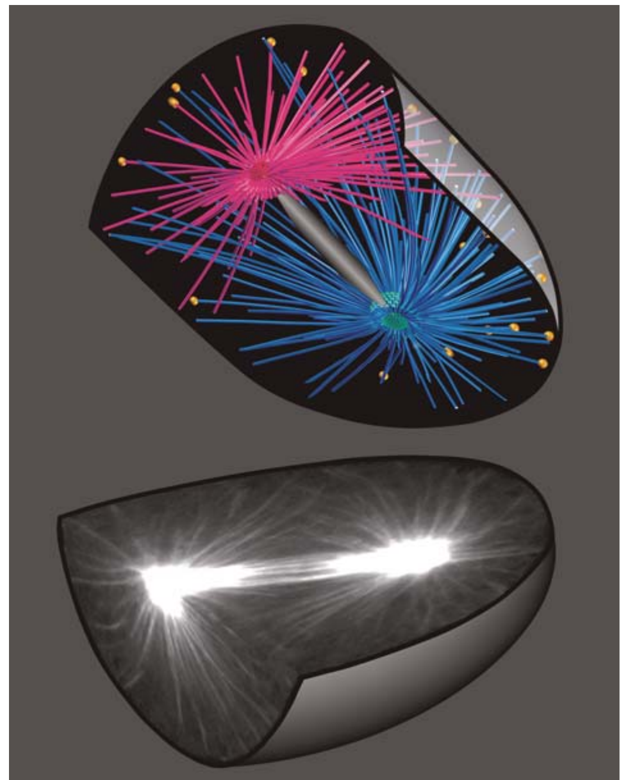
Besse F, Mertel S, Kittel RJ, Wichmann C, Rasse TM, Sigrist SJ, Ephrussi A (2007) The Ig cell adhesion molecule Basigin controls compartmentalization and vesicle release at Drosophila melanogaster synapses. *J Cell Biol* **177**: 843–55





Zooming into a *Drosophila* larva to find a neuromuscular junction.

In for the long haul



A combination of computer simulations (top) and microscopy studies (bottom) reveals the mechanism of asymmetric division of a nematode worm embryo. Microtubules (pink and blue) branch out from the spindle poles until they touch the cell cortex to generate forces that position the spindle.

CELLS EMPLOY AN ARMY of molecular dockers to moor their internal skeletons in the right places, scientists at EMBL Heidelberg have discovered. The work helps to explain how a cell organises the highly complex structure of its interior and how this affects important functions such as cell division.

Group leader François Nédeléc and his team have been studying the behaviour of microtubules, tiny tube-like structures that play many different roles within the cell. They form, for example, an ever-changing internal skeleton that governs a cell's shape. Microtubules also help to organise the cell's tiny internal organs, or organelles, and are vital for cell division, in which they haul newly copied chromosomes into the correct daughter cells. The microtubules are made of building blocks called tubulin, which are constantly being added or taken away. The behaviour of the microtubule – whether it grows, shrinks, or remains static – depends on the balance between these two processes.

The question is, how does the cell get microtubules to the right place at the right time? "It's extremely important to organise the microtubules in order to organise the cell on a large scale," says François. To find out more, his team turned to the embryos of a tiny worm called *Caenorhabditis elegans* to look at how microtubules position themselves for a special kind of cell division.

Many cells, including those of *C. elegans* embryos, don't divide equally, straight down the middle. Instead, they divide asymmetrically, with one daughter cell ending up smaller than the other. The asymmetry helps convey information to the worm's cells about what cell types they are destined to become.

During cell division, the cell's chromosomes line up on a microtubule structure called the mitotic spindle. This looks like a set of guy ropes tethered at either end of the cell. The chromosomes assemble down the middle in preparation for division. In the first cell division of the *C. elegans* embryo, the spindle moves towards the rear end of the egg to make the division asymmetric.

By labelling microtubule components with fluorescent molecules, the team was able to watch the microtubules at work. They found that when their ends touch the inner lining of the cell, the cortex, the balance between the building and removal of the tubulin blocks shifted so that they began to shrink. With the help of computer modelling, the team showed that these dynamics were enough to shift the spindle backwards in the cell, most likely with the help of molecules in the cortex. "There are proteins that can take advantage of the microtubule shrinkage to generate force," says François.

These proteins are known to exist in other organisms, and work by grasping the frayed end of a shrinking microtubule and pulling – rather like a docker hauling on the ropes of a tanker as it moors in a harbour. The next task for François and his team is to unpick the molecular details of how this mooring takes place, and so reveal new insights into how cells know which strings to pull to get things done.

Kozlowski C, Srayko M, Nedelec F (2007) Cortical microtubule contacts position the spindle in *C. elegans* embryos. *Cell* **129**: 499-510 ■

Safety in numbers

LIKE HUMAN BEINGS, cells can move as individuals, in small clusters or even in large, organised crowds, depending on their purpose and the environment in which they find themselves. Also like human beings, their behaviour can alter depending on whether they are alone or in the presence of fellow travellers. Scientists are interested in what causes those behavioural differences, or, in other words, what defines crowd behaviour in cells.

The question is important, not only because of the key role played by cell migration in development, but also because it continues to occur later in life – when sheets of cells migrate during wound healing or cancer metastasis, for example. Historically, cell migration has been studied in single cells, and relatively little is known about their collective behaviour. To learn more about collective cell migration, developmental biologist Pernille Rørth and her group at EMBL Heidelberg have been studying the process in a simple biological system – the egg chamber of the fruit fly *Drosophila*.

This egg chamber contains not only the egg, or oocyte, but also a number of giant nurse cells whose job is to nourish the egg, follicle cells that line the chamber and eventually form its hard outer casing, and a small population of border cells. These border cells detach themselves from the chamber's follicular lining and move in a cluster towards the oocyte. Their function is twofold: to ensure that a corridor is left to the outside world when the chamber seals over, so that a male fly's sperm can enter and fertilise the egg; and to deposit signalling molecules around the egg that direct its later growth.

Pernille is not particularly interested in the egg chamber *per se* – except as a substrate across which the border cells migrate. "It's a substrate in a positive sense, in that it gives them something to crawl over," she says, "but it's also a hindrance, because the egg chamber is a completely compact environment and there is no free space inside it." She is more interested in what triggers migration in the border cell cluster (see box), and how the cells find their way once they have started migrating.

The cluster consists of two inner cells and six outer, migratory cells. The outer cells respond to guidance cues, or signals, which are emitted from the region of the oocyte. When Pernille arrived at EMBL in 1998, none of those cues were known, but by systematically overexpressing different genes in *Drosophila* egg chambers, her group has since found a number of them. They have also identified two receptors which read the cues and instruct migrating cells how to position themselves in space. These receptors in turn activate a protein called ELMO, which is critical for migration.

It was in investigating ELMO that the group made a puzzling discovery: the protein is only important in early stages of migration. Ambra Bianco, a PhD student in the lab, created genetic mosaics in which mutant border cells lacking ELMO were generated in a heterozygous animal, and then observed how those mutant cells behaved. She found that they remained at the back of the cluster in the early stages of migration, indicating that they had lost their migratory sense and were being 'carried' by the others. Later on, however, they were just as likely as the nor-

Former EMBL group leader Pernille Rørth now heads a group at the Temasek Life Sciences Laboratory in Singapore.

mal cells to be at the front of the cluster, which suggested that successful migration no longer depended on ELMO. In the later stages, the migrating cells seemed to make use of a different signalling pathway to find their way. In a sophisticated experiment using mixed clusters, Ambra identified two pathways, referred to as Raf-MAPK and PLCγ, which are required only for the late stage of migration. A puzzle remained, however: how and why do these molecular changes occur halfway through migration?

Only when the group succeeded in imaging the migration of border cells, by labelling them with green fluorescent protein and viewing them under the confocal microscope, were they able to make sense of this genetic conundrum. That took time, because egg chambers are extremely sensitive to environmental perturbations, and to begin with they were unable to persuade them to grow outside the living insect. "The egg chamber doubles its volume in about six hours, within the female abdomen," Pernille says. "That means it's constantly pumping in and synthesising materials, and if conditions aren't right to support this fantastic growth rate, the whole process is arrested."

Through the dogged persistence of postdocs Minna Poukkula and Adam Cliffe – Team Poukkula as they are affectionately known in the lab – they eventually stumbled on a set of conditions that was favourable to the uninterrupted growth of the egg chamber. "To work out how to culture the egg chambers took the two of us a month, during which time we tested 123 conditions," say Adam and Minna. For each condition, they had to dissect the fly with two fine pins and extract the egg chambers without damaging them, which they describe as "like trying to shell a hard-boiled egg with a pair of pool cues".

With the help of the Advanced Light Microscopy Facility in Heidelberg, headed by Rainer Pepperkok, they were finally able to see the border cells migrating in real time. "Adam and Minna came up to me with big round eyes, saying, 'You won't believe it, they change their behaviour in the middle of the movement!'" Pernille recalls, laughing, "and that's exactly what they do. Early on you see the cluster moving fast, with a couple of cells at the front pulling on the back cells in a streamlined, torpedo-like fashion. Later on the whole cluster slows down, the cells appear a little more round and they kind of shuffle forward." The changes in cell shape and migration shape coincide precisely with the observed molecular changes.

This two-speed migration posed a conceptual problem for them, because classically cells have been thought to move as a result of differential sensitivity to a guidance cue between the front and back of the cell. By virtue of signalling more strongly at the front, the cell knows which way is forward. Early in border cell migration, the researchers saw this front-to-back gradient in the requirement of ELMO in the cell. Later on they observed the reverse gradient for the requirement of Raf-MAPK and PLCγ. But the signalling pathways necessary only in the later phase of migration are uniformly active across every cell in any organism in which they have been studied, so how did those cells know which way was forward?

"The penny dropped when we realised we were not dealing with one cell, but with a cluster in which each of the

outer cells is pointing in a different direction with respect to the centre," Pernille says. "Perhaps information was stored in each cell, not just about which is the front of the cell and which is the back, but also about which cell is at the front of the cluster and which is at the back."

She suggests that this novel kind of guidance might be based on small differences in the cells' ability to respond to the cue: "The bias is created by their relative proximity to the source of the signal, the oocyte, and over time it is enough to create movement." The lab's experiments seemed to support this hypothesis, because although the cells in 'slow' migration mode showed no within-cell differential in signalling strength, a differential was detectable across the cluster in a front-to-back fashion.

Pernille speculates that as the cells approach the oocyte, the early, classical form of signalling gives way to the later, novel form. Both make use of the same signals and receptors, but downstream of those receptors they are mediated by different molecular pathways. If she is right, her findings could throw light on the phenomenon of metastasis, or the spread of cancer cells beyond a primary tumour site. This has long been blamed on individual cells that stray, but recent evidence suggests that cell collectives could be involved too. The Rørth group's work indicates that the two models need not be mutually exclusive: the same cells may be capable of autonomous movement, and of what Pernille refers to as "collective cell decisions".

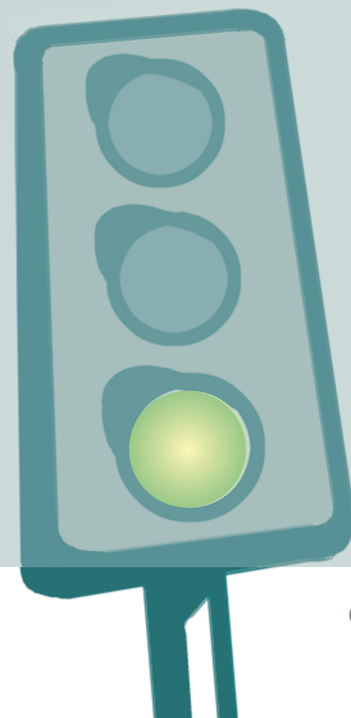
Bianco A, Poukkula M, Cliffe A, Mathieu J, Luque CM, Fulga TA, Rørth P (2007) Two distinct modes of guidance signaling during collective migration of border cells. *Nature* **448**: 362-66

A green light for migration

What persuades stationary cells to start migrating? In the case of border cells in the egg chamber of the fruit fly *Drosophila*, which travel in clusters, it seems to start with a message sent from cells at the heart of the cluster to the outer, migratory cells. That message in turn triggers a signalling cascade which sets the cluster in motion.

One of the proteins activated in that cascade is Slbo. Pronounced 'slobo' and short for 'slow border cells', the protein takes its name from the behaviour that Pernille Rørth and her team observed in a fly mutant that lacked it. But how does Slbo regulate migration? Do the cells move actively, or are they passively released from constraints that normally keep them in place? And are there universal rules governing cell migration?

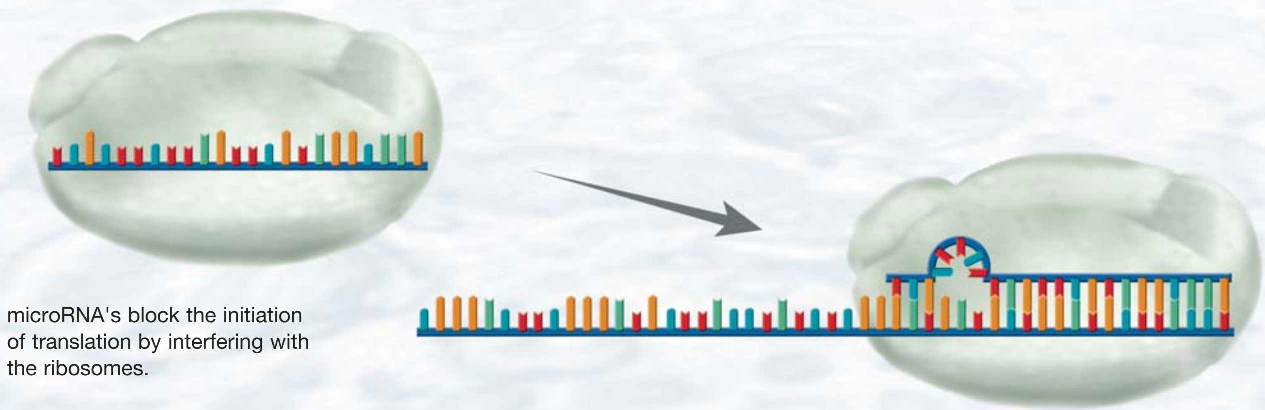
To try to answer some of these questions, Pernille's team compared the gene expression profiles of two sets of cells – normal border cells versus mutant border cells that lacked Slbo, and normal border cells versus the non-migratory follicle cells from which they are derived. Their aim was to identify a subset of genes that is active only in migrating cells, and they found 300 in all.



Those 300 genes provide a reference set against which similar sets from other organisms or cells can now be compared, to search for migration-related patterns. Looking more closely at the 300 genes, the researchers found a number of unexpected ones, including a collection of genes which are normally active in muscle cells – raising the intriguing possibility that the same instructions that coordinate movement in animals also coordinate movement in border cells.

Borghese L, Fletcher G, Mathieu J, Atzberger A, Eades WC, Cagan RL, Rørth P (2006) Systematic analysis of the transcriptional switch inducing migration of border cells. *Dev Cell* **10**: 497-508 ■

Cell-free supermodel



microRNA's block the initiation of translation by interfering with the ribosomes.

BIOLOGY DEMANDS COMPROMISES. Real life is complex, and not often amenable to experimentation. So to answer biological questions, scientists often choose 'model' systems that they can easily manipulate, and then extrapolate the results of those experiments to real-life systems.

Matthias Hentze, EMBL Associate Director, and his co-worker Rolf Thermann knew this when they became interested in some very tiny, but very powerful, molecules called microRNAs. microRNAs have important roles in gene regulation, and control many important cellular processes, but how they do this is not very clear. Matthias and Rolf wanted a better understanding of how microRNAs control gene expression, but in order to carry out experiments to investigate this, they had to develop a new model system.

The processes of translating DNA code into proteins require two important steps. During the first step, the DNA sequence is transcribed into 'messenger RNA' (mRNA). In the second step, machines called ribosomes bind to the mRNA and translate those short pieces of genetic code into proteins. microRNAs are small pieces of RNA that stick to the ends of mRNA and inhibit protein translation. Matthias says that microRNAs are extremely important for the regulatory systems that affect human physiology. "They are thought to regulate the expression of up to 30% of vertebrate genes," he says.

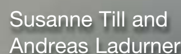
But once they have stuck to the mRNA, how do microRNAs inhibit protein translation? Some studies suggest that microRNAs affect the translation process after it has been initiated, but others say that microRNAs inhibit the initiation step itself. The question is hard to resolve because mRNAs and microRNAs are fragile molecules, and thus are difficult to manipulate in cell culture. "The cell membrane is a barrier to experimentation," Rolf explains. "We needed a system that would be easy to manipulate and easy to modify – so we developed a cell-free system, which meant we could add or remove components that we were interested in."

Matthias and Rolf based their system on extracts from fruit fly embryos. To track the effects of the microRNAs on gene expression, researchers chose a handy 'reporter' protein that would allow them to see what was going on. They decided on a bioluminescent protein from the firefly, called luciferase. The researchers took the DNA sequence encoding luciferase and attached to one end of it a piece of sequence to which microRNAs are able to bind, and at the other end, a 'cap' sequence – which mediates ribosome binding. When ribosomes have found their target mRNA, they form aggregates called polysomes, which is where protein translation happens. The researchers were then able to add or remove different components of the system – such as the microRNA binding sites – and watch the consequences of this on the amount of luciferase produced. They also developed a way of 'arresting' the translation process, so that they could analyse the kinds of intermediates formed during the process.

From the results of their experiments, the researchers suggest that microRNAs interfere with the binding of ribosomes to the mRNA. They also saw that instead of forming polysomes, the mRNAs are aggregated into what the researchers have dubbed pseudo-polysomes – which might also be involved in the repression of translation.

Matthias says, "The development of this model system is a major step forward in the understanding of the basic mechanisms underlying protein synthesis and its regulation by microRNAs and RNA-binding proteins in cell metabolism, differentiation and development."

Thermann R, Hentze MW (2007) Drosophila miR2 induces pseudo-polysomes and inhibits translation initiation. *Nature* **447**: 875–8 ■


Susanne Till and Andreas Ladurner

Hooking up gene expression

FIFTEEN YEARS AGO, a remarkable discovery changed biology. RNA had always been thought of as a messenger – a conduit for the flow of genetic information from DNA to protein. But then came hints that RNA might be more than just a go-between, that it might interfere with the delivery of the message itself. Today, it is clear that tiny, transient RNAs can powerfully influence where and when genes are expressed.

One of the latest discoveries is that small interfering RNAs (or siRNAs) can affect gene expression by altering the structure of chromatin – the material that chromosomes are made of. It was this discovery that caught the attention of Andreas Ladurner, group leader in the Gene Expression Unit at EMBL Heidelberg.

Andreas has long been fascinated by unusual interactions between proteins and their binding partners (or 'ligands'). He explains that in some yeasts, siRNAs help the formation of 'heterochromatin' – the chromatin found in silent regions of the genome. siRNAs bind to Argonaute 1 (or Ago1), one of the proteins in the RITS complex, and under guidance from the siRNAs, RITS changes a selected region of the chromosome from active chromatin into silent heterochromatin.

Andreas and his student Susanne Till set out to investigate how RITS remodels chromatin. By inducing mutations in the RITS proteins, they found a short sequence of amino acids (in a protein called Tas3) that directly binds to Ago1 and is absolutely required for heterochromatin silencing.

Andreas explains that this sequence, called the 'Ago hook', is remarkable in several respects. First, it is highly conserved, and even found in humans. Second, it has a flexible, open structure, very different to the highly structured domains that proteins normally adopt. "It is like a loose piece of thread, contrasted with the structured fabric of woven cloth," Andreas says. Third, the Ago hook interacts with a region of Ago1 that normally binds to siRNAs. In fact, the Ago hook even looks a little like a string of nucleotides, so the researchers think it is possible that the Ago hook may mimic some features of siRNAs.

These observations prompted Andreas to investigate the role of the Ago hook in other short-RNA processes, and he found valuable expertise next door. Matthias Hentze, EMBL Associate Director, studies a gene silencing mechanism that involves another type of short RNA – microRNAs. Protein synthesis happens in two steps: first, DNA is transcribed into messenger RNA, then messenger RNA is translated into protein – and microRNAs affect this translation process. They guide an Argonaute-containing protein complex called RISC to specific messenger RNAs, and either chop up, or silence, messenger RNAs before translation. Matthias' postdoc Rolf Thermann found that the Ago hook is able to relieve such microRNA-mediated translational repression. In fact, the functions of the Ago hook don't stop there. A recent study from another lab shows that, in plants, the Ago hook couples Argonaute proteins to a special RNA polymerase (responsible for transcribing DNA into RNA).

Andreas says that if proteins show conserved features over evolutionary time, then these features must be important for protein function. Today's era of biology is sometimes referred to as 'The Age of RNA', so it's an exciting time for the Ago hook, which appears to be relevant to several regulatory processes in the gene expression pathway. Andreas says, "We look forward to figuring out how these short Ago hooks regulate the function of short RNA-mediated gene silencing."

Till S, Lejeune E, Thermann R, Bortfeld M, Hothorn M, Enderle D, Heinrich C, Hentze MW, Ladurner AG (2007) A conserved motif in Argonaute-interacting proteins mediates functional interactions through the Argonaute PIWI domain. *Nat Struct Mol Biol* **14**: 897–903

Through the kaleidoscope

IF YOU WANT TO SEE how simplicity can create complexity, look no further than a child's toy box. In it you may find a kaleidoscope, a toy that uses a simple arrangement of mirrors to reflect the images of beads or other coloured objects within a tube. As mathematicians in ancient Greece discovered, setting up mirrors to reflect one another creates multiple duplicate images. These create beautifully symmetric and complicated forms from the random placing of simple objects in the tube – a feature reflected in the translation of the word *kaleidoscope*: 'beautiful form viewer'.

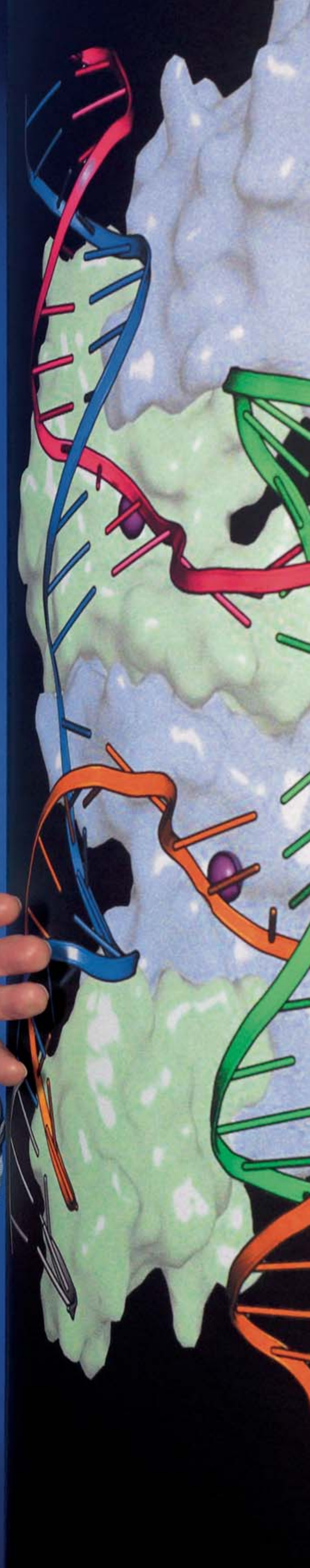
Beautiful forms abound in nature, yet from the outside, they seem far too complex to understand in detail. The multitude of molecules that somehow comes together to create our bodies, minds and behaviour seems almost anarchic. But just as a kaleidoscope brings beauty and symmetry to arbitrary patterns of objects in a cardboard tube, so science can reveal order and patterns in this apparent confusion.

Perhaps the finest level of order you can get in biology comes from studying how atoms are arranged within a molecule to give it its 3D structure and so deduce how it might work. By shining X-rays on molecules, researchers can work out where the atoms that build them lie. Several groups at EMBL are dedicated to structural biology research, from those studying particular molecules to those working to develop the computational and hardware resources and new methods needed to meet the challenges of this fast-developing science.

Although information on an individual molecule's structures is invaluable, it doesn't really let you see how all the molecular parts fit together to build the big picture – the whole cell. Several groups at EMBL are working on piecing this puzzle together. Some efforts focus on developing ways to study the structures of molecules as they interact in three dimensions to form large, machine-like assemblies, or complexes. One of these is 3D Repertoire, an international project that aims to unite a large range of different techniques to describe these complexes.

Another way to see the bigger picture is to image the behaviour of cells or the molecules within them with high precision. Many such techniques are being pioneered at EMBL, including those that allow scientists to watch how individual molecules behave within living cells, or those that let them screen vast numbers of cell biology experiments quickly and accurately.

Admittedly, this kind of science is rather more complicated than gluing mirrors to the inside of a tube, but it still works as a kind of intellectual kaleidoscope – a viewer that lets us understand the beautiful forms of living things. ■



Dietrich Suck

Into the crystal ball

IT'S NOT WHAT ONE EXPECTS from the normally sober environment of an international research centre – walls covered in a dazzling array of pictures and desks littered with stacks of multicoloured illustrations. But this is the office of Dietrich Suck, group leader in EMBL Heidelberg's Structural and Computational Biology Unit. Structural biologists study the architecture of biological molecules. And the pictures on Dietrich's wall are of molecular structures inferred through a delicate combination of physics, chemistry and biology.

One of the most powerful tools in the structural biologist's toolkit is X-ray crystallography – a technique for determining structures that are smaller than the wavelength of light. Dietrich has been working with X-ray crystallography at EMBL for nearly 30 years. And recently he has used the technique to get to the heart of a long-standing question in biology: how is DNA repaired?

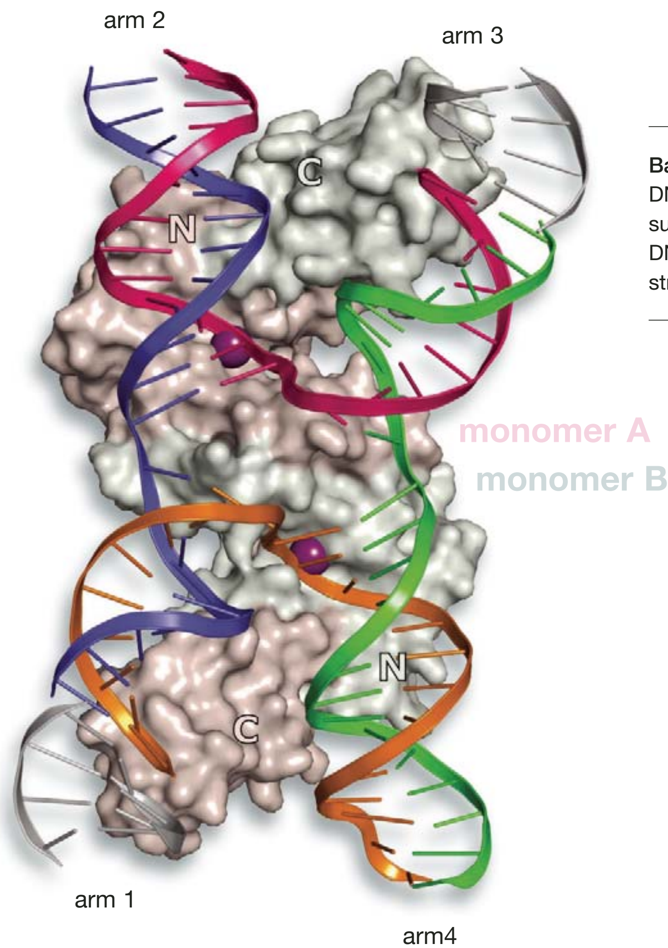
X-ray crystallography works by taking advantage of the special properties of high-frequency spectrum wavelengths of energy called X-rays. Like light, X-rays are scattered as they pass through matter. As Dietrich explains, when an X-ray strikes the electrons within a crystal, such as salt, it makes secondary ripples, which reveal the crystal's size and shape. "By bombarding the crystal with X-rays from many different angles," he says, "we can use the scatter to work out the arrangement of atoms within it."

When X-ray crystallography was first invented, the technique was used to determine the structures of inorganic crystals, such as salt or diamond. But as it became more sophisticated, it was applied to simple biological molecules, such as cholesterol, and eventually to proteins, most famously myoglobin and DNA. In fact, Rosalind Franklin used X-ray crystallography to produce some of the early images that describe DNA's double helical shape.

But not all structures are equally easy to visualise. One group of molecules that have proven to be particularly tricky are 'DNA-protein complexes', which are structures formed when proteins bind to DNA. The crystallographer's greatest challenge is to coax crystals to form, and to do this for a complex of DNA and protein is not easy.

It was Dietrich's interest in DNA-protein interactions that led him to think about DNA repair. DNA repair proteins are recruited whenever strands of DNA break. Sometimes this happens randomly during DNA replication or transcription. Sometimes cells break their DNA on purpose. Most organisms engage in some form of gene swapping, switching, conversion or crossing over. Plants and animals use a process called recombination, in which DNA from sister chromosomes (one from the mother, one from the father) swap sections of DNA, mixing up the genes for the next generation. Yeast and bacteria do something similar when they exchange genetic material with their peers, and viruses use similar DNA-snipping mechanisms to get themselves in and out of host genomes.

But whether by accident or design, DNA breaks need to be repaired efficiently and quickly. Many details of this repair process, however, have remained mysterious. Dietrich decided to apply his crystallographer's skills to



Background: the molecular surface of the DNA repair protein Endo VII with the two subunits coloured differently. **Foreground:** DNA of the Holliday junction, with each strand in a different colour.

unravelling the mystery of a DNA repair protein called Endonuclease VII (or Endo VII).

Endo VII is an enzyme involved in resolving DNA branch points, such as those formed during recombination. In the 1960s, a biologist called Robin Holliday suggested a new model for the gene switching between matching pairs of chromosomes. He proposed that the four strands of DNA on the two matching chromosomes become cut and joined together into a four-way temporary braid. This intermediate structure – called the Holliday junction – then slips along the chromosome, allowing genetic material to switch from one matching chromosome to another, before the DNA strands are snipped again and resolved back into two separate double helices.

This is where Endo VII comes in. Endo VII was first identified in a phage – a type of virus that infects bacterial cells. When the T4 phage makes copies of itself, it needs to sort out its newly replicated branched DNA and package it into a capsid shell. Endo VII is the protein that resolves those branches. But scientists soon realised that Endo VII could also resolve Holliday junctions and other branched DNA structures, so this protein quickly became an important protein for answering questions about the mechanisms of recombination. How does Endo VII recognise the four-way junction? How does it bind to its DNA target? How does it manipulate the DNA during repair?

Dietrich thought that some important insights might be gained from using X-ray crystallography, but it wasn't going to be easy. "Crystallising Endo VII together with its DNA target is not an easy task," he says.

Together with colleagues Christian Biertümpfel and Wei Yang (who is based at the National Institutes of Health in the USA), Dietrich set out to create a stable interaction between the Endo VII protein and DNA. First, they mixed together small pieces of DNA to make the Holliday junctions. "By carefully heating and slowly cooling the mixture," Dietrich explains, "we were able to make the four-way junctions, with DNA arms of just the right length to crystallise with Endo VII." They then made a type of Endo VII molecule that would bind to the Holliday junctions, but not cleave the DNA strands. And the resulting structure was stable – ideal for X-ray crystallography.

Their work yielded a structure that has given Dietrich some fascinating insights into the way in which Endo VII interacts with DNA. It has long been held that the four-

way junction could have one of two structures: either a flat 'square planar' form with the four DNA strands forming a crossroad, or a 'stacked-X' structure, where the two helices are stacked on top of one another, crossing at an angle. Previous studies suggested that Endo VII formed a stacked-X structure with the DNA. Instead, Dietrich and his colleagues found that it forms a hybrid between a stacked-X and a square planar structure.

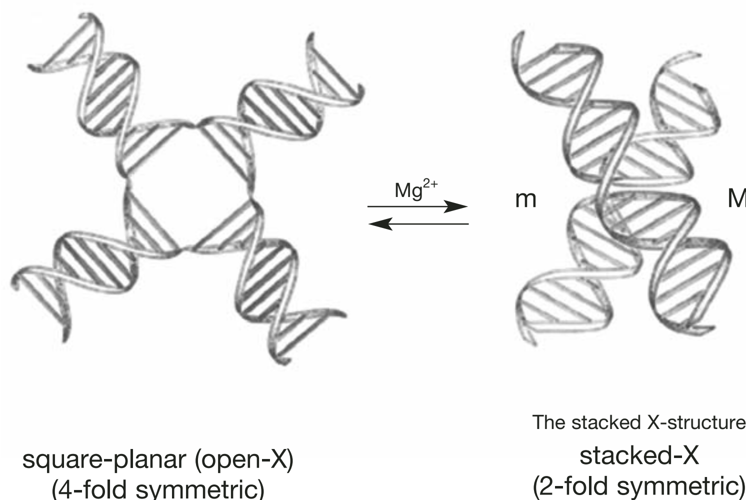
"By bombarding the crystal with X-rays from many different angles we can use the scatter to work out the arrangement of atoms within it."

And what does the structure tell us about the way in which Endo VII recognises its target? Endo VII is a protein that recognises structures, not sequences. So Dietrich was surprised to see that as it nestles itself within the four-way junction, it severely distorts the structure of the DNA. He says, "Although it is structure-specific, Endo VII has to change the structure of its target before it can perform its function." This mechanism of recognition is known as 'induced fit'.

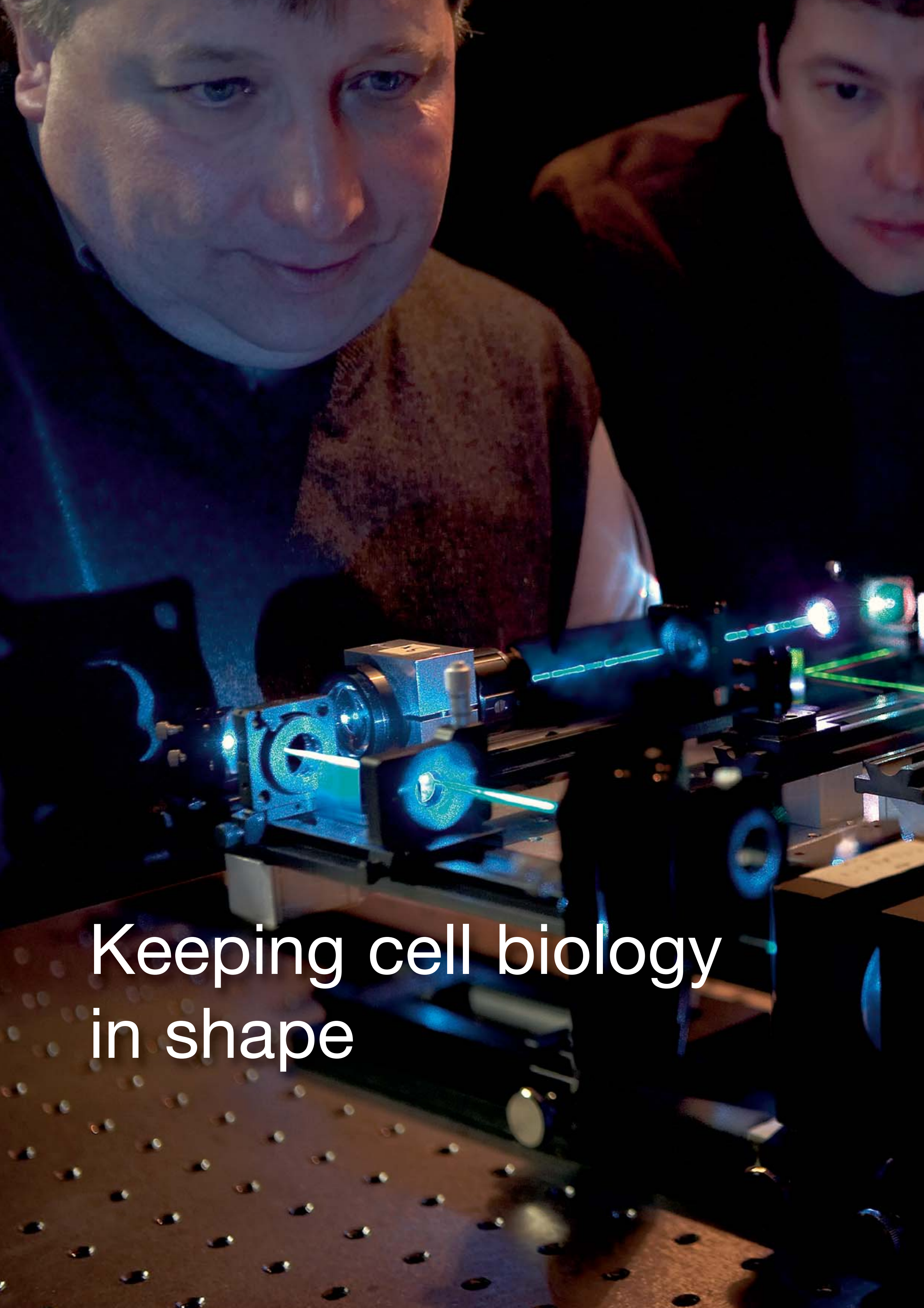
And Dietrich says that it is interesting to compare the structure of Endo VII with other kinds of similar repair enzymes that bind DNA, a group of proteins known as resolvases. In this way, structural analysis is offering new insights into the differences between proteins. "Different resolvases have different levels of specificity and this seems to be reflected in their structures," he says. And when it comes to Endo VII, Dietrich has found that it has enormous shape-shifting flexibility, probably because it is a very general resolvase, capable of recognising many different kinds of junction.

It also looks as though resolvases from different species might have very different structures, but many bind to DNA in similar ways. Yet, true to his roots, whether this is actually the case "has to be confirmed by X-ray analysis", Dietrich says.

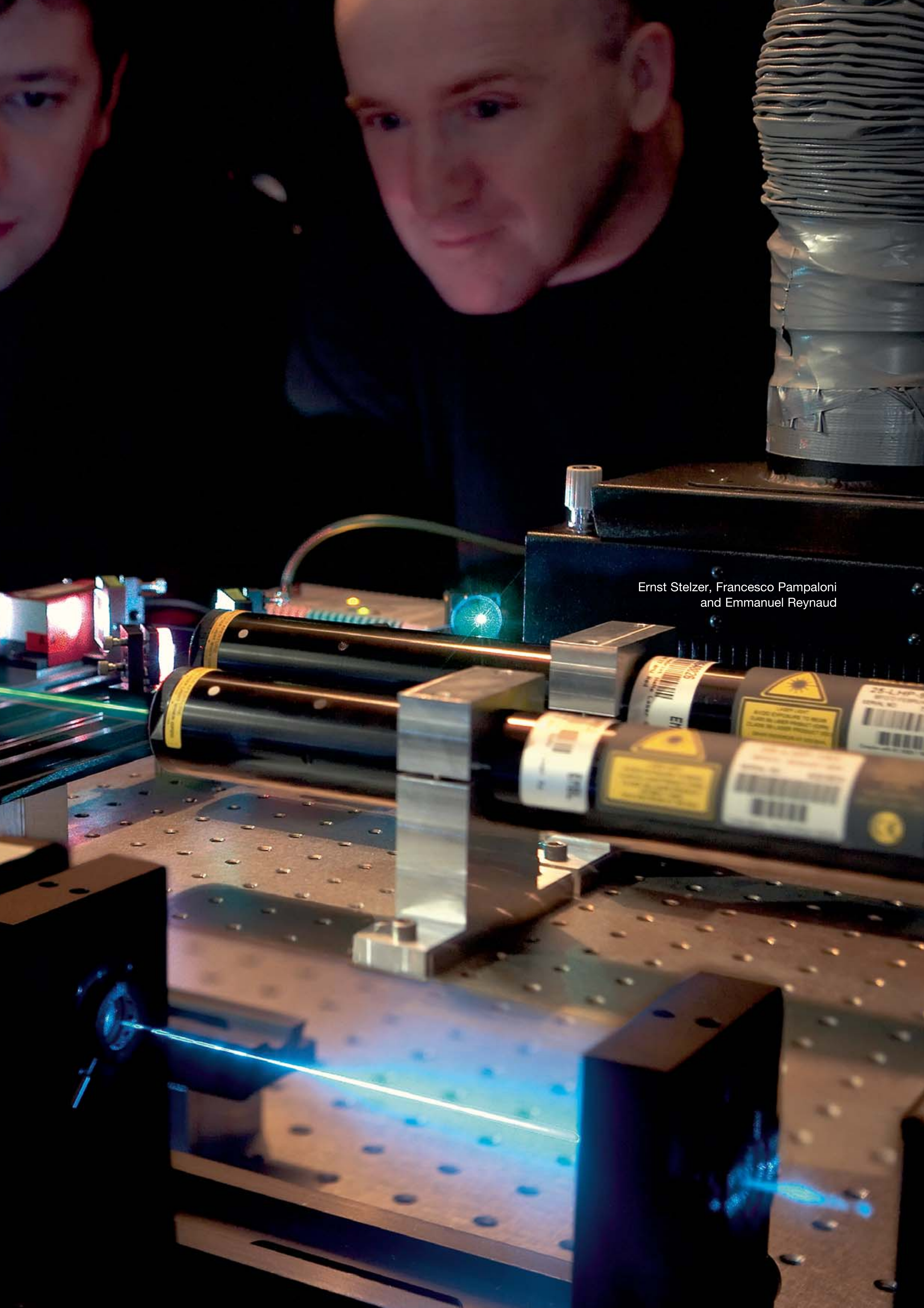
Biertümpfel C, Yang W, Suck D (2007) Crystal structure of T4 endonuclease VII resolving a Holliday junction. *Nature* **449**: 616-20 ■



For a long time scientists wondered if the Holliday junction has a 'planar square' of 'stacked-X' confirmation. Now it turns out that the truth, as always, lies somewhere in-between.



Keeping cell biology in shape



Ernst Stelzer, Francesco Pampaloni and Emmanuel Reynaud

IMAGINE WHAT LIFE WOULD BE like if everything in the world were completely flat. Confined to a mere two dimensions, we would be extremely limited in what we would be able to do. In fact, the very concept of a third dimension would be alien and near impossible for us to grasp. It's an idea that inspired nineteenth century schoolteacher Edwin A. Abbott to write *Flatland: A Romance of Many Dimensions*, a story about a 2D inhabitant of Flatland called A. Square, who was visited by a 3D being trying to convince him of the existence of a solid world called Spaceland.

A schematic drawing of the cell culture chamber which allows cells to be kept at 37 °C...

The story highlighted how radically different life would be for the people living in worlds of different dimensions. Coincidentally, around the time Abbott was penning his novella, biologists were taking the first steps towards a 2D adventure of their own. By growing slices of tissue in glass bowls, they had started a line of experimentation that would eventually lead scientists to isolate cells from their natural homes in solid bodies and grow them in flat sheets in the lab dish.

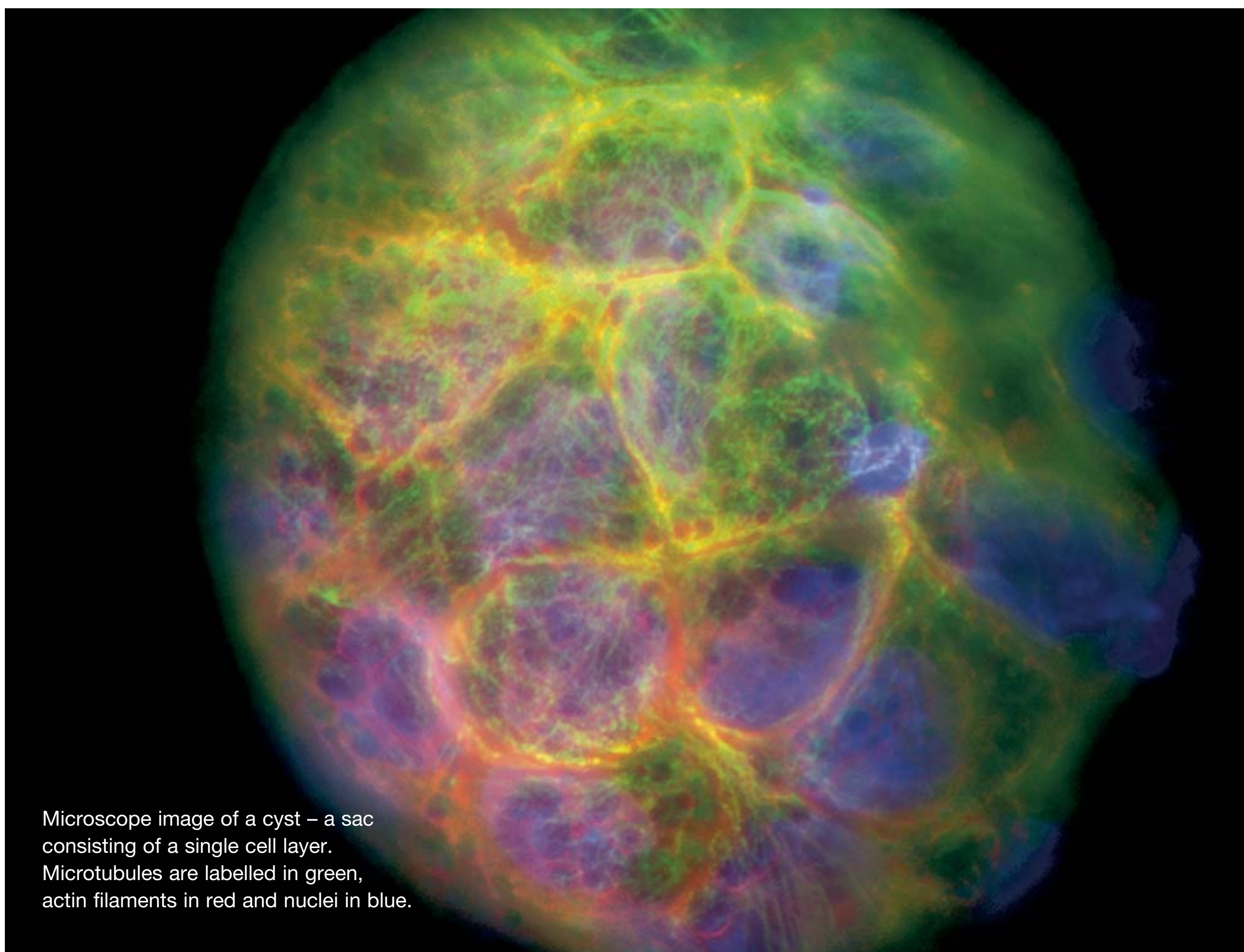
When this method – now referred to as cell culture – was first developed, many biologists rejected it as an unnatural and misleading way to study cell behaviour. But it had its advantages: scientists were able to control the conditions of cultured cells much better than pieces of tissue, for example. Another advantage is that culture cells can be observed in a microscope directly, whereas tissue requires special preparation. Therefore cell culture currently dominates the study of cells. Now, however, researchers at EMBL are developing new technologies and techniques to let biologists grow and study cells in soft, 3D environments, rather than on hard and flat surfaces. They think this 3D approach will let biologists study cell behaviour in a more lifelike setting, thus making their findings more lifelike.

The cells in our bodies are very social creatures. They talk to each other constantly, monitor their environment and adapt their behaviour accordingly. Taken out of this context and placed in a cell culture dish as a layer only one cell thick, they are deprived of much of this vital communication. In flat culture, for example, only 16 per cent of a cell's membrane is in contact with other cells. Within epithelia, the most abundant type of tissue in the body, 50 per cent of the membrane connects with other cells. "In a tissue, what is important is the collective behaviour of the cells," says Emmanuel Reynaud, a project leader in Ernst Stelzer's lab at EMBL Heidelberg.

Both Emmanuel and his colleague Francesco Pampaloni, also in the Stelzer lab, emphasise that they are not deriding flat-cell culture, pointing out that it offers advantages. "It is a powerful approach," says Francesco. "It makes cells easily accessible and it allows biologists and biophysicists to get a lot of data." However, now it is time to add the benefits of 3D cell culture to the mix, he says. "They are two complementary approaches."

The key advantage of 3D cell culture is that it creates more lifelike conditions for cells. In the body, for example, a large part of the cell surface is in contact with a gel-like

...and how it is mounted inside the microscope.



Microscope image of a cyst – a sac consisting of a single cell layer. Microtubules are labelled in green, actin filaments in red and nuclei in blue.

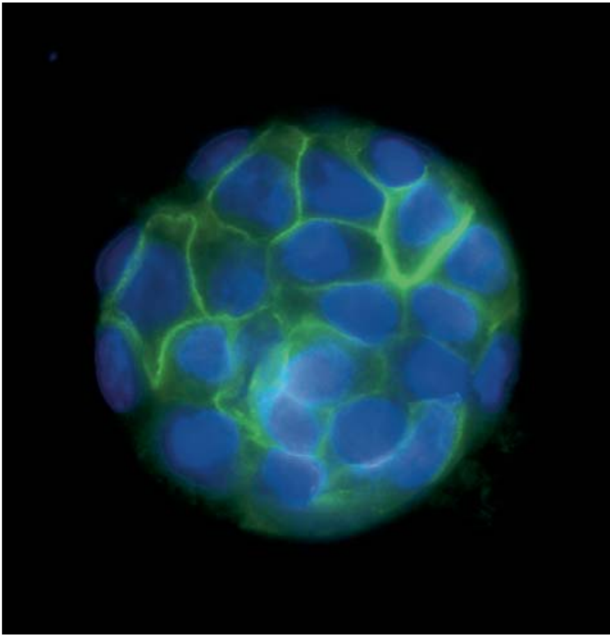
substance called the extracellular matrix. Together with the signals it receives from its neighbours, this positional cue allows a cell to work out its top from its bottom and adapt its structure accordingly. This structure, or polarity, is important in tissues, such as the kidney, where cells secrete fluids into a cavity.

In 2D cultures, however, cells are flattened onto a plastic dish, with no extracellular matrix to guide them. They often fail to form a proper polarity, have abnormal shapes and don't completely develop their specialised functions. Some die, and others fail to divide or behave like cancer cells. What's more, studies have shown that cells in 2D culture switch on a different suite of genes compared with those in 3D culture. "We see a striking difference between the two situations," says Francesco.

This makes it hard to extrapolate many research findings from cell culture to entire organisms. It is a particular problem for researchers trying to test new drugs. Only 8 per cent of the candidate drugs that enter clinical trials ever make it to the bedside. Being able to culture cells in more realistic conditions could improve the reliability of methods designed to find promising new compounds before they reach the clinical testing stage. Currently many animals are killed in drug and toxicity tests. The European Union is now enforcing the testing of all chemical compounds ever marketed. "There is a strong hope that 3D cell cultures could reduce the number of animals sacrificed," says Francesco.

A number of researchers have now switched to using 3D cell cultures. "Very much like us, more and more scientists are becoming interested because they see how it can lead us back to the physiology of the tissue," says Francesco. They can do this in a number of ways: growing cells in a hanging drop of fluid, for example, encourages them to clump together. Growing clusters of kidney cells in a block of isolated natural extracellular matrix encourages them to polarise and form hollow structures reminiscent of the tiny tubules found in living kidneys.

Part of Emmanuel's and Francesco's work focuses on developing new 3D culture systems. Emmanuel has a particular interest in developing cultures that let him study epithelia: a tissue type composed of layers of cells that line the cavities and surfaces of structures throughout the body, including the kidney and the gut. Epithelial cells are polarised, with their top, or apical, ends pointing away from the base of the epithelium.



Cells in a cyst interact closely with each other. Membranes are labelled in green and nuclei in blue.

Emmanuel has developed a technique for growing such cells on the surface of beads made from a gel based on the sugar dextran. As well as mimicking the extracellular matrix, this allows chemical signals to diffuse, so cells can communicate with each other. Because they are growing on the surface of the bead, the cells' apical ends point outwards, making the epithelium much easier to study in full.

So far, so good, but the main drawback of 3D cell culture is that the technologies used to study them are still geared for flat specimens. This is especially true of light microscopy. Most light microscopes are set up to accommodate thin specimens placed on hard and flat glass slides, and have only a limited ability to focus up and down through thick objects like spheres of kidney cells. "Optically, they are really challenging structures," says Francesco.

This is where a revolutionary new microscopy technique pioneered by Ernst and his team comes into play. Dubbed SPIM (for single plane illumination microscope), it allows biologists to obtain clear, high-magnification images from within soft living specimens for the first time. "We are able to go well beyond the surface of large 3D objects," says Francesco.

SPIM is based on a kind of microscopy called light sheet-based fluorescence laser microscopy, which uses a laser light sheet to illuminate single planes of a sample and does not require any technology that eliminates out-of-focus light. This allows scientists to record high-quality pictures directly. Sets of images can even be recorded along different directions and, with a little help from a computer, can be used to reconstruct a 3D image of a specimen.

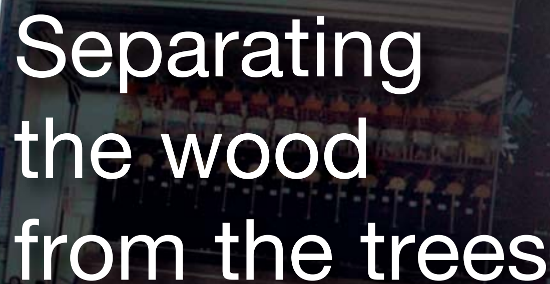
Conventional and confocal fluorescence microscopes focus on a single plane inside a specimen but they still illuminate the entire specimen. "Thus all fluorophores and all other non-fluorescent molecules that absorb the light risk light-induced damage," says Ernst. So, for example, recording information from 20 planes exposes the specimen to 19 times more electromagnetic energy than should be necessary.

SPIM implements several key innovations: one is to use different lenses for illumination and light collection, another is to illuminate only the sample with a thin sheet of light from the side and to observe the emission at an angle of 90°. The specimen is moved through this light sheet and is thus optically sliced. This greatly reduces the photo-induced damage in cysts by a factor of around 25 and in zebrafish embryos by around 200. Biologists can study living samples for hours or even days at a time. "We are not discussing a meagre 10 per cent improvement, we are talking about two to three orders of magnitude," points out Ernst. Since it uses cameras and looks at all pixels in parallel, SPIM is also faster than confocal microscopy. An additional feature is that the setup allows scientists to rotate their sample vertically within the microscope. "You can acquire different views along different directions of the same object," says Francesco.

Francesco has developed a chamber that allows biologists to culture cells in the microscope for several days, by continuously pumping fresh culture solution through the medium. He is also using SPIM to study how physical forces affect the development of cells within spheres grown in culture. The system allows him to study large numbers of cells and even to study how the structures within them change. "Only SPIM allows you to get this kind of data," he says.

As well as developing his epithelial system, Emmanuel helps other EMBL scientists and collaborators prepare their samples for SPIM, from tiny marine plankton to artificial cartilage pads for use in knee surgery. It surely will not be long before many more biologists appreciate the benefits of the third dimension and echo the sentiments of A. Square, when he said: "My mind has been opened to higher views of things."

Pampaloni F, Reynaud EG, Stelzer EH (2007) The third dimension bridges the gap between cell culture and live tissue. *Nat Rev Mol Cell Biol* **8**: 839–45 ■



Victor Lamzin

Separating the wood from the trees

STUDYING THE LARGE molecular structures that govern our cells' behaviour provides some of the fine detail for biology, in the same way that the study of individual trees can lead to an understanding of an entire forest. Although experimental tools now exist to study these molecules, the results obtained often require much individual effort to yield precise structures. Victor Lamzin and his team at the Hamburg Outstation have developed software that addresses this problem in an automated fashion, making the analysis of structural biology data faster, more accurate and more efficient.

When researchers want to study the structure of a protein or other biomolecule, they can crystallise it and expose it to X-rays. The arrangement of the atoms within the crystal causes the X-rays to produce diffraction patterns, which scientists can use to deduce the structure of the molecule.

Modern computing techniques in macromolecular crystallography have reduced some of the manual labour required to obtain 3D structural information, but Victor's software, called ARP/wARP, was the first to automatically produce accurate protein structures and has been in continuous development for a number of years. Together with colleagues at the Netherlands Cancer Institute in Amsterdam, Victor and his team have extended and improved the package to meet the demands of advanced structural biology techniques and to make it easily accessible to non-specialist users. "It has an enormous number of innovations," says Gerrit Langer, a postdoc in Victor's group who plays a key role in the software development.

Many of the innovations address a central problem faced by modern structural biology: scientists want to examine large assemblies or complexes of molecules to see how they interact, but the physics of X-ray diffraction means that larger objects tend to yield weaker and lower-resolution – or fuzzier – data, which are very difficult to analyse.

The new software has more sophisticated pattern-recognition abilities, and can model the stereo-chemical features of biomolecules, including secondary structure elements (a-helices and b-strands), for low-resolution data (down to 4 Å). Importantly, the program is able to make its own decisions on how to build a model of a structure, meaning that it is faster, more accurate, more automated and less biased than ever before. "Nowadays it makes very few mistakes," says Victor, "However, we are not merely aiming to improve ARP/wARP, but rather to capitalise on new scientific approaches and the demands driving research."

For scientists working on drug development, ARP/wARP's ability to decide which molecules or 'ligands' interact with their target structure is very useful. Researchers can download ARP/wARP to their desktop computers, but they can also submit data for remote analysis on the EMBL server. The web interface is designed to be as user-friendly as possible. "Simplicity is very important as more and more of our users are non-experts in structural biology," says Victor.

The latest release of ARP/wARP – version 7 – is already a huge hit amongst academic and industrial scientists. Since July 2007, it has been downloaded by more than 1000 researchers from more than 600 institutions worldwide. The number of computational jobs submitted through the online web portal has doubled. Victor now has more than 70 industrial customers (a third of whom have updated to the latest version), and the software has been cited in more than 3500 scientific publications. Thanks to the new innovations, ARP/wARP is well placed to help push back the boundaries of modern structural biology. "There are new features, new possibilities and new opportunities," Victor says.

Langer G, Cohen SX, Lamzin VS, Perrakis A (2008) ARP/wARP version 7: automated macromolecular model building for X-ray crystallography. *Nature Protoc*, in press

POPCORN
POPCORN

Christoph Müller, Joint Coordinator of the Structural and Computational Biology Unit, and Rob Russell

3D vision

"It is an alien world, with no sunlight, and temperatures far beyond boiling. It is called Earth. Join Academy Award-winning director James Cameron for a once-in-a-lifetime journey…two and a half miles straight down."

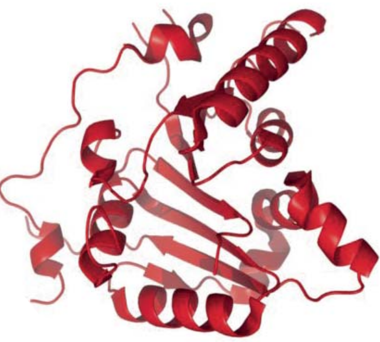
THE DOCUMENTARY FILM *Aliens of the Deep* wowed audiences worldwide when it hit cinema screens in 2005. Part of this was due to the fascinating subject material: the bizarre creatures that live around scalding volcanic springs lying thousands of metres under the sea. But director James Cameron had another ace up his sleeve: he filmed the documentary in 3D. He wanted to give his audiences a realistic feeling of what it was like to be down there, and to inspire their enthusiasm for science and exploration. As he told *New Scientist* magazine: "There are places on Earth that are so strange you can't believe it and we haven't even begun to find all of them yet."

But you don't have to go to the bottom of the ocean to explore strange new worlds. Perhaps one of the strangest, and trickiest, places to study lies inside our own bodies: the cell. Scientists at EMBL are now surveying the inside of the cell by undertaking their own voyage in 3D – albeit one that doesn't involve IMAX screens, popcorn or wacky 3D glasses.

At EMBL Heidelberg and the Grenoble and Hamburg outstations, the groups of Peer Bork, Bettina Böttcher, Anne-Claude Gavin, Darren Hart, Christoph Müller, Rob Russell, Klaus Scheffzek, Dietrich Suck and Matthias Wilmanns are part of an international project called 3D Repertoire. This aims to determine the structures of the large, 3D assemblies that result when certain proteins stick together to form massive, machine-like clusters known as complexes. These complexes are interesting because they perform many of the biological functions within the cell, and seeing how they work together in three dimensions will, like Cameron's movie, give scientists a much more life-like impression of what is going on.

Although structural biologists know a fair amount about the structures of the individual proteins within a complex, they lack detailed information about how these components fit together to make the whole. Piecing together the complex puzzle of how proteins come to work together will give scientists new insights into how the cell works, and also offer new avenues for developing drugs to tackle disease. The work is being done in yeast because it is well understood and easy to manipulate genetically. But because many of our basic cellular functions are similar to those of yeast, the researchers hope to learn a great deal about human biology too. "The more things we know at the atomic level, the better handle we have on all aspects of the biology," says Rob Russell, the group leader who heads the bioinformatics side of the EMBL efforts.

But there's more to it than that. Understanding protein interactions in this kind of detail will help researchers realise the full potential of a relatively new discipline called systems biology. This aims to show how proteins and other molecules work together to produce something bigger than their sum: a living cell. "When you have structure, you can ask very intelligent questions about the nature of the processes that it mediates," says Rob. If you are studying disease mutations, for example, seeing how they affect a complex's structure will help explain how such mutations affect the functions of these large protein machines.

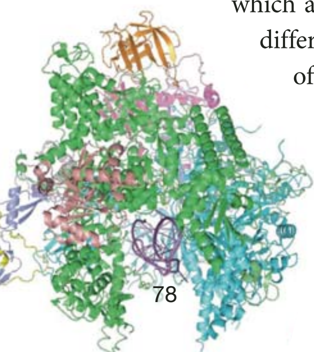


3D Repertoire is a four-and-a-half-year EU Integrated Project, with €13 million of funding that began in February 2005. It grew from an earlier project performed by Cellzome, a biotech company that was spun out from EMBL in 2000. Researchers at Cellzome had isolated and described all the protein complexes present in yeast cells, but could reveal little about their 3D structure. The next step was to try to produce detailed structures for all these complexes. "That was certainly very ambitious," says Christoph Müller, Joint Unit Coordinator of the Structural and Computational Biology Unit at EMBL Heidelberg, who together with Rob, is coordinating EMBL's contribution to 3D Repertoire. "The idea was to analyse the structures of the complexes using all kinds of techniques."

Each structural biology technique has its own benefits and drawbacks. X-ray crystallography, where scientists bombard a crystallised protein with X-rays to look at the placing of atoms within it, gives a very detailed picture of a protein's structure. But it often doesn't work on complexes, because they don't form crystals easily. Nuclear magnetic resonance spectroscopy can provide you with dynamic information but has its limitations when studying very large complexes. Electron microscopy, on the other hand, gives an overall picture of a complex's shape, but no fine detail. By combining the strengths of several approaches, the researchers hope to glean as much information as possible.

A key feature of the project centres around a new structural biology technique called intact complex mass spectrometry, or ICMS, which is being conducted by Carol Robinson, a 3D Repertoire collaborator at Cambridge University in the UK. Unlike conventional mass spectrometry, ICMS doesn't destroy the proteins in the sample. Rather, it peels the complex apart into smaller pieces, which themselves are made of two or more proteins. The team can then use existing information about the structure of these smaller pieces to deduce how the whole complex is put together in three dimensions. "It gives you hints about how they are interacting with each other," says Christoph.

Another important technique is electron tomography, which allows scientists to look at complexes from different angles, and then reconstruct 3D images of them. "That's very powerful if you want to look at cells," says Christoph. Ultimately, the hope is that researchers will be able to identify large molecular structures within cells themselves, rather than removing them from cells and studying them in the artificial environment of the lab test tube.

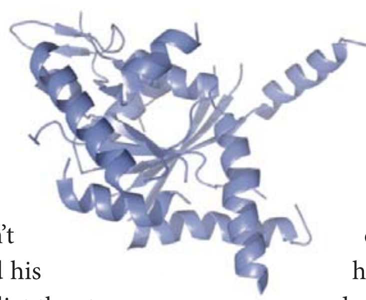


EMBL's contribution to 3D Repertoire is essentially divided into two parts. The classical structural biology component is carried out by Christoph and his colleagues Bettina, Klaus, Dietrich and Anne-Claude at EMBL Heidelberg, Darren at the Grenoble outstation and Matthias at EMBL Hamburg. Together with Peer, Rob works on the other, bioinformatics-oriented, half of the project. The overall coordination of 3D Repertoire lies with Luis Serrano, the former Joint Coordinator of the EMBL Structural and Computational Biology Unit who is now based at the Centre for Genomic Regulation in Barcelona.

Each of the participating EMBL groups approaches the project from a different angle and contributes different types of data to build up a complete image of the cell. Christoph, for example, is working on the structure of a complex known as RNA polymerase III. This massive enzyme is made of 17 smaller pieces, or subunits, and produces some of the small RNA molecules – in particular transfer RNA – that are essential for a cell to function. Despite its importance, however, relatively little is known about its structure. By studying its shape with electron microscopy and comparing it with a related enzyme whose X-ray crystal structure was known, Christoph was able to gain new insights into the structure of RNA polymerase III and how it starts and finishes making RNA molecules.

Although this sounds simple enough on paper, the experimental challenges in work such as this are considerable. Huge amounts of yeast cells had to be grown to extract protein complexes and prepare them for study. Yeast fermentation carried out in Anne-Claude's group has proved invaluable to this project.

Luis Serrano, Head of the CRG-EMBL Systems Biology Unit, coordinates 3D Repertoire.



As a bioinformatician, Christoph's colleague Rob doesn't have to get his hands dirty in the lab. This doesn't mean he has it easy, however. Rob and his team develop and use software to predict the structures of proteins and complexes. One way they can do this is to draw on information that already exists. This is possible because, thanks to having a shared evolutionary history, many of the protein complexes found in one species, say mice, will have similar structures to those found in another species, such as humans. The team can use this relationship to develop predictions about a complex's structure, which lab scientists can then go out and test – a considerable saving of time and money compared with studying the complex from scratch.

When Rob's team has information about the structure of a whole complex, modelling it with the computer is relatively straightforward. More often, however, they only have information about a fragment of a complex. The challenge is piecing together the complex from these bits of information. Often, there are many thousands of such pieces of information, many of which may not be accurate. "It becomes rather computationally difficult," says Rob.

As well as developing new methods to address these problems, Rob and his team are using their software to help other researchers solve the structures of their target complexes. A particular interest of the group is electron microscopy. Rob's team can develop a best guess for the detailed atomic structure, based on the somewhat fuzzy electron microscopy image. "It's been pretty successful," says Rob.

In addition to helping researchers study their pet complexes, Rob's team plans to drive structural biology research in new directions by identifying and highlighting interesting complexes. "We don't just want to help people, we want to lead projects ourselves," he says. His team is working on one complex, called the eIF4–eIF4b complex, which is involved in protein production in cells. Together with Bettina Böttcher and Carol Robinson, Rob's team is drawing on data from ICMS and electron microscopy to predict a structure. Ultimately, the goal is to minimise the amount of experimental data needed to produce a reliable prediction. "Can we actually determine structures just using, say, mass spectrometry and modelling?" asks Rob. "This isn't always possible, but there are many cases where it is."

Since 2006, the project has succeeded in obtaining structural information using X-ray crystallography, NMR or electron microscopy for a considerable fraction of the complexes on the list, and the teams hope to complete more before the funding runs out in 18 months. Looking to the future, as well as extrapolating 3D Repertoire to human cells, the teams hope eventually to add a fourth dimension: studying how these complexes change over time. And despite the challenges facing them on their 3D adventure, the scientists say they are enjoying the journey. "There's a good relationship between people," says Christoph. "It's actually quite fun."

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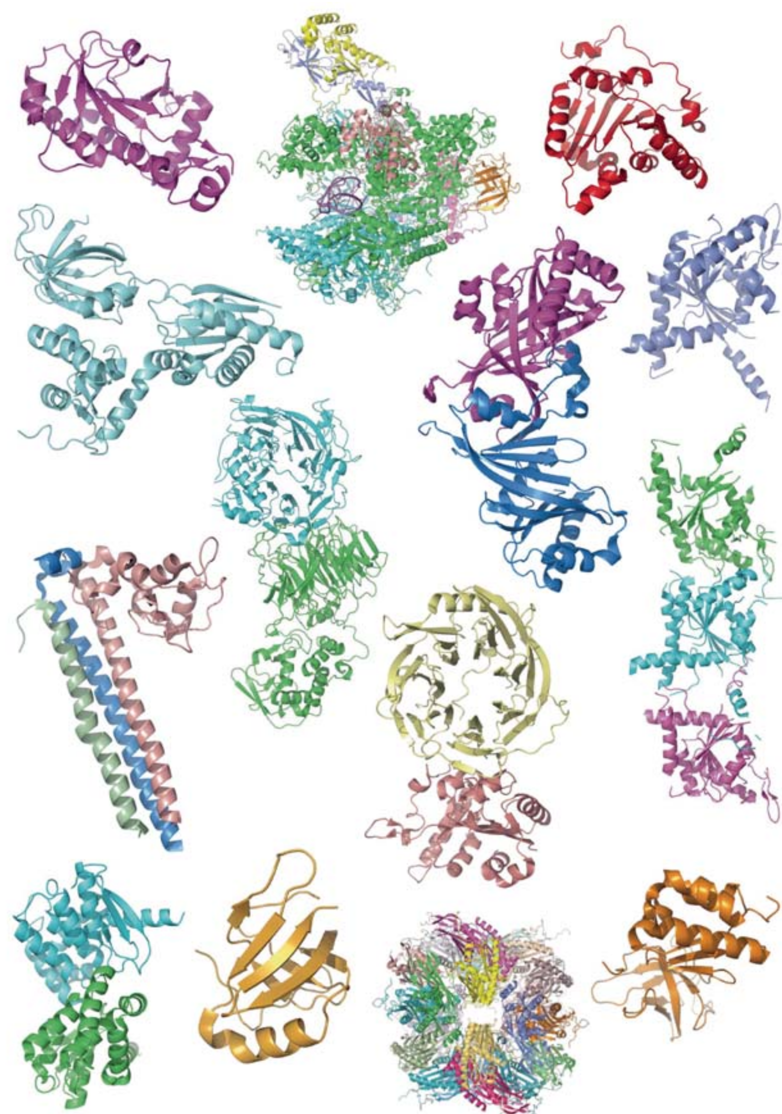
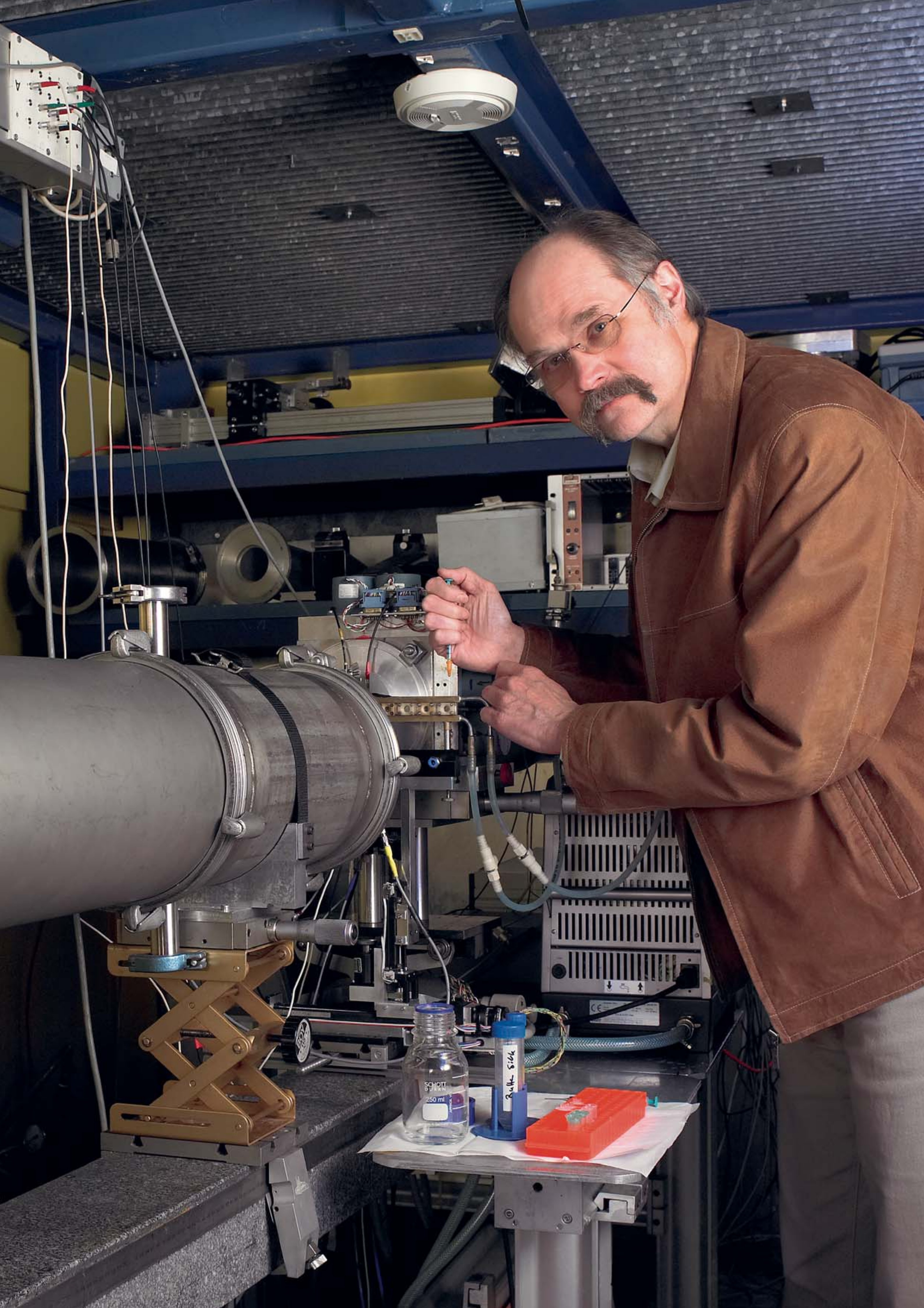
Taverner T, Hernández H, Sharon M, Ruotolo BT, Matak-Vinkovic D, Devos D, Russell RB, Robinson CV (2008) Subunit Architecture of Intact Protein Complexes from Mass Spectrometry and Homology Modeling. *Acc Chem Res*. doi: 10.1021/ar700218q

A collection of yeast proteins and protein complexes that have already been solved as part of 3D Repertoire.

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Dmitri Svergun at the SAXS beamline at EMBL Hamburg

The joy of SAXS

IT WAS ONLY SUPPOSED TO BE a routine experiment. Danish pharmaceutical company Novo Nordisk A/S, together with the Faculty of Pharmaceutical Sciences from the University of Copenhagen, had approached Dmitri Svergun, a group leader at EMBL Hamburg, to ask for help with a problem. It is well known in the pharma community that many protein-based drugs – often referred to as 'biodrugs' – are prone to developing long, insoluble fibrils, formed by inactive protein. Insulin potentially has the same problem. And with insulin being a major biodrug, used all over the world by people suffering from diabetes, companies such as Novo Nordisk A/S have an obvious wish to understand and control this process.

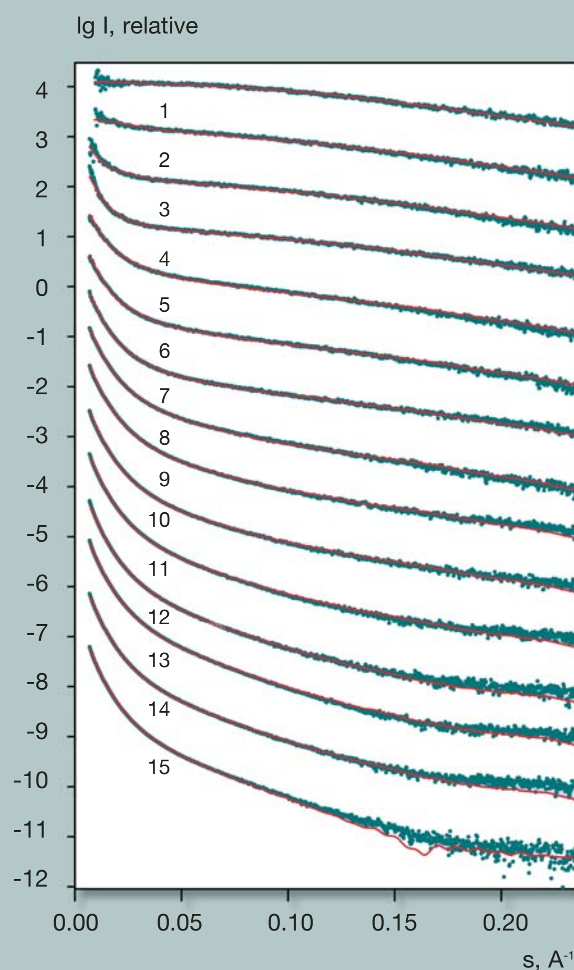
Biologists had already developed a theory of how proteins do this, and Novo Nordisk just wanted the teams from Copenhagen and EMBL to work out the mechanistic details of how their insulin followed this theory. Instead, the teams made an unexpected discovery that suggested this theory might be wrong. "When we tried to do this, it didn't work," Dmitri says. "We had to recognise that there had to be something else there."

This 'something else' has proved to be a clue that could change the way scientists think about the course of development of diseases such as Alzheimer's, Parkinson's and mad cow disease. It offers a new insight into how the long protein fibres involved in these conditions might form, and how scientists might develop drugs to target them. The discovery was made possible thanks to the technologies developed at EMBL Hamburg by Dmitri and his group.

The technologies centre on a technique called small angle X-ray scattering, or SAXS for short. In principle, it is similar to X-ray crystallography, where scientists shine X-ray beams on crystals of molecules, such as proteins. The atoms in the molecules spread, or diffract, the X-rays in a characteristic way and by collecting the patterns of light and dark cast in this way, scientists can work out the structures of the molecules in the crystals.

SAXS differs from X-ray crystallography in that it cannot offer a detailed picture of the atomic structure of molecules in a crystal, only an idea of their sizes and shapes. But SAXS's key advantage is that it can be used on solutions of molecules, sparing researchers the painstaking crystallisation step. As well as saving time and effort, SAXS allows biologists to study molecules that simply cannot be crystallised. This feature is becoming increasingly important as researchers tackle ever larger and more complex proteins.

Another key advantage of SAXS is that researchers can take successive snapshots of a molecule's shape over time. It also allows scientists to change the conditions of an experiment quickly and easily. "This means you can study the structural responses of biological molecules to changes," says Dmitri. "It's a huge advantage." This means researchers can, for example, see what happens if they make the solution containing the molecule of interest more acid or alkaline, add in a collection of different molecules to see how they interact, or, as in the insulin study, just see how the molecule behaves by itself over time. And unlike other methods, SAXS allows biologists



Can you see the structure in these graphs? This is the output of a SAXS experiment. The slope of the graphs tells skilled structural biologists what the structure of a protein complex looks like.

to study anything from small protein fragments to entire viruses. “The range is really huge,” says Dmitri. “You can do it for practically any macromolecular object.”

In particular, for the insulin story, this was crucial. Although insulin is a small protein, about 3 nanometres in size, the fibrils it forms are huge collections of protein threads more than a thousand times its length. SAXS can be used to study a mixture of the two forms, so scientists were able to probe the relationship between them.

In spite of its advantages, however, problems with interpreting SAXS data meant it languished in the background for many years. The physics of small angle scattering means that it yields a very limited amount of data, which are extremely difficult to convert into a three-dimensional model of a molecule. For many years, researchers resorted to trial and error to interpret these patterns: building models of possible structures and seeing whether they fitted the patterns. But in the 1970s and 80s, other, faster methods of looking at molecules, such as electron microscopy, took over. It wasn’t until the late 1990s that SAXS really came into its own, thanks to the efforts of EMBL Hamburg scientists.

The renaissance was pioneered by Heinrich Stuhrmann, who headed EMBL Hamburg from 1976 to 1981 and by Michel Koch, who headed it from 1981 to 1985. They developed mathematical methods and new experimental techniques to make SAXS faster and more versatile. Starting in the 1990s, Dmitri and his team built on this work, refining the practical applications of the technology and making it widely available to scientists around the world.

One key application developed by Dmitri’s team is *ab initio* analysis. This involves deducing information about a molecule using nothing but the data contained in the one-dimensional scattering pattern produced by a SAXS experiment. This is possible thanks to software developed by the team, which is used to compute millions of possible molecular shapes that could cause a pattern. Hundreds of them would give excellent fits to the experimental data, and the program selects the most compact of these best-fit models. “The trial and error method is now delegated to the computer, which has been taught human perception,” explains Dmitri.

Another pivotal development involves combining data from SAXS and X-ray crystallography to exploit the advantages that both techniques offer. Many large proteins consist of several different sections, or subunits. They are extremely hard to crystallise as a whole, so structural biologists have taken to breaking them up into the individual subunits and deducing the structure of each at a high resolution. Thanks to the new methods developed at EMBL Hamburg, they can now study the overall, albeit low-resolution, shape of the entire protein to see how these subunits combine. “Using SAXS, you can put them together,” says Dmitri.

For Dmitri, however, the most important contribution of his group has been to make SAXS technology and software widely available to the international scientific community. “We brought all these methods to the public,” he says. “I think that is extremely important.” For example, a SAXS data interpretation program suite called ATSAS has been freely available to download from the EMBL website since 2000. Maxim Petoukhov and his colleagues in Dmitri’s team designed it with broad accessibility in mind. It is easy for biologists to use, even those who are not SAXS specialists.

Over the past five years, ATSAS has been downloaded more than 15 000 times by about 1000 users in over 500

different labs around the world. “For small angle scattering, that is a lot,” says Dmitri. The ATSAS programs are cited in at least half of all journal publications that include biological SAXS data. These days, researchers no longer have to download ATSAS. As of August 2007, they have been able to use the major ATSAS programs directly online at the EMBL website.

As well as developing the software, Dmitri’s team has completely refurbished the EMBL SAXS beamline X33 and recently installed an automated sample changer using a liquid-handling robot. The overall productivity of the station was improved about tenfold, and, at the moment, a SAXS experiment at EMBL Hamburg takes a mere two minutes. When the projected BioSAXS beamline at the third-generation synchrotron PETRA III comes online, this will be reduced to seconds.

The results speak for themselves. The X33 beamline now has five times more users than it did five years ago. Not one to rest on his laurels, Dmitri continues to push the development of new SAXS software and technologies: a recent example is a novel technique that reveals flexible regions within proteins.

So when Novo Nordisk approached Dmitri, they expected his SAXS setup to give a quick and easy answer to their question. Previous studies had predicted that when insulin formed protein fibres or fibrils, it began when a small collection of single insulin proteins acted as a ‘nucleus’ that attracted first one other single insulin molecule to stick to it, then another, and so on. The fibre grew by adding a single insulin molecule at a time to each end.

As well as causing problems for producing and storing synthetic insulin, these fibrils can spell trouble in the body. If you use fibrillated insulin against diabetes, there is a risk that your immune response reacts very fiercely. Other proteins can form similar kinds of fibrils and play an important role in a number of diseases including Parkinson’s, Alzheimer’s and the prion diseases bovine spongiform encephalopathy (BSE) and Creutzfeldt-Jakob disease. Apparently all these proteins form fibrils via similar pathways. Thus, it is expected that results from examples such as the insulin fibrillation pathway can also be used to understand what causes these other fatal diseases.

To find out how insulin formed fibrils, Dmitri’s team, together with Bente Vestergaard from the University of Copenhagen, studied how the molecules behaved over a given time period. They expected the SAXS experiment to produce a graph revealing the presence of two different structures: the single insulin molecules and the fibres, and to reveal how the ratios between the two changed as the fibres grew.

But the graph simply didn’t fit the bill for having just two components, and Dmitri realised there had to be a third structure present in the solution. He and Bente worked out that this structure consisted of six insulin molecules arranged together in a helix. Importantly, they also discovered that these helices were in fact the building blocks of the fibrils, and not the single insulin molecules as previously thought.

If the same is true of other protein fibril diseases, researchers will have a new target for developing drugs against such conditions. The insulin study is a testament to the potential of SAXS, says Dmitri. “It is a really nice project.”

Vestergaard B, Groenning M, Roessle M, Kastrup JS, van de Weert M, Flink JM, Frokjaer S, Gajhede M, Svergun DI (2007) A helical structural nucleus is the primary elongating unit of insulin amyloid fibrils. *PLoS Biol* **5**: e134 ■

Dmitri’s SAXS beamline is one of Hamburg’s bestsellers. It is fully booked by users many months in advance.

Imaging the big picture

'MANY HANDS MAKE LIGHT WORK', or so the old adage goes. This is especially true of modern biology, much of which relies on generating huge amounts of data. Scientists from all over the world come together to collaborate and analyse the vast reams of information gathered by such projects. Now, however, some projects are producing so much data that it is simply impossible for any team, no matter how large, to process it in a realistic timeframe.

One area where this is particularly problematic is large-scale cell biology projects that collect their data as images or movies. It would take a scientist longer than his or her lifespan to study them all. "Looking at all those movies isn't even theoretically possible for anyone because it would just take too long," says Wolfgang Huber, group leader at EMBL-EBI. "And that's before you even think about doing analysis."

Wolfgang is one of several researchers at EMBL, including Jan Ellenberg and Rainer Pepperkok at EMBL Heidelberg, whose team is developing new software that can perform this kind of data collection and analysis quickly and efficiently. But there is much more to this work than sparing biologists the Sisyphean task of analysing millions of images by hand. Human judgement is subjective and varies from person to person, making it hard to compare results collected by different scientists. Handing the job over to a computer will ensure more precise, consistent and reproducible results. "To do science, you really want something you can write down in a formula or similar," explains Wolfgang. "Our interest is in making this a more objective discipline."

Until recently, biologists hadn't really considered performing large-scale experiments to collect data in the form of images. True, enterprises such as the Human Genome Project had shown it was possible to do 'high-throughput' projects, in which scientists quickly perform millions of experiments. But such projects generated comparatively straightforward sets of data, such as biochemical measurements, or information about the sequence of chemical bases within a stretch of DNA. Scientists were still just about able to manage such large sets of data with the help of ever-growing computing power.

Images, on the other hand, present their own sets of benefits and challenges. Taking photographs or videos of cells down a microscope lets biologists asses the effects of, say, a drug on the physiology, shape and behaviour of an entire cell, as opposed to studying its effects on one or two biochemical reactions in isolation. This makes the experimental findings much more relevant to what actually happens in the body.

The downside of images, however, is that until now, they could only be analysed by people. This makes the analysis time-consuming, meaning that only a relatively small number of images can be dealt with in any given study. The data they deliver are also subjective, depending on the senses and experience of the scientist analysing them.

Now, however, there is a growing demand from scientists who want to perform high-throughput studies on whole cells. To help them deal with the staggering number of images and movies involved, Wolfgang and his colleagues have developed a computer program called EBImage, which is already being used in labs around the world. The program is an extension of an existing software package called R, which is widely used by bioinformaticians who want to perform a range of statistical analyses. Wolfgang is co-developing EBImage with Michael Boutros and his team at the German Cancer Research Centre in Heidelberg, who are producing images of cell biology experiments.

Michael's project centres on a method called RNA interference, or RNAi. This is a technology that allows scientists to turn down the activity of a gene almost to zero, by adding a relatively simple chemical. This 'knock-down' approach can be used to study the function of any given gene: by knocking it down, a biologist can see what goes wrong in a cell or organism, and so deduce the function of the gene.

Instead of knocking down individual genes, the project is taking a high-throughput approach. It involves knocking down all the genes in a genome, one at a time, within one huge experiment, to look for genes involved in cell shape and behaviour. At EMBL Heidelberg, Jan and his colleagues are also conducting a high-throughput RNAi screen. This international project, named Mitocheck, is looking for genes that are specifically involved in cell division, or mitosis, and is producing time-lapse recordings of the cells' behaviour.

The researchers on both projects are doing this by placing cells in rows of tiny wells in plastic dishes, or in spots on a glass slide. In each well or spot, a specific RNAi reagent knocks down a different gene. After a while, they record the cells' reactions by taking pictures of them down the microscope. The idea is to connect each cell's reaction, or phenotype, with the particular gene that has been knocked down.

The amount of information they are producing is staggering. Michael alone is generating more than 170 000 images. Mitocheck is creating even more. This project monitors the cells under a microscope for 48 hours, taking a still video image every 30 minutes to make a time-lapse film. Jan, whose role in Mitocheck involves developing automated microscopy systems, has produced more than 200 000 movies, or a total of 20 million images. His team is developing image-processing methods in parallel with the EBImage work, tailoring them in particular to recognise cells with abnormal division phenotypes.

The first problem Wolfgang faces is how to teach the computer how to spot the difference between a normal cell and one whose process of interest has been disrupted.

Then he has to get the computer to classify them. He does this by grouping the phenotypes of the cells together into classes – for example, those whose shape has become elongated – and then using these to classify the function of the genes involved. A key benefit of his system is that it makes allowances for experimental error. "You can understand it and adjust for it," says Wolfgang. "This allows you to have more confidence in your results."

Such large-scale studies of cellular behaviour have finally become a reality for biologists. It offers them the opportunity to dissect the relationship between genes and cell behaviours in much greater detail – information that will be vital for building a better understanding of how healthy cells work and what happens when they go wrong in diseases such as cancer.

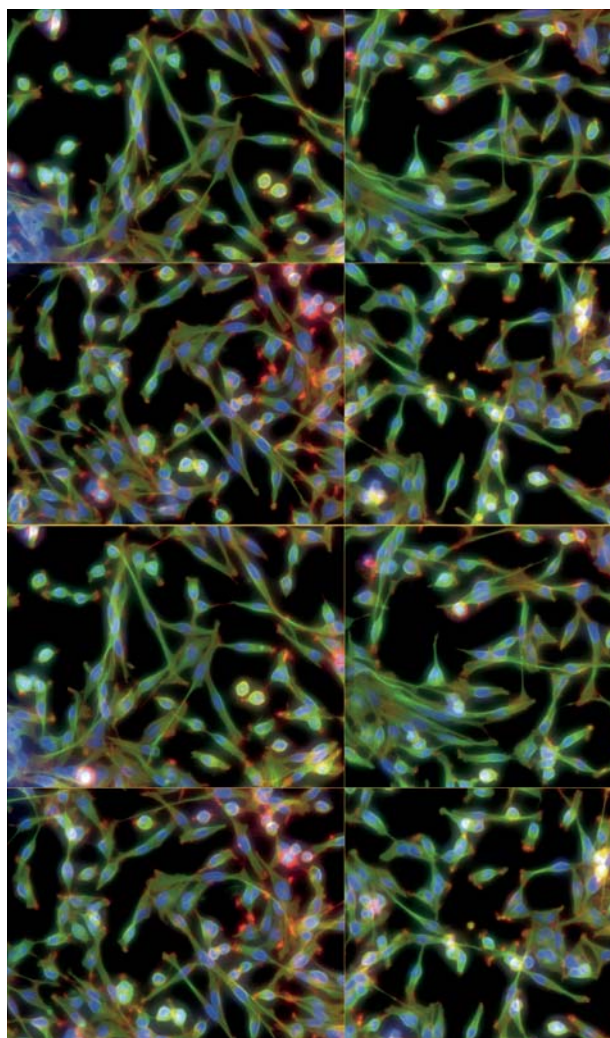
But there's no chance that this will put biologists out of a job – they will still have to set up the system and tweak it to suit each experiment. "There will not be an out-of-the box solution which works for every new experiment," explains Wolfgang. "The approach is to build a general toolbox of software modules, which you can then combine to do this kind of analysis, but which you have to plug together and adapt to each individual new analysis."

EBImage has been available online since mid-2007 and researchers can download it from a website called Bioconductor. Bioconductor is an 'open source' resource for bioinformatics analysis – one that provides data analysis software free of charge for anyone anywhere to download and use. It is a collaboration between Wolfgang and several other groups, including one at the Fred Hutchinson Cancer Research Center in Seattle. "The goal is to provide software to people to do good and technically competent analysis of their data," says Wolfgang.

Bioconductor also acts as a kind of online journal for publishing details of new technological methods. This ensures that as many people as possible benefit from them. "Just writing about how great your method is, is not that satisfying," says Wolfgang. "You really want to give it to people and see it be used." Consistent with this goal, Wolfgang and his colleagues undertake a programme of teaching scientists how to use Bioconductor.

But once the results of Jan's and Michael's studies are published, they plan to make all the data freely available on public websites. "The idea is to allow people to go and look at all the data and all the analysis we performed," explains Wolfgang. A long-term goal is to extend the scope of the software and the website to include screens done by other labs.

So where will this kind of technology take biology in the future? "The experiments will get more sophisticated," says Wolfgang. For example, EBImage could be adapted for different kinds of microscopy, such as 'confocal' microscopy, a technique that allows scientists to get sharp, high-magnification images that can be reconstructed into a 3D image of a solid object such as a cell. It would also let scientists look at more complex cell behaviours, such as differentiation, the process by which a young cell gradually develops a specialised function. "In the future, scientists will be doing more and bigger experiments like this," says Wolfgang. "So this is all building stepping stones to being able to do more."



Microscope images like these pictures of human HeLa cells can be analysed with the help of EBImage. Different fluorescent dyes label the DNA (blue) and cytoskeleton components actin (red) and tubulin (green).

Skylar O, Huber W (2006) Image Analysis for Microscopy Screens. *R News* **6**: 12-16

Hahne F, Huber W, Gentleman R, Falcon S (2008) *Bioconductor Case Studies (Use R)*. New York, NY, USA: Springer ■

Andrew McCarthy and David Flot at the microfocus beamline in Grenoble

Pole position

"IT'S LIKE MAKING A FORMULA One car available for anyone to drive." David Flot, staff scientist in Andrew McCarthy's group at EMBL Grenoble, proudly shows off his charge: a state-of-the-art X-ray beam machine that is helping scientists uncover the structures of molecules that have so far resisted their efforts. Over the past two years, David has been developing the machine and its surrounding setup, the only one of its kind in the world, to make it easier for researchers to use. The result? A clutch of high-profile discoveries and some very happy structural biologists. "Some groups are getting addicted to it," says David.

The machine in question is known as a microfocus beamline, and this one is the European Synchrotron Radiation Facility (ESRF) ID23-2 Gemini Microfocus Beamline. Beamlines channel X-rays from a synchrotron so that structural biologists can shine them on crystals of molecules to determine the molecules' structure.

Problems arise, however, if the molecule a researcher wants to study does not crystallise well, something that is becoming increasingly common as ambitious biologists tackle ever more complex proteins. These can often only be coaxed into forming tiny crystals that are too small to give a clear picture with conventional beamlines. Solving this problem requires a much thinner and more intense beam – a microfocus beam – to match the tiny crystals.

ID23-2 is one of a few microfocus beams in the world, and the only one wholly dedicated to protein X-ray crystallography. At less than 10 micrometres in diameter – a tenth of the width of a human hair – the ID23-2 beam is about ten times smaller than conventional beams and lets researchers tackle crystals that measure a mere 5 micrometres across. Studies on large crystals can benefit too: X-rays damage molecules, so scientists can normally only gather one set of measurements from each crystal. But a microbeam means researchers can perform several experiments on the same crystal, by probing different areas each time.

ID23-2 is the culmination of microfocus work pioneered by Stephen Cusack and the ESRF in the 1990s, and built upon by subsequent researchers such as Florent Cipriani's group, who developed part of ID23-2's experimental setup. The optical elements were designed and built by the MX group of the ESRF. David's contribution has been to integrate the hardware and software to ensure that users, who come from labs all over the world, can get the results they need easily and efficiently.

The results speak for themselves: since ID23-2 went online in November 2005, it has helped crack many previously recalcitrant proteins and led to a flurry of publications in high-ranking journals such as *Nature*. Other synchrotrons are now starting to build microfocus beams or propose setups for small crystals, and are looking to ID23-2 for inspiration and to buy its technology, including its super-accurate diffractometer, a machine that places crystals in the correct position within the X-ray beam.

Far from sitting on his laurels, David is striving to make the "driving experience" of ID23-2 even smoother in future, to ensure it retains its pole position in the world of structural biology.

Hadden JM, Déclais AC, Carr SB, Lilley DM, Phillips SE (2007) The structural basis of Holliday junction resolution by T7 endonuclease I. *Nature* **449**: 621–4

Rasmussen SG, Choi HJ, Rosenbaum DM, Kobilka TS, Thian FS, Edwards PC, Burghammer M, Ratnala VR, Sanishvili R, Fischetti RF, Schertler GF, Weis WI, Kobilka BK (2007) Crystal structure of the human beta2 adrenergic G-protein-coupled receptor. *Nature* **450**: 383–7

Ringleaders get ready to rock

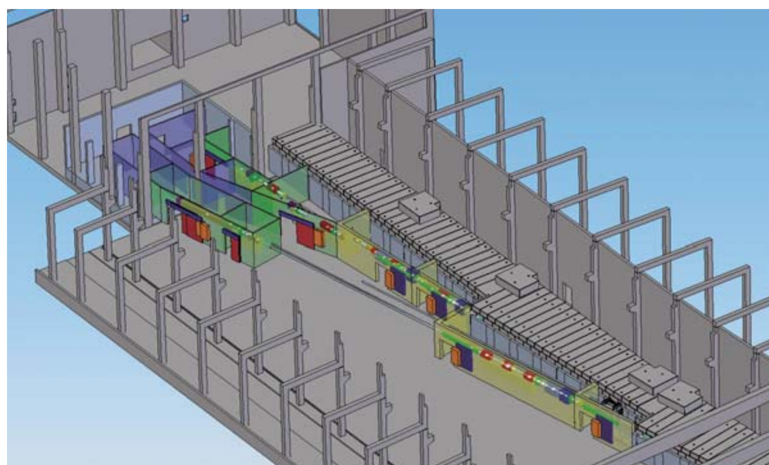
IN 2010–2011, EMBL Hamburg will open its doors to a new state-of-the-art synchrotron facility called EMBL@PETRA3. Designed to meet the demands of the next generation of structural biology research, EMBL@PETRA3 will provide world-leading integrated support facilities for international researchers. The Hamburg researchers have been busily upgrading facilities and developing the new infrastructure as building work in the new PETRA III ring progresses apace. "The construction is going at an incredible speed," says Thomas Schneider, a group leader who heads the project at the outstation.

"Our goal is to build an integrated facility making use of our unique expertise," says Matthias Wilmanns, Head of the outstation. Much of the new infrastructure is being developed bilaterally with EMBL Grenoble. "It's important that we develop a common base of expertise," says Thomas. Each outstation will, however, still maintain a distinct area of specialist practice. "Hamburg and Grenoble will both complement and synergise with each other," says Matthias.

One important issue at Hamburg is keeping communication open between the EMBL scientists who will use the beamlines, and the physicists at the German Synchrotron Research Centre (DESY) who are building the synchrotron. Matthias and Thomas have been developing links with the physicists to make sure this happens. "Things are growing together," says Matthias. "It's a very complex endeavour," adds Thomas. Thomas and Matthias are also actively maintaining contact between Hamburg and its current and future users.

In this context, running a limited number of beamlines at the older ring, DORIS III, is an attractive option for the user community while the new beamlines at PETRA III are being set.

A number of technical development projects for EMBL@PETRA3 are underway as well. One of these is a new robot being developed by Dmitri Svergun's group, which automatically changes liquid samples in the SAXS beamline. Another is the new PILATUS detector, which collects the X-rays after they have been scattered by a sample. A prototype module was purchased by EMBL, with the help of partial funding from SAXIER, an EC-funded project for new infrastructures in SAXS. This detector module is presently being tested on beamlines in both Grenoble and Hamburg.



Sneak preview of what EMBL@PETRA3 will look like from the inside.

Some of the other innovations will help tackle some long-standing problems of crystallography. One is a new multi-layer-based monochromator, which modulates the energy dispersion of the X-ray beam before it hits a crystal. Developed in-house at Hamburg, this will increase the intensity of the beam – by up to 60 times with reference to recent test measurements. Together with a new data-collection strategy, this could help researchers deal with the problem of radiation damage, which limits the number of experiments they can do on a given crystal. A new, versatile sample changer for changing crystals on the beamlines is also under development and could become a future key link between automated crystallisation and automated data collection.

The ultimate aim of the Hamburg team is to provide a unique integrated facility that brings together biological sample preparation, data collection and data evaluation, as well as providing expert support to users. "It's like a jigsaw puzzle," says Thomas. "The challenge is to grow things together on the right timescale." ■

The molecular basis of disease

"THERE DOES NOT EXIST a category of science to which one can give the name applied science. There are sciences and the applications of science, bound together as the fruit to the tree which bears it." Louis Pasteur, the famous nineteenth-century French scientist, counted the invention of pasteurisation, the first rabies vaccine and providing proof for the germ theory of disease among the many achievements that mark him as one of the founding fathers of modern medicine. But although many of his discoveries had applications that profoundly altered medical practice, he never lost sight of the importance of basic, curiosity-driven research. Indeed, as his quotation shows, he believed the two to be inseparable.

Much of the work carried out at EMBL falls under the umbrella of what is commonly regarded as basic research, but which nonetheless has many practical benefits. The first benefit is increasing understanding: if scientists know how a particular part of our biology works, such as cell division, they are more able to understand how it breaks down in diseases, such as cancer. Another benefit is that studying basic biology means scientists can also manipulate it for therapeutic purposes: the stem-cell research performed at EMBL is a case in point.

What's more, the techniques that researchers develop to carry out this curiosity-driven research can also be used in projects with a more applied goal in mind. One example is the technologies used to remove, or knock out, the function of a gene, or to switch it on or off over the course of an experiment – both of which are now widely used in biomedical research.

But there are many other projects at EMBL that focus specifically on understanding the molecular underpinnings of the disease process. Some are focusing on mistakes or defects within our own bodies that cause disease, such as gene mutations that cause kidney disease, or faulty neuronal wiring that can result in chronic anxiety. Others target the viruses, bacteria or other microorganisms that make us ill. One team, for example, has uncovered the trick used by influenza to fool our cells into making more of the virus.

But there's another reason why basic research is so important. History tells us that many of the great scientific discoveries – such as the discovery of penicillin – happen entirely by accident. Without detailed knowledge of how our cells and bodies work, we risk missing the significance of any unusual results that might crop up. As Pasteur once said, in yet more words of wisdom: "In the fields of observation, chance favours only the prepared mind." ■

It’s a knockout

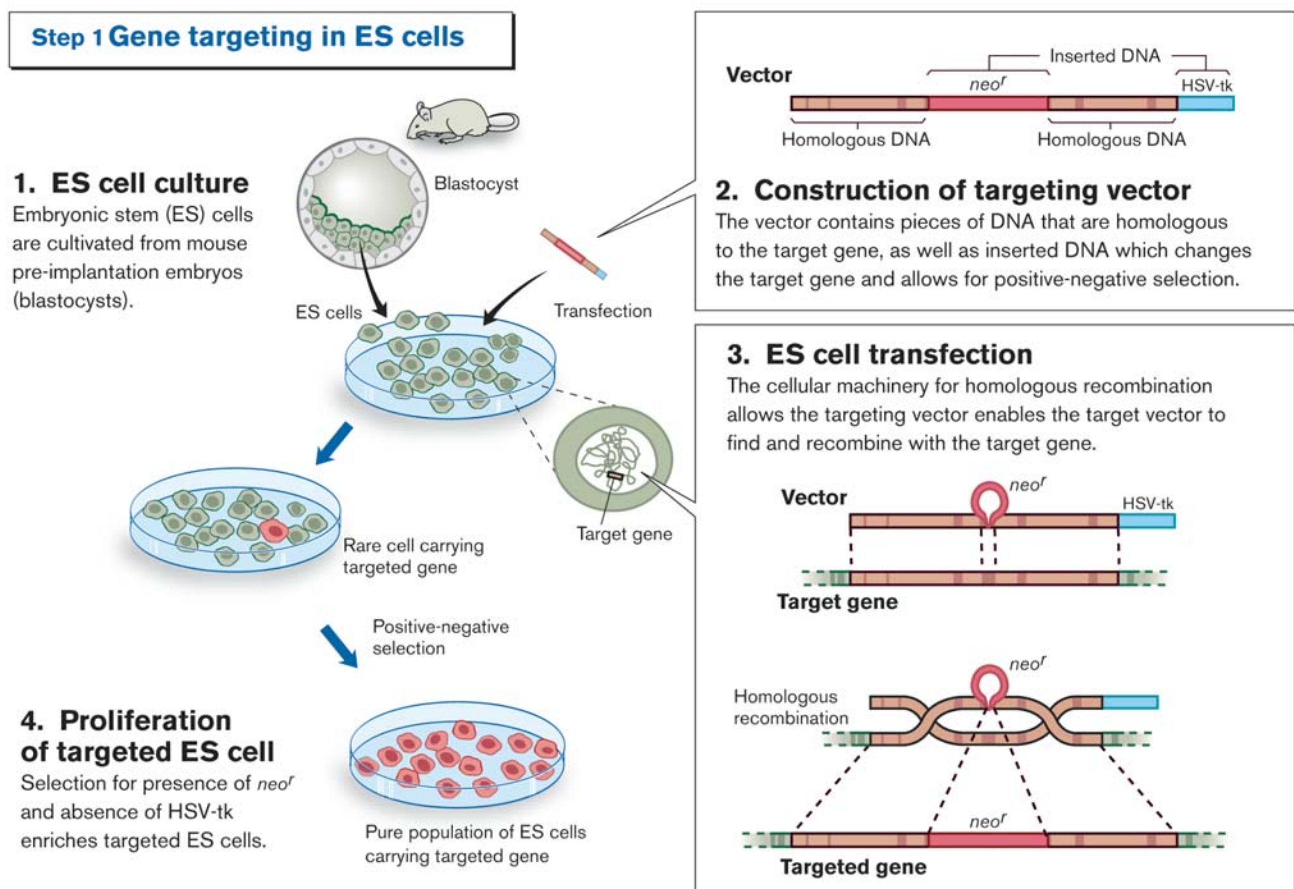
The team at EMBL Monterotondo

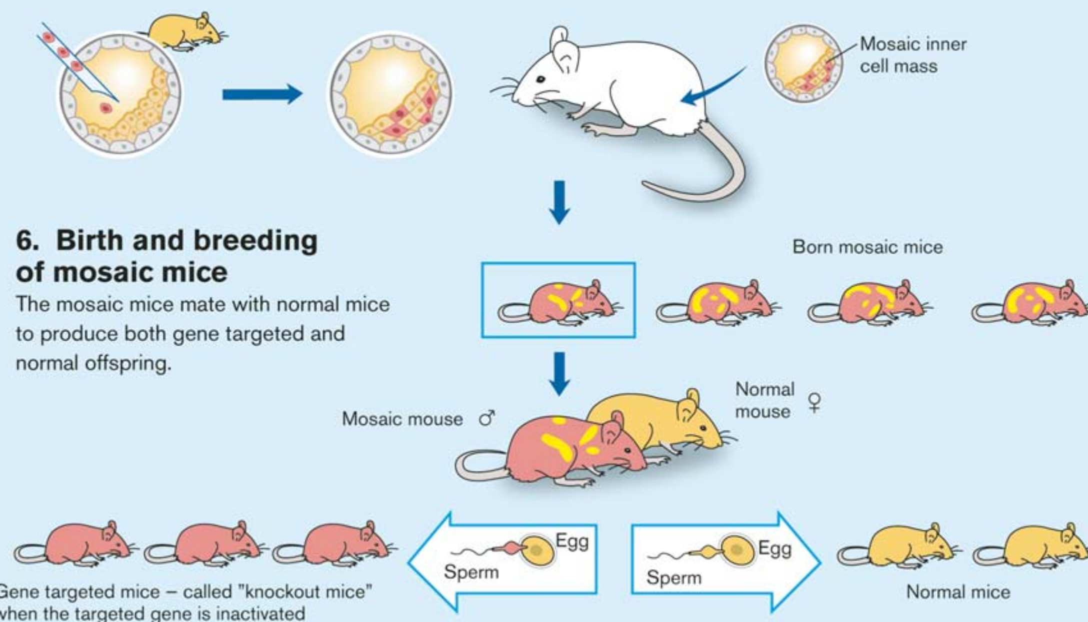
"THE 2007 NOBEL PRIZE in Physiology or Medicine is indeed a real knockout." With these deliberately ambiguous words Christer Betsholtz of the Nobel Assembly paid homage to Mario Capecchi, Martin Evans and Oliver Smithies in Stockholm last year when awarding them with the prize that undoubtedly marked the climax of their scientific careers. The three researchers were honoured for their discoveries of "principles for introducing specific gene modifications in mice by the use of embryonic stem cells". Even if this official wording sounds less exciting than Betsholtz's catchy speech, the discovery behind it was clearly one of the biggest breakthroughs ever achieved in genetics and one to completely revolutionise the field within a few years.

But what exactly is this technique that in insider circles is only referred to as 'knockout'? How does it work? Why is it so special and where will it take biology and medicine in the future?

Someone who is bound to have answers to some of these questions is Nadia Rosenthal, Head of EMBL Monterotondo's Mouse Biology Unit. She is a real specialist in knockouts and has been using the technique from its early days. "Today knockouts have become the standard for genetic studies in mice. But back then they were revolutionary." It is thanks to the knockout technique that mice – and with them mammals as a group – finally became genetically tractable. Before then, scientists could only guess what genes were doing in the mouse. They introduced random mutations somewhere in the genome, through radiation and chemicals. Observing the resulting symptoms, they then tried to track back which gene they had hit – a very tedious and unreliable procedure at the time. The alternative was to consider the mouse as an evolutionary extension of lower organisms that were amenable to systematic genetic investigation, such as flies and worms. But since mammals differ from insects and worms in many fundamental ways it is difficult to transfer the insights gained to mice.

General strategy for gene targeting in mice





In 2007, the Nobel Prize for Physiology or Medicine was awarded for the discovery of 'principles for introducing specific gene modifications in mice by the use of embryonic stem cells'. How these principles are used to generate knockout mice is explained in this graphic by the Nobel Committee.

So, researchers were longing for a way to switch off or change the activity of individual genes in the mouse directly. All this became possible when knockouts entered the scene in the early 1990s and with them an enormous new potential for biology and medicine. Suddenly scientists could see with their own eyes what a gene was needed for in the mouse.

"In that sense, gene targeting completely changed our idea of what is possible with mice. But we are rushing ahead. Why don't we start at the top and first talk a little bit about what knockouts actually are," Nadia suggests, picking up a pen and drawing genes, cells and mice. The biological principle that forms the basis of gene knockouts is called homologous recombination. It denotes the process by which fragments of DNA can be exchanged between chromosomes. Most commonly that happens during the formation of sperm and egg cells between the chromosomes donated by mother and father, but homologous recombination can occur between any segments of DNA as long as they are similar enough in sequence.

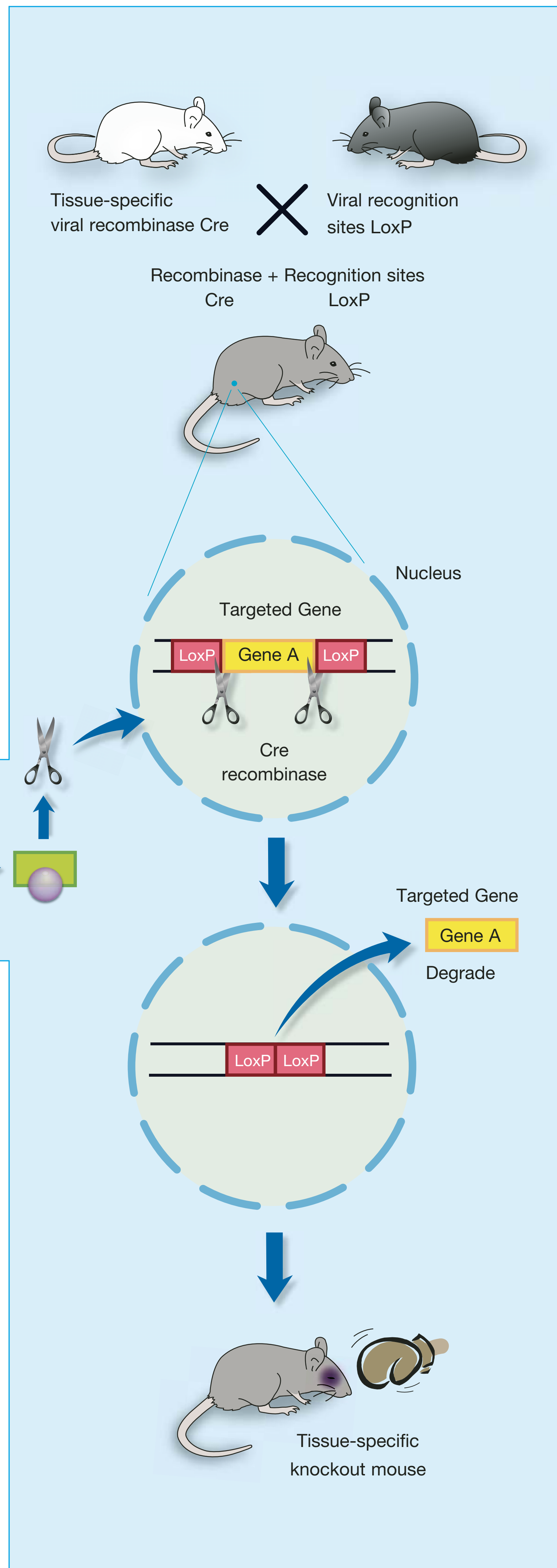
Mario Capecchi and Oliver Smithies worked out how they could use this property of DNA to make specific changes in any gene in a mammalian cell. They capitalised on the components of the homologous recombination system and engineered DNA constructs flanked on either side by the same sequence as the gene that should be replaced by them. Enzymes called recombinases then cut the DNA molecule at these flanking sequences and excise the fragment enclosed by them, which can then be replaced by the engineered construct. Injecting a corrected version of a non-functional gene into the nucleus of a cell the technique can be used to repair a genetic defect. More common, however, is the so-called knockout – in which a functional gene is replaced by a non-functional copy, shutting down the production of the protein it encodes.

Not every type of cell, however, can then be used to generate a whole gene-targeted animal. For that it requires cells that can give rise to germ cells. This is where the work of Martin Evans comes in. He had developed ways to isolate and culture embryonic stem cells – pluripotent

cells that can give rise to every cell type in the body. Genetic changes introduced into these cells can be inherited by the next generation of mice when the altered stem cells injected into the embryo form germ cells. A mouse with an altered gene in its germ cells can give rise to offspring that carry the genetic modification in all their cells.

Combining Evans' finding with the gene targeting technology developed by Capecchi and Smithies, the first knockout mouse was born in 1989. Since then the technique has been continuously advanced, many useful features have been implemented and most of its initial shortcomings have been overcome. "One of the biggest problems with knockouts was that they were not controllable in space and time," Nadia remembers. "Many of the knockout mice died before they reached adulthood, because the altered gene had a vital function during development. Without more control over the knockout process you could never find out what the gene is doing in the adult animal." Similarly, it was impossible to assess a gene in the context of only one specific organ or cell type.

With the advent of conditional knockouts in the middle of the 1990s, all of this changed. One of the pioneers of this technique was Klaus Rajewsky, Nadia's predecessor and head of the Mouse Biology Unit in Monterotondo from 1995 to 2001. Klaus developed the first conditional knockout system based on a recombination set-up used by viruses called phages. His trick was to flank gene constructs with sequences that are recognised only by viral recombinases. Mice with the construct integrated in their germ cells were then crossed with mice that were engineered



The conditional knockout system is based on a recombination set-up used by viruses. A mouse, engineered to carry a gene flanked by two viral sequences in the germ cells, is crossed to a mouse that carries the viral recombinase in its germ cells. In the offspring, the recombinase is expressed only in one cell type and is engineered to carry domains of a hormone receptor. When the mice are fed with a synthetic hormone, the conformation of the recombinase changes and it is admitted into the nucleus, where it excises the gene flanked by the viral sequences from the genome and creates a tissue-specific knockout mouse.

to carry the phage recombinase. But the viral recombinase gene had been inserted at a very specific location in the genome where it is under the control of a tissue specific promoter that allows its expression only in one organ or cell type. On top of that the recombinase can be engineered to carry domains of a hormone receptor, which influences its shape so that it cannot move into the nucleus to cut the DNA. Only when the mice are fed with a synthetic hormone that acts on the receptor domain does the conformation of the recombinase change and it is admitted into the nucleus. In this way scientists gained control over where and when a gene was switched off.

"Our vision is to produce a complete catalogue from which researchers around the world can pick and order the knockout they need."

"Thanks to Klaus' pioneering work, scientists at EMBL Monterotondo were some of the first to take on the knockout technology when it became available. Things haven't really changed since then. There is not one group here that does not use it," Nadia says. She herself tries to identify genes and pathways involved in the regeneration of tissues, especially skeletal and heart muscles, with the help of knockouts.

Of similar medical relevance is the research that Nadia's colleague, Walter Witke – group leader and deputy head of the Mouse Biology Unit – undertakes with knockout mice. He is interested in the cytoskeleton, the framework of protein filaments that gives cells their shape. More precisely he studies one type of protein filament called actin. "The connection between actin and disease is not immediately obvious," Walter explains, "but the protein is involved in many fundamental processes like cellular transport, cell division and migration. If these are disturbed a variety of medical conditions can develop."

Walter's group systematically knocked out various proteins involved in assembly and breakdown of actin filaments in different tissues. They were surprised how many of them reproduced symptoms of human diseases in mice. For example, switching off n-cofilin, a protein that binds to and disassembles actin filaments, in the brain leads to the same anatomical defects and symptoms as shown by patients who suffer from lissencephaly, a form of mental retardation. The intricate cerebral cortex architecture, which is organised into many distinct cell layers, is distorted in these mice. The reason is a defect in cell migration and stem cell differentiation early on in development – both processes depend on the remodelling of actin filaments by n-cofilin. The protein is conserved between mice and humans and so likely plays a similar role in the development of the human cortex, lissencephaly and possibly other neural migration disorders, such as epilepsy and schizophrenia. "Knockout mice like ours are powerful tools to investigate disease mechanisms and even suggest potential drug targets for new therapeutic approaches," says Walter. This attitude is shared by fellow group leader Claus Nerlov, who tries to unravel the molecular basis of leukaemia by targeting genes involved in blood development.

But the value of gene targeting stretches even further. The technique grants insights into the realms of behaviour that are normally hardly accessible by conventional wet-lab methods. Liliana Minichiello and Cornelius Gross are interested in how molecules bring about behaviour – normal behaviour like learning and memory, emotional behaviour, and abnormal behaviour as caused by mental disorders. With the help of knockout mice, Liliana came close to deciphering the molecular underpinnings of learning and memory and proved that the selective strengthening of connections between neurons in a brain structure called the hippocampus underlies the ability of mice to form memories. Interference with this process has adverse effects on memory formation similar to those that occur in disorders like Alzheimer's. Cornelius' knockout mice, on the other hand, revealed molecules and neural circuits that are responsible for fear and excessive anxiety.

Since every group in the Mouse Biology Unit relies heavily on gene targeting, a method that is technically challenging and very time consuming, EMBL Monterotondo runs an expert service that centrally produces knockout mice. José Gonzalez, Pedro Moreira and the other five staff of the transgenic services are experts in handling embryonic stem cells and microinjecting them with DNA. "Both of these steps are very difficult and require a lot of patience and skill," says José. His team can produce a knockout mouse in around six months. The same exercise would take a less-experienced scientist up to two years. The transgenic services produce around 80 knockout animals per year for the groups at EMBL and on top of that they have recently started to take orders from the international scientific community.

The facility does more than classic gene targeting via stem cell manipulation. They generate transgenic animals – which have been genetically modified in some way – by various techniques. Genetic material can be injected directly into a fertilised embryo, for example, where it integrates randomly into the genome. The newest and most cutting-edge addition to the catalogue of genetic

manipulations performed by the EMBL transgenic services is called ICSI, or intracytoplasmic sperm injection. A sperm cell is coated with a DNA construct and then injected into an unfertilised egg cell. During fertilisation the manipulated DNA integrates into the chromosomes and generates random mutations. "Mouse ICSI is extremely tricky because you are working with a very big injection pipette that can easily hurt the oocyte. But it allows you to use large constructs that also incorporate regulatory sequences of a given gene and facilitates its complete integration into the host genome. In this way you can achieve very natural expression patterns," says Pedro, outlining the advantages of ICSI.

With these kinds of services and expertise at hand, the scale of knockout projects is growing. For example, EMBL Monterotondo is part of a big, international consortium called the European Conditional Mouse Mutagenesis Project – EUCOMM for short. Funded by the European Union, the initiative brings together the nine major European players in mouse biology to generate an extensive archive of conditional knockouts. The goal is to systematically knock out all 20000 genes of the mouse and archive them as modified embryonic stem cells that can be turned on demand into transgenic animals. "Our vision is to produce a complete catalogue from which researchers around the world can pick and order the knockout they need. Such a system would save a lot of time and cost. It would speed up biomedical research and drug development and also provide less well-equipped labs with access to knockout animals," Nadia says.

Large-scale efforts like EUCOMM prove that knockouts are not just a flash in the pan of the fast moving field of genetics; they have come to stay. "Of course, at some point we will reach saturation when all mouse genes have been knocked out. But advancing the technique further we will be able to work more and more specifically," Walter comments. Already now there are more subtle ways to influence a gene than switching it off completely. Scientists can place point mutations or just interfere with the level of gene expression. More sophisticated knockout systems will soon allow to generate multiple knockouts, mice that lack several genes in different subpopulations of cells. So, even if the Nobel Prize might be the biggest accomplishment of knockout-discoverers Cappechi, Evans and Smithies, the biggest triumph of the technique they invented may be yet to come.

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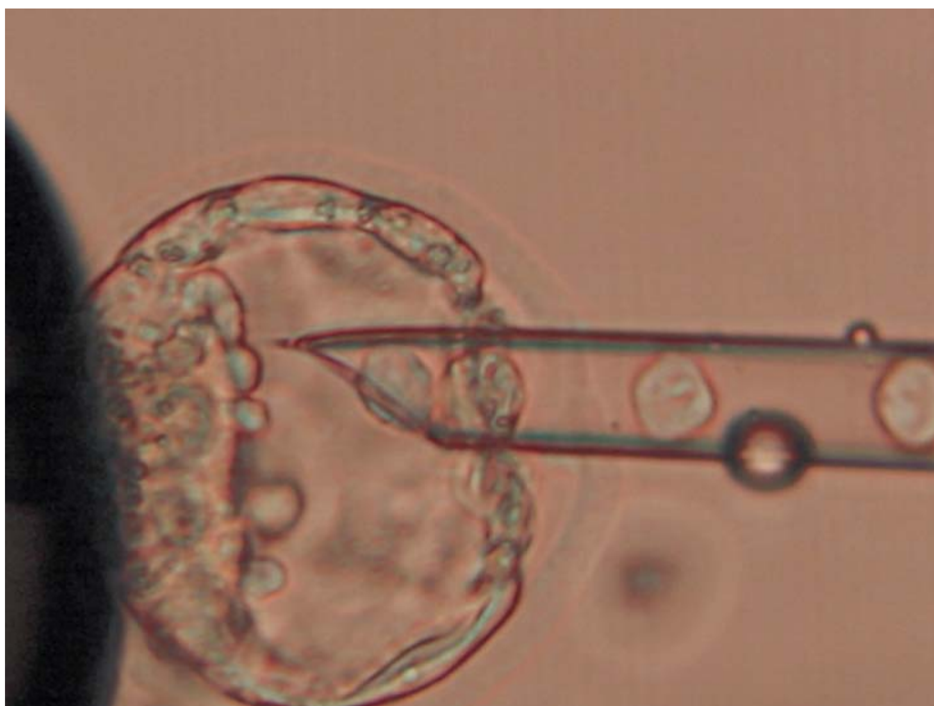

With a sharp pipette, embryonic stem cells are injected into mouse oocytes in EMBL Monterotondo's transgenic facility.

Reading the signs

Andrew McCarthy faces navigation problems

TRYING TO FIND YOUR WAY to a country wedding is never easy, but if it weren't for road signs or SatNav, most guests would never make it. Nerve cells in a developing brain face a similar problem. They must navigate to the right place and form the right connections with other nerve cells, and they rely on the molecular equivalent of SatNav to guide them. Now, scientists at EMBL Grenoble have discovered the structure of part of one of these signals, a result that will help scientists overhear the messages cells receive and perhaps understand how it goes wrong in diseases such as cancer.

Andrew McCarthy and his team have been studying a signal called Slit2 and its receiver Robo1. Slit2 is used to guide developing neurons to their destinations, but it also plays a role in the development of blood vessels. It can be hijacked by tumour cells as a means to spread through the body. "A lot of these development proteins are being triggered in cancer," says Andrew.

Andrew became involved in studying these proteins when he first joined EMBL as a member of Stephen Cusack's group. Stephen had long been interested in proteins that contain structures rich in the amino acid leucine. Such structures, called leucine-rich repeats, are sticky and play a key role in the interactions between many proteins.

Slit2 caught Andrew's attention because it contains four of these leucine-rich repeats – a very unusual structure. But the protein proved problematic to produce in the lab, so he turned to colleagues at the Bijvoet Center for Biomolecular Research at Utrecht University in the Netherlands, who are developing a new system for getting mammalian cells to produce proteins. With their help, he soon had Slit2 protein.

Once the structure of a leucine-rich section of Slit2 was determined, the team turned their attention to its receptor, Robo1. Slit2 interacts with the part of Robo1 that contains structures known as immunoglobulin domains. These are common protein structures, also found in the antibodies our bodies use to fight infections. Slit2 interacts with two immunoglobulin domains, and Andrew's structure revealed that the domains are linked with a flexible hinge.

By crystallising these fragments of Slit2 and Robo1 together, the researchers discovered that Slit2 interacts primarily with just one of the immunoglobulin domains. Intriguingly, however, it is possible for the other to bend around via the hinge and also interact with Slit2, a feature that could form part of a signalling mechanism. The structure also hints that a third protein could interact with Robo1 and Slit2 via a molecule called heparin.

As well as offering new insights into how cells read Slit2's directions, the structure reveals potential strategies for anti-cancer therapies. "An idea would be to design a drug to block this interface," says Andrew. A key question for the future is whether two Robo receptors club together to relay Slit2's signal inside the cell. Andrew is now trying to study larger fragments of Robo and Slit to answer this and to learn more about how our cells choose their directions in life.

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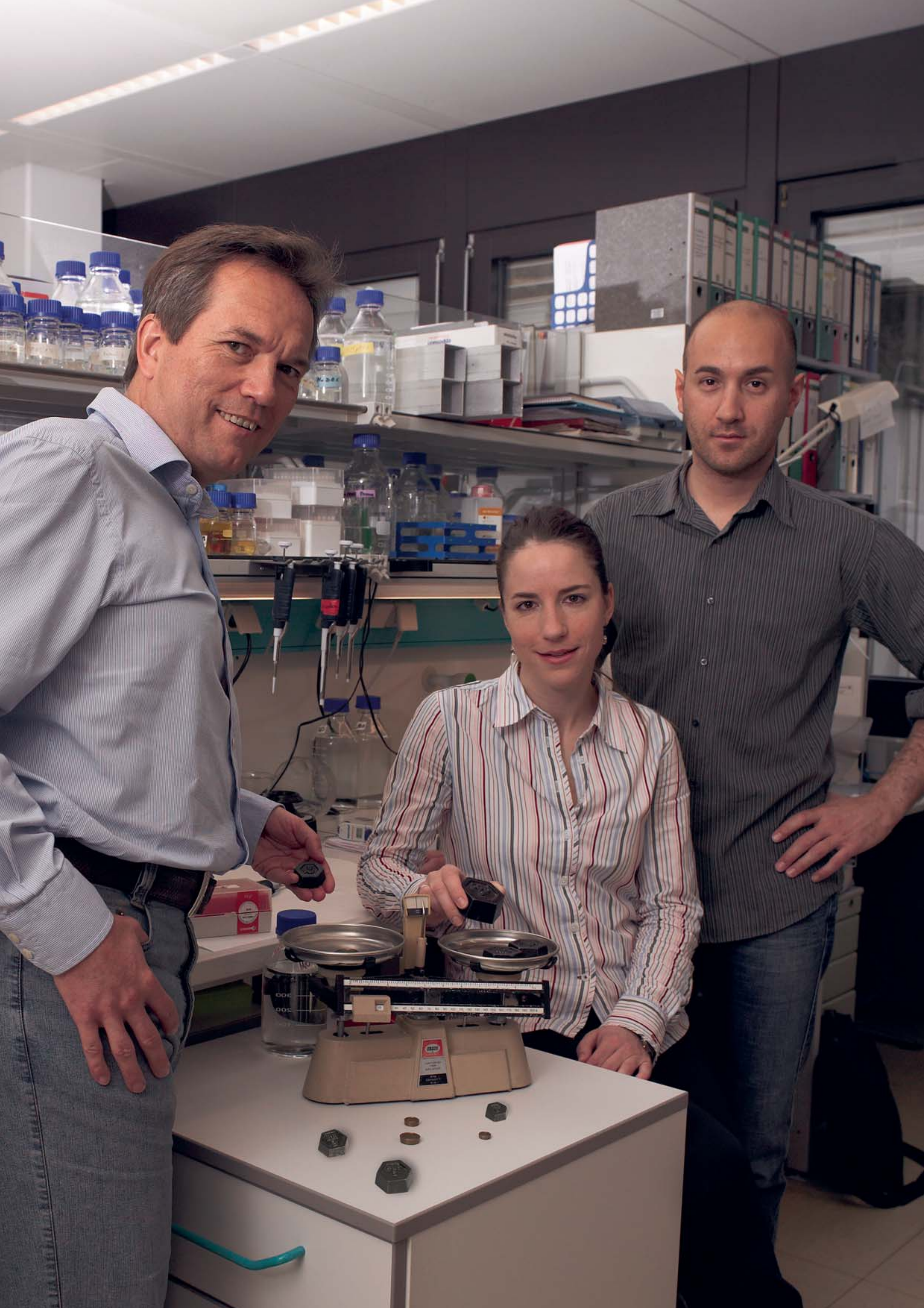


Matthias Hentze, Dunja Ferring-Appel and Bruno Galy

Iron's balancing act

IRON DEFICIENCY IS A COMMON PROBLEM in many parts of the world. But a diet packed with spinach and steaks can be overkill. Striking the delicate balance between too much and too little is crucial for essential – but potentially toxic – nutrients such as iron. Iron is a central component of red blood cells and is needed to transport oxygen around the body, but too much can damage internal organs. In fact, once the body has absorbed iron, it is impossible to get rid of it, so the mechanisms that regulate its absorption are absolutely critical.

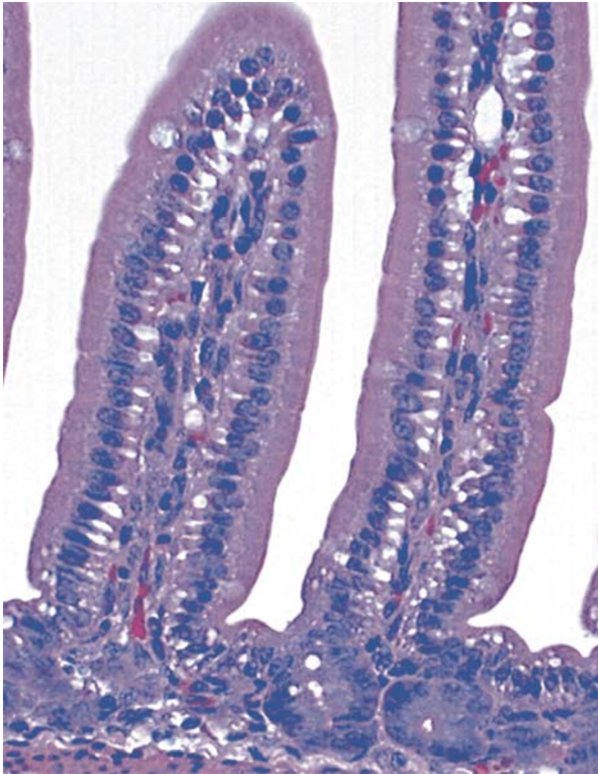
Matthias Hentze has spent many years studying iron – how we get it, how we use it, and how we maintain a balanced amount of it in our bodies. Matthias, who started his career as a medical doctor, says that disorders of iron metabolism are some of the most common nutrition-related diseases around the world. Now Associate Director of EMBL, Matthias studies the causes of iron overload in a genetic disorder called hereditary haemochromatosis, which affects about one in every 300 Europeans. These investigations have led to some crucial insights into how a balance of iron is maintained in the body. Remarkably, recent experiments by Matthias and his colleagues suggest that a drug commonly used to treat angina and hypertension might also be used to control iron levels.

This journey began when Matthias was approached by an alumnus of the lab, Günter Weiss of the University of Innsbruck in Austria. Günter had become interested in a protein called divalent metal transporter 1 (DMT1), one of an array of proteins involved in carrying iron around the body. DMT1 is essential for absorbing dietary iron from the gut and helping to transport it into the bloodstream. Günter, Matthias and their colleagues wanted to find ways to help patients with iron overload, and they wondered whether controlling DMT1 might help.

A previous study hinted at a surprising connection between the activity of DMT1 and the activity of a channel responsible for shuttling calcium in and out of cells, and they decided to test the drug nifedipine, chosen because it was already known to affect the activity of calcium channels. The results were dramatic. When tested on mice, nifedipine sharply increased the rate at which iron was transported away from the liver. It also enhanced the rate at which iron was excreted into the urine. The researchers don't yet know how the drug affects DMT1 function, "but whatever the mechanism," Matthias says, "this drug with a known clinical safety profile could provide an effective therapy for iron overload disorders."

Besides DMT1, a host of other proteins are involved in iron regulation. In fact, the balance of iron depends crucially on the balance between proteins that enhance the uptake of iron, and the proteins that reduce it. A major focus of Matthias' lab is to understand the control system responsible for setting this balance.

The system that he has been studying – called the IRP/IRE system – depends on a process that has only recently come to the attention of biologists. A basic 'dogma' of biology is that protein production happens in two steps. The genetic code is first 'transcribed' into pieces of code called messenger RNA (mRNA), then the mRNA is 'translated' into protein. But it was only relatively recently that biologists realised that the quantities of protein produced are pow-



Close-up of mouse intestinal tissue that absorbs iron from the gut.

erfully influenced by mechanisms that regulate events after transcription – so-called post-transcriptional control. For example, the amount of protein synthesised is heavily influenced by the accessibility of the mRNA template by the ribosome – the machine that makes proteins. Protein production is also affected by mRNA stability: if an mRNA strand is very stable, then lots of protein is translated, but if it is unstable, then only a small amount of protein will be produced before the mRNA disappears.

Mechanisms that affect translation, the process of protein synthesis, or mRNA stability strongly influence protein levels. As a postdoc at the National Institutes of Health in the USA, Matthias discovered that the mRNAs of important components of the iron regulatory system are tagged with small sequence elements that were dubbed 'iron responsive elements', or IREs. These response elements attract so-called 'iron regulatory proteins' (IRPs), which bind to IREs, altering the stability of the mRNA or its translation. Because both steps strongly affect how much protein is translated, the IRE/IRP system is a powerful regulator of the production of proteins that have important roles in iron metabolism. It turns out that the IRP/IRE system is involved in the regulation of nearly all processes that are important for handling iron in the body, affecting blood cell formation as well as iron recycling, absorption and storage. Although many details of this system have been understood for some time, most of the work has been carried out only on cultured cells. There was an important scientific reason for this: normally, researchers determine a gene's function by knocking it out and working backwards to see what went wrong. But animals cannot survive without the two essential iron-regulatory proteins, IRP1 and IRP2. This confirms that these proteins are very important, and it also makes finding out exactly what they do in a living organism very tricky.

To get around this problem, Matthias and his colleague Bruno Galy turned to the mouse facility at EMBL Heidelberg, which is dedicated to making and looking after transgenic mice. With their help, they made a mouse that lacked both IRP1 and IRP2, but only in specific tissues – something known as a 'conditional knockout' (see also page 96). The tissue that the researchers decided to target was a part of the gut called the duodenum, which is the main site of iron absorption, and expresses important mRNAs that possess IREs. This conditional knockout mouse allowed Bruno and Dunja Ferring-Appel in Matthias' group to study the roles of the IRE/IRP system at the primary site of iron absorption, in action.

They found that the lack of IRPs caused a widespread disruption of the major components regulating iron absorption. In the conditional knockout mice, the positive and negative parts of the control system were way out of kilter: some proteins such as DMT1 were downregulated, while others were upregulated. Intriguingly, whether a particular protein is upregulated or downregulated by an IRP depends on which end of its mRNA is tagged by the IRE.

The IRP/IRE system is clearly an important force in the delicate balance between proteins that help the absorption of iron and those that prevent it. Matthias says that these new results illustrate just how crucial the IRP/IRE system is in living organisms. "We've been waiting for 20 years for an *in vivo* assessment," Matthias says. "Our doubly IRP-deficient mice finally show that the IRPs are essential for intestinal function and survival."

One of the exciting consequences of this work is its potential for understanding numerous human diseases. Bruno adds: "Now that we have found ways to examine the role of the IRP/IRE system in living animals, we can begin to explore its role in a diverse spectrum of diseases from heart attacks to Parkinson's disease. It's a long journey ahead, but now we have all that we need to get going."

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A gene that protects against kidney disease

THE KIDNEYS ARE THE ORGANS that help our body dispose of potentially harmful waste and excess water. Diseases that affect this fundamental function are very serious but only poorly understood. Nephronophthisis (NPHP) is such a disease: starting in early childhood, it causes the kidneys to degenerate and shrink, often leading to renal failure by the age of 30. So far, kidney transplantation in early age has been the only way to save patients suffering from NPHP and effective, non-invasive therapies are desperately sought.

To address this problem and study the molecular mechanisms underlying NPHP, Mathias Treier and his group developed a mouse model of the disease. "Our mice showed striking similarities with NPHP patients," says Henriette Uhlenhaut, the postdoc in Mathias' lab who carried out the research. "Very early on in their lives their kidney cells die and the mice develop all the characteristic disease symptoms. Increased cell death appears to be the mechanism underpinning kidney degeneration in NPHP."

The genetic cause of these symptoms is a mutation that the mice carry in a gene called GLIS2. GLIS2 normally prevents cell death and fibrosis in the adult kidney. It does so by shutting down genes that encode the blueprint for the development of the organ. Such a blueprint, which is crucial for generating and shaping the kidneys during embryonic development, becomes harmful when reactivated in an adult animal. That is why GLIS2 makes sure that genes required during development are silent in adults. A mutation interfering with GLIS2 function, however, reactivates these genes and ultimately leads to the death of large numbers of kidney cells. The organ shrinks as changes in its architecture occur which affect normal kidney function.

Even if mice and humans are closely related and share many genes, insights gained from a mouse model cannot simply be transferred to humans. The only way to find out if GLIS2 plays the same role in humans and might be involved in causing NPHP is to conduct patient studies. For this reason, Mathias and his group teamed up with human genetics expert Friedhelm Hildebrandt of the University of Michigan, who carried out a genetic screen of patients suffering from NPHP. As was predicted by the mouse model, they found that some NPHP patients carried mutations in the same GLIS2 gene. The findings confirm that GLIS2 is also a crucial player in NPHP in humans.

"This is an excellent example of how combining basic and medical research can help to uncover mechanisms of human disease," says Mathias. "The next step will be translating the insights gained into new therapeutic approaches to develop alternatives to kidney transplantations. With GLIS2 we have already identified one promising candidate drug target and our mouse model will help us to find many others."

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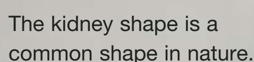


The kidney shape is a common shape in nature.

Cornelius Gross from EMBL Monterotondo

Of mice, men and scaredy-cats

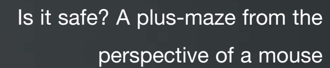
YOU ARE WALKING THE STREETS of your neighbourhood when you become aware of a dog lurking in a corner. As you come closer, it jumps to its feet, eyeing you suspiciously. What do you do? Most people would probably take a deep breath, approach carefully and mentally prepare to start running. The more fearful of us, or those who have had bad experiences with dogs in the past, might decide to take another road, but would quite comfortably walk the same street again a day later. Not so an over-anxious person. At the actual encounter they might be paralysed with fear or panic and run away, and it is unlikely they would ever use the same road again.

"Of course the risk of actually being attacked by the dog is small and the chance of having the same experience several weeks later is even smaller. But people who suffer from anxiety overreact to such ambiguous situations – situations that could be dangerous but most of the time are not. This behaviour is a hallmark of clinical anxiety," explains Cornelius Gross. His group at EMBL Monterotondo studies the molecular and neural basis of anxiety behaviour. Since this is very difficult to do in humans, they use the mouse as a model organism.

Mice and humans not only share most of the same genes and the same fundamental brain architecture, but also are remarkably similar in their behaviour. Anxiety is one example. Humans and mice both show avoidance and escape responses when they anticipate danger. Both species also show individual variations in emotionality, with some being inherently more anxious than others. Anxious mice, for example, are more hesitant to explore unfamiliar areas, especially exposed areas where they might be attacked from above. Less anxious mice explore such places more easily.

Sometimes there is only a fine line between healthy anxiety and pathological anxiety. All it takes to turn mice into scaredy-cats is a genetic change to just one molecule in their brains. The molecule is called serotonin, a chemical signal used for communication between brain cells. Serotonin has been implicated in various mental disorders in humans, most markedly in anxiety and depression, and antidepressants like Prozac affect its action in nerve cells. Cornelius and his group genetically engineered mice that lack serotonin receptor 1a, one specific receptor molecule that senses and responds to serotonin in the brain. Without the receptor, mice are a lot more anxious, explore their surroundings less and avoid novel situations.

To find out if the mice suffered from a condition similar to human anxiety disorders, the scientists tested if they overreacted in ambiguous situations. For that they used a common behavioural paradigm called fear conditioning. In fear conditioning, a mouse comes to associate a neutral stimulus, such as a flashing light, with an aversive stimulus, such as a weak electric shock, through repeated exposure. After some time the mice start to show fear responses to the neutral stimulus even before the shock is delivered, because they have learned the contingency between the two events. It is just like when a child who has been bitten by a dog learns to avoid dogs before they even get the chance to bite.

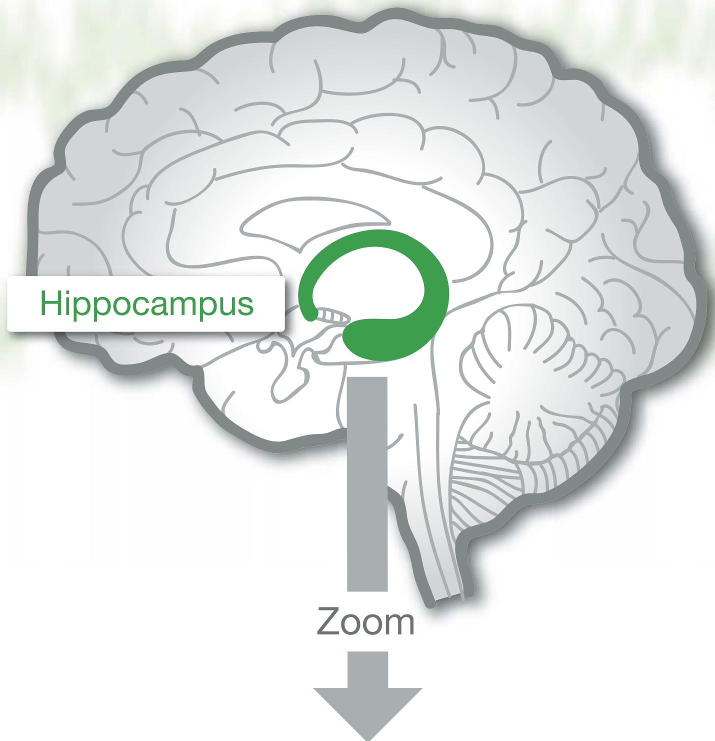

Is it safe? A plus-maze from the perspective of a mouse

But since not every dog will bite every time, Cornelius and his team also rendered the scenario for the mice a bit more realistic. In their study they introduced a second neutral stimulus, a tone, which was only sometimes followed by a shock and which otherwise occurred without consequences. Normal mice froze to the perfect cue, the light, but showed less freezing to the ambiguous stimulus, the tone. This intermediate fear reaction corresponds to us walking past the dog in our neighbourhood feeling uneasy and being alert. Without serotonin receptor 1a, however, mice showed the same fully fledged fear responses to both tone and light and didn't take the reduced contingency into account. It seemed as if these mice had lost their capacity to assess the risk inherent in the situation. The symptoms are reminiscent of those of anxiety patients and Cornelius hopes that these similarities will make the mice a good model for studying the molecular basis of human anxiety disorders.

"Before we can start to think about applying this knowledge, for example to develop new potential therapies against anxiety disorders, we first have to understand the precise role of the receptor and when and where it is needed in the brain," says Cornelius. Using a drug that blocks the activity of the receptor, the scientists treated animals at different stages of their lives and observed that serotonin receptor 1a is required between day 14 and day 35 of life, a period that in humans corresponds roughly to early childhood, from birth until age 10. In this period, many of the essential connections are established in the brain. In mammals, most brain cells are formed and send out connections to neighbour cells before birth, but the actual contacts are only established after birth. In this way brain connections can be shaped by experience. Cornelius suspects that serotonin receptor 1a might be involved in this experience-dependent shaping of connections. Its absence might lead to faulty wiring of the neural circuit underlying anxiety later in life.

Which neural circuit that is and which brain structures are involved in generating anxiety was the next question that Cornelius and his lab set out to answer. Several facts pointed them in direction of the hippocampus, a structure deep inside the brain that is known mostly for its role in learning and memory. In one particular part of the hippocampus, called CA1, serotonin receptor 1a is very abundant and studies had shown that lesions in the area affect anxiety-related behaviour in mice. Thanks to a new technique developed by Theodoris Tsetsenis, a PhD student in Cornelius' lab, the scientists were able to zero in further. They inhibited neural activity selectively in one cell type in the hippocampus, called dentate gyrus granule cells. "The result was really unexpected and very specific. Our over-anxious mice became absolutely normal. They froze only moderately to ambiguous cues, but still showed full-blown fear responses to the perfect cue," Cornelius summarises. Blocking the activity of the dentate gyrus cells counterbalanced the bias introduced by the lack of serotonin receptor 1a and rescued the anxiety.

With this approach, the scientists seem to have hit a central component of the neural circuit underpinning anxiety. The hippocampus has two major inputs, one of which is the dentate gyrus. They suspect that the effect of inhibiting the dentate gyrus granule cells is accomplished by selectively shutting down one of these inputs. Within the hippocampus, CA1 acts as a comparator and integrates information from the two inputs. It is likely that



A graphic of the hippocampal circuit involved in anxiety. Sensory information enters the hippocampus directly from the cortex and from the dentate gyrus. The two inputs are integrated and compared by the hippocampal area CA1, which then feeds back to the cortex where a response is triggered. Shutting down the dentate gyrus abolishes one input and throws the comparator CA1 off balance.

Hippocampus Circuit

Cortex

Comparator

Dentate Gyrus

CA3

CA1

Cortex

Shutting down dentate gyrus

Cortex

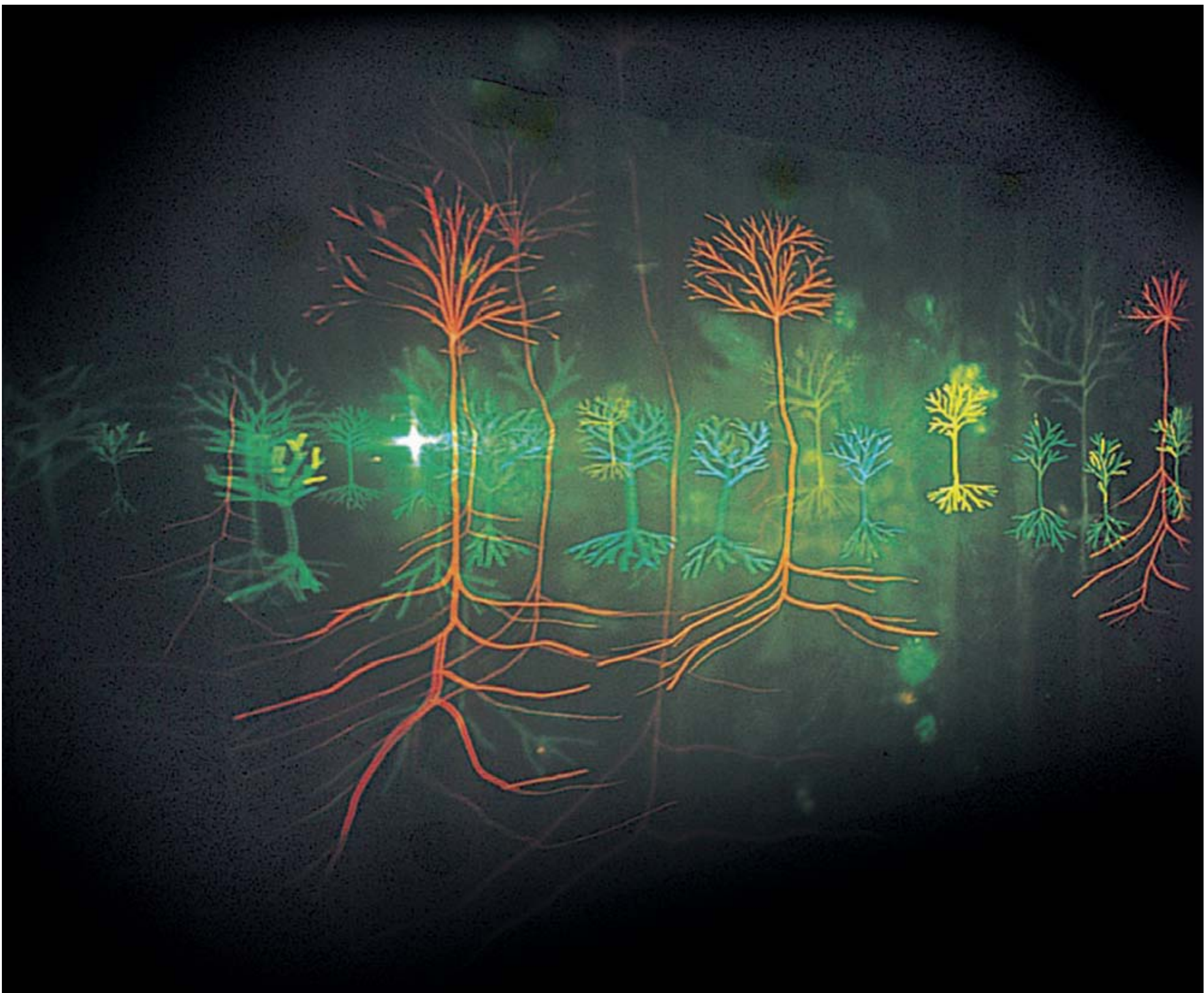
Comparator

Dentate Gyrus

CA3

CA1

Cortex



The picture tracks the development, proliferation and organisation of neurons in the growing brain as it becomes capable of holding memories. The colours reflect the fluorescence used to see individual neurons with a confocal microscope.

lack of the serotonin receptor might result in excessive input from the dentate gyrus, which may make animals overestimate sensory contingencies. Reversing this deficit by inhibiting this input makes the animals normal again. Cornelius's group is now trying to find out if there is in fact an imbalance in the inputs received by the CA1 in their anxious mice. By studying slices of brain tissue taken from the anxious mice, they have been able to show that cells in CA1 respond more to stimuli coming via the dentate gyrus than do cells from normal animals. They have also seen that the shape of CA1 cells is different and they are currently investigating the possibility that serotonin acts as a signal to alter cell shape while they are growing. This could explain why blocking the receptor during development is sufficient to make anxious mice.

The findings suggest that the functions of the hippocampus go beyond learning and memory. As a centre for the evaluation of information and risk assessment it is also a key component of the neural circuit that controls fear and anxiety in mice. Very likely the hippocampus plays a similar role in humans. "Mice and men are genetically very similar and the processing of fundamental, essential emotions is likely to be very conserved between species," explains Cornelius. This makes the serotonin signalling system and the hippocampal circuit identified by his group promising drug targets that scientists should look into for new treatments that fight anxiety disorders.

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Spotlight on phenotyping

At EMBL Monterotondo, scientists use mice as model organisms to study human diseases. This is fairly straightforward when you are working with a disease that manifests itself through clear physiological symptoms that can be measured, like an increase in body temperature for example. But how does one realise if a mouse is anxious or has memory problems as opposed to being unmotivated or suffering from a motor impairment? EMBL Monterotondo has a dedicated facility to address these questions.

Mumna Al Banchaabouchi of EMBL Monterotondo's phenotyping facility

In the phenotyping facility, Mumna Al Banchaabouchi and her team try to categorise the effects of mutations on the physiology and behaviour of a mouse. In accordance with ethical regulations, they first monitor the general health of the mutant mouse and examine physiological changes, such as temperature, heart rate and reflexes. Afterwards they assess the performance on specific behavioural tasks, related to learning, memory and emotional processes. "One way to find out if a mutation induces anxiety-related responses is to study mouse behaviour on an elevated plus-maze," says Mumna.

The plus-shaped maze is located 60 cm above the ground and two of its four arms are surrounded by walls providing a secure environment; the other two are open. Mice are not very comfortable with bright, open spaces and the more anxious the mouse the more time it spends in the safe, closed arms of the maze.

Another clear indicator of fear is freezing – a complete cessation of all movement except breathing. Memory, on the other hand, is often assessed through tasks that exploit the natural curiosity of mice. Such tests can be based on olfaction or involve a food-search task. If a mutation affects memory, mice have difficulties recognising an odour or need more time to find their way back to a food source.

While for some behavioural tests scoring is automated, for subtle details it still needs a human eye. Of course this introduces subjectivity into the scoring and raises questions about the robustness of the data. To minimise these influences and to make behavioural data comparable and reproducible, EMBL Monterotondo is part of a consortium called EUMODIC. The initiative, funded by the European Commission, aims to standardise protocols and scoring methods within and between different research centres and laboratories.

"Evaluation of behavioural responses is an important component for the *in vivo* screening of mutations, drugs or potentially toxic compounds in mice. Behavioural phenotyping provides a better understanding of the symptoms observed in disease. Ultimately it is the symptoms that we want to understand and eventually treat in humans," says Mumna. ■

Outmanoeuvering influenza's tricks

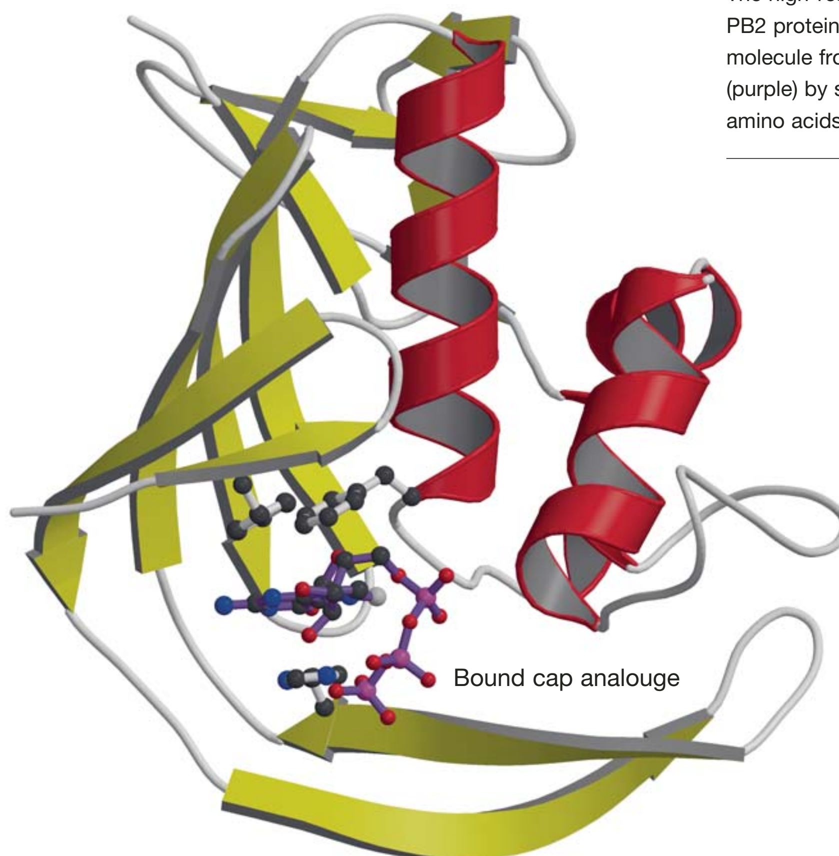
Stephen Cusack, Head of EMBL Grenoble, and Darren Hart

IT SEEMS LIKE A CONSTANT BATTLE. No sooner have you downloaded a security upgrade for your computer than a hacker somehow finds a weak point. Once again, your machine is open to attack by viruses, spyware and other malicious programs, until the next upgrade patches the flaw.

The cells in our bodies face similar problems. They too have a raft of security systems to prevent their biochemistry from being hijacked by viruses. But these systems are not perfect and many viruses have evolved sneaky strategies to hack into them. Now, thanks to work by structural biologists at EMBL Grenoble, one of the tactics used by the flu virus has been laid bare, opening the way for new drugs to combat future influenza pandemics.

Influenza is a grave concern for governments and health organisations around the world. Although common human strains of the virus are rarely deadly to healthy people, they can prove fatal to the elderly or those with existing illnesses or weakened immune systems. Worse, new strains of the virus are constantly evolving which could prove to be more virulent than their predecessors. Most worrying of all is the potential for 'bird flu' – the influenza virus that infects birds – to develop the ability to infect humans easily. In the past, viruses that have made this leap have been extraordinarily deadly. The 1918 influenza pandemic, for example, originated in birds and killed between 50 and 100 million people as it swept around the world.

Recently, fears have been mounting over a strain of bird flu called H5N1, which has infected and killed a few hundred people worldwide. If it eventually acquires the ability to spread from human to human, we could face a new, deadly pandemic. Flu vaccines do exist, but those that work against one flu strain often have limited effect against others. Vaccines against new strains take a long time to produce, and so may come too late to curtail an H5N1 pandemic. So new drugs to halt the spread of the virus are badly needed.



The high-resolution image of the influenza virus' PB2 protein shows how the virus steals a 'cap' molecule from its host. PB2 binds the cap (purple) by sandwiching it between aromatic amino acids.

Stephen Cusack, Head of EMBL Grenoble, and colleague Darren Hart, a team leader at the outstation, are cracking the structure of the protein that influenza uses to hack into our cells' protein production machinery. The protein in question forms part of the viral 'polymerase' – the enzyme that copies its genetic material and helps produce more viruses. Its biochemistry is probably very different from human proteins, which makes it an attractive target for drugs aimed at reducing the spread of infection in the body.

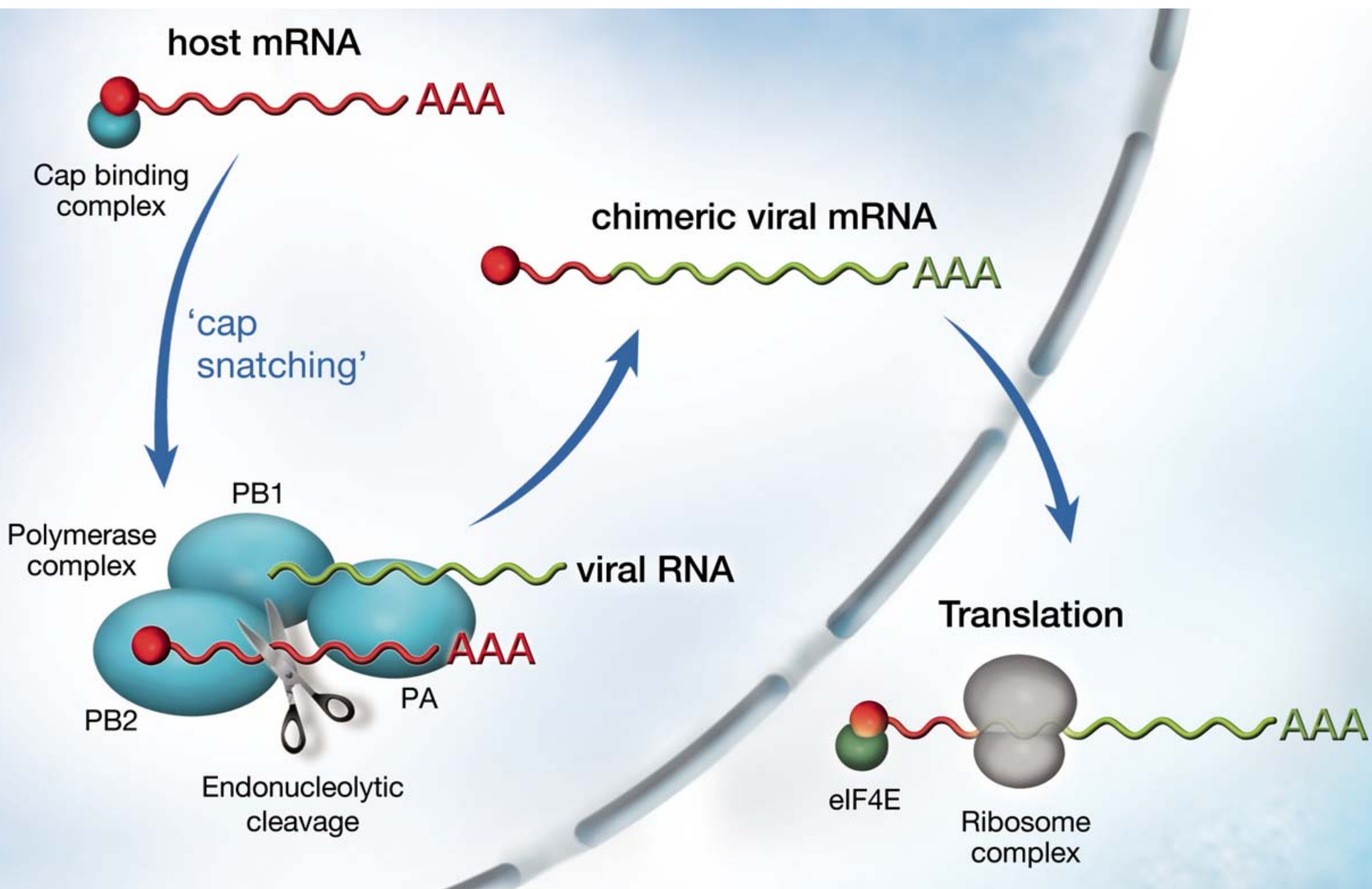
But drugs are not the only imperative. A number of the mutations that enable bird flu to adapt to life in a human host occur within its polymerase protein, and finding out what they do will be crucial for understanding how influenza leaps between species. This is no easy task, and demands a wide range of expertise. Stephen and Darren, together with colleagues from the Unit of Virus Host Cell Interactions in Grenoble, have joined other European teams in Madrid, Lyon, London and Marburg as part of a European Union initiative called FLUPOL, formed to meet this challenge. "We have a multidisciplinary approach to studying this enzyme, from bioinformatics via structure to mouse models of interspecies transmission," says Stephen.

"If you can inhibit cap-snatching, you can greatly reduce the severity of an influenza infection and allow the body to better fight the virus."

The polymerase, however, does not yield its secrets easily. For many years, it was impossible to get any detailed information on its three-dimensional structure. To do this, researchers needed to produce the proteins that make up the polymerase in a soluble form and get them to form crystals. By shining X-rays on the crystals, they would be able to deduce the atomic structures of these proteins and better understand how they worked.

No one, however, had managed to get the all-important crystals. Whenever they tried, researchers found that either the proteins formed useless, insoluble clumps, or they could not produce enough protein to work on. Frustratingly, the researchers were unable to resort to their usual strategy for solving this problem: chopping the proteins up into their individual working parts, or domains, in the hope that these might be soluble. This method relies on being able to identify domains in advance, something biologists usually do by comparing the amino acid sequence of the target protein with those of other proteins whose structures are known. But as well as being painstakingly slow, this approach did not work with the flu polymerase, because it is unlike any other known protein.

That all changed thanks to a new automated system, invented by Darren, that allows scientists to screen all the possible fragments of a protein for suitable soluble domains quickly and easily. The system, called ESPRIT (Expression of Soluble Proteins by Random Incremental Truncation), is set to revolutionise the study of previously intractable proteins. Only last year, Darren and Stephen used ESPRIT to obtain the first soluble fragment of PB2, one of the three proteins, or subunits, that unite to form influenza's polymerase. Having determined the structure



The RNA of the influenza virus steals the cap of host mRNA with the help of protein PB2, which makes part of the polymerase complex. With the cap attached, the viral RNA can be translated into proteins by ribosomes in the cytoplasm and more copies of the virus are produced.

of this fragment, they discovered how it helps the subunit to hitchhike into the cell's nucleus, where it assembles with the other two subunits to form the functional polymerase.

Stephen and Darren were particularly interested in another region of PB2 which plays a key role in stealing an important tag from host cell RNA molecules to direct the protein production machinery towards the synthesis of viral proteins. Without this password, the virus would be unable to reproduce itself. The password relates to the way cells turn instructions encoded in genes into proteins. In the nucleus, the correct stretch of DNA is selected and a copy made of it using a chemical relative of DNA called RNA. At the beginning of the copy, a short extra piece of RNA – actually a modified RNA base, called a cap – is added. The copy is then sent out of the nucleus to the cell's protein-synthesising centres, where machines called ribosomes read the so-called messenger RNA (mRNA) and make the correct protein. The cap directs the ribosome to the start of the mRNA.

The flu virus must ensure that the mRNAs made by its polymerase, coding for viral proteins, also have caps on. This is where PB2's hacking talents come into play. The PB2 subunit binds to host cell mRNAs via the cap and then the PB1 subunit cuts the cap off and adds it to the beginning of the viral mRNA – a process called 'cap snatching', first described nearly 30 years ago. The capped viral mRNA can then be recognised by the host cell machinery allowing viral proteins to be made at the expense of host cell proteins.

Without a cap, an mRNA cannot be turned into protein. So drugs that block PB2's cap-binding activity could stop the flu virus reproducing. Until now, however, little was known about how PB2 worked. "This has been studied for many years but no one was sure exactly where the cap binding domain was," says Stephen.

Thanks to ESPRIT, Darren, Stephen and colleagues found a PB2 fragment that bound to the cap. With the help of the high-throughput crystallisation facility at EMBL Grenoble run by team leader Josan Marquez, and the

intense synchrotron X-ray beamlines at the European Synchrotron Radiation Facility in Grenoble, they determined the structure of the PB2 cap-binding domain bound to cap. This revealed that the domain contained a structure never seen before. “It’s completely unlike any other protein in its fold,” says Stephen.

But although the structure was entirely new, the way the domain interacted with the cap uses the same basic idea as observed with other cap-binding proteins: the central interaction is a sandwich with two PB2 amino acids stacking either side of the cap. “It’s a nice example of convergent evolution, where proteins from completely different origins come up with the same solution,” says Darren.

To prove that their fragment was indeed the culprit cap-stealer, Darren and Stephen created fragments containing mutations of amino acids in contact with the cap. Sure enough, most of these mutations stopped the fragment from interacting with the cap. What’s more, as shown by FLUPOL collaborators at the Centro Nacional de Biotecnologia in Madrid, the same mutations blocked the ability of the whole polymerase to make new viruses. “We were able to display the critical importance of this for the virus as a whole,” says Stephen.

Now that the structure is known, scientists can use it to design relatively simple, small molecules that block PB2’s action. “If you can inhibit cap-snatching, you can greatly reduce the severity of an influenza infection and allow the body to better fight the virus,” says Stephen. He and Darren are now gearing up to look for potential drugs that do just that, with the help of EMBLEM, EMBL’s technology transfer company. As well as using the structure to actively design an inhibitor, they are exploiting their ability to produce large amounts of the PB2 fragment to screen extensive chemical compound collections for new leads, with the help of Joe Lewis, Head of the Chemical Biology Core Facility at EMBL Heidelberg. “We are the first people to be able to make enough of this to take this brute force approach for identifying small molecule inhibitors,” says Darren.

The two researchers are also in the process of using ESPRIT to crack the rest of the flu polymerase’s structure and hope that, in the near future, they will uncover new ways of countering influenza’s dirty tricks.

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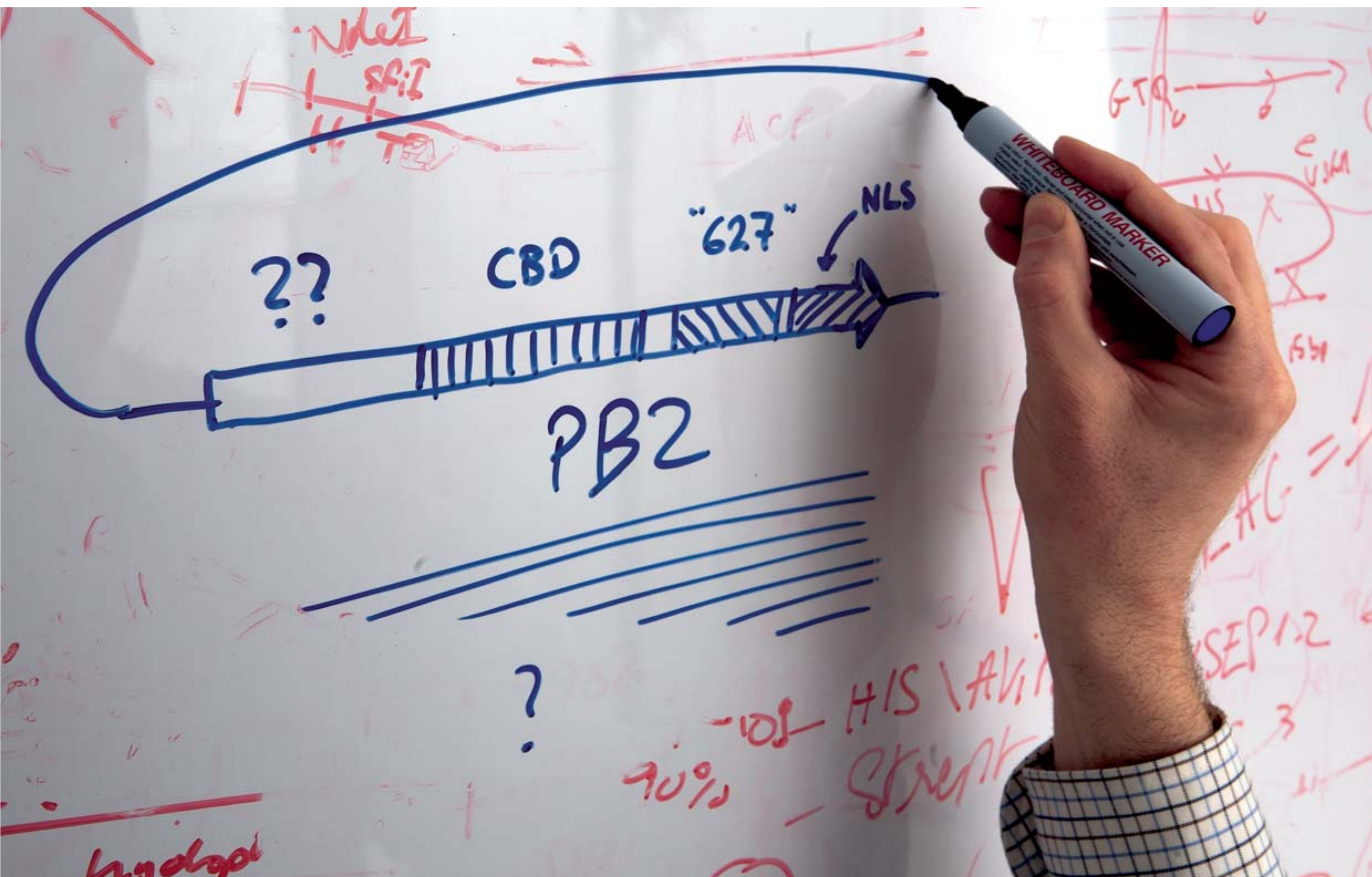
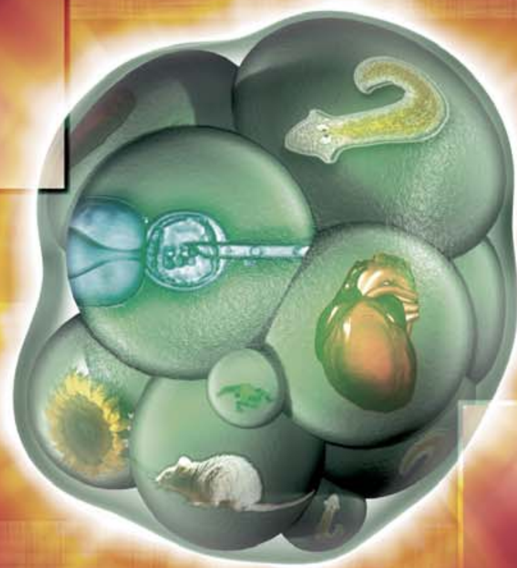
Darren explains the genetic structure of the influenza virus protein PB2.





Demystifying stem cells

ENTHUSIASTICALLY CELEBRATED as a miracle cure and at the same time condemned as an assault against human rights and life in general, stem cell research is clearly one of the most controversial topics of new millennium biomedicine. In the past ten years, no other scientific issue has been so constantly at the centre of the public eye or has stirred bigger debates in the media and governments and on the street. Scientific breakthroughs share the front pages of newspapers with reports about scandals, fraud and false promises. But while everybody is busy trying to decide if stem cell research is a blessing or a curse for modern science and society, many people do not know much about stem cell science. What are stem cells? How are, can and should they be used in research and medicine? And what is their true potential?

"Stem cell research is essentially the study of all life. It is about solving the mystery of how one cell can give rise to a whole organism. It is incredibly exciting," says Nadia Rosenthal, Head of EMBL's Mouse Biology Unit in Monterotondo. Like several other research groups at EMBL, her team is working with mouse stem cells. Fascinated by their unique capacities and their role in regeneration and tissue repair, Nadia has advanced to become a spokesperson for stem cell research over the recent past. In 2006 she delivered the prestigious Howard Hughes Medical Institute Lectures on stem cells, cloning and regeneration together with scientist colleague Douglas Melton from Harvard University.

What makes a stem cell special is its unique ability to self-renew and at the same time give rise to a variety of specialised cell types. When normal cells divide, they produce two identical daughter cells. Stem cells, on the other hand, can divide asymmetrically. They produce one daughter that is an exact copy of the mother cell, but the other daughter cell undergoes a maturation process called differentiation and acquires specific properties and functions. This process is crucial during embryonic development, in which a single fertilised egg cell generates a whole organism consisting of hundreds of different cell types. But adults also have stem cell pools.

"It is important to realise that not all stem cells are the same. In the public debate embryonic and adult stem cells are often lumped together, but biologically they are very different," explains Nadia.

Embryonic stem cells are taken from developing embryos when they are still a ball of dividing cells. At these early developmental stages, stem cells are pluripotent. This means they have the ability to generate every specialised tissue of an organism, ranging from bone and skin to muscle, nerve and blood.

Mathias Treier, group leader in the Developmental Biology Unit of EMBL Heidelberg, investigates which molecular factors bring about this extraordinary ability of embryonic stem cells in mice. With the help of genetic engineering he systematically removes different components from the cells to observe which ones are essential for a stem cell to remain undifferentiated. One crucial molecule has already surfaced in these experiments: a transcription factor called Sall4. Without this important

regulatory protein, embryonic stem cells can no longer self-renew indefinitely.

Over the course of development, stem cells gradually lose their pluripotency and become more specialised and so adult stem cells can give rise only to a limited range of cell types. A haematopoietic stem cell, for example, can generate red blood cells, white blood cells, platelets and all the other cell types that constitute blood, but it is not normally programmed to form a nerve or a kidney cell. It is multipotent as opposed to pluripotent. Unlike embryonic stem cells, adult stem cells can be extracted without harm from adult organisms and are thus not subject to the same ethical concerns. They also fulfil a different purpose than their embryonic counterparts. Rather than tissue generation they are responsible for tissue maintenance and regeneration. Adult stem cells are recruited as a self-repair kit in response to injury, inflammation or degenerative disease. They replenish dying cells and ensure the integrity and function of vital tissues and organs.

Nadia and her team are trying to find ways to enhance this inherent regenerative capacity of the body. "Stem cells are not standalone agents, they always act in the context of a specific environment. It is the environment of a tissue that instructs a stem cell with the help of molecular signals to take on a given identity," Nadia explains. Her goal is to modify the molecular signals that influence the behaviour of adult stem cells in mice in order to improve tissue healing. The strategy has proven successful: the team has identified a molecule, called IGF-1, that, when overexpressed in skeletal and heart muscle, makes these normally slowly regenerating tissues heal better and faster by mobilising local stem cell populations.

There are many other ways in which scientists are trying to harness the enormous therapeutic potential of stem cells. Alzheimer's and Parkinson's diseases, muscle dystrophy and heart attacks are conditions either caused by or involving tissue degeneration. Injecting pluripotent or multipotent cells into degenerating tissues or organs, where they divide, differentiate and replenish damaged cells, might help slow down the progression of these diseases or even cure them.

Mathias is currently investigating if stem cell transplants could be used to treat a degenerative kidney disease called nephronophtisis. His team has generated a mouse model of the condition in which a mutation causes kidney cells to die, leading to renal failure. They are now testing if the injection of embryonic stem cells can help regenerate parts of the organ and restore its function (see page 103).

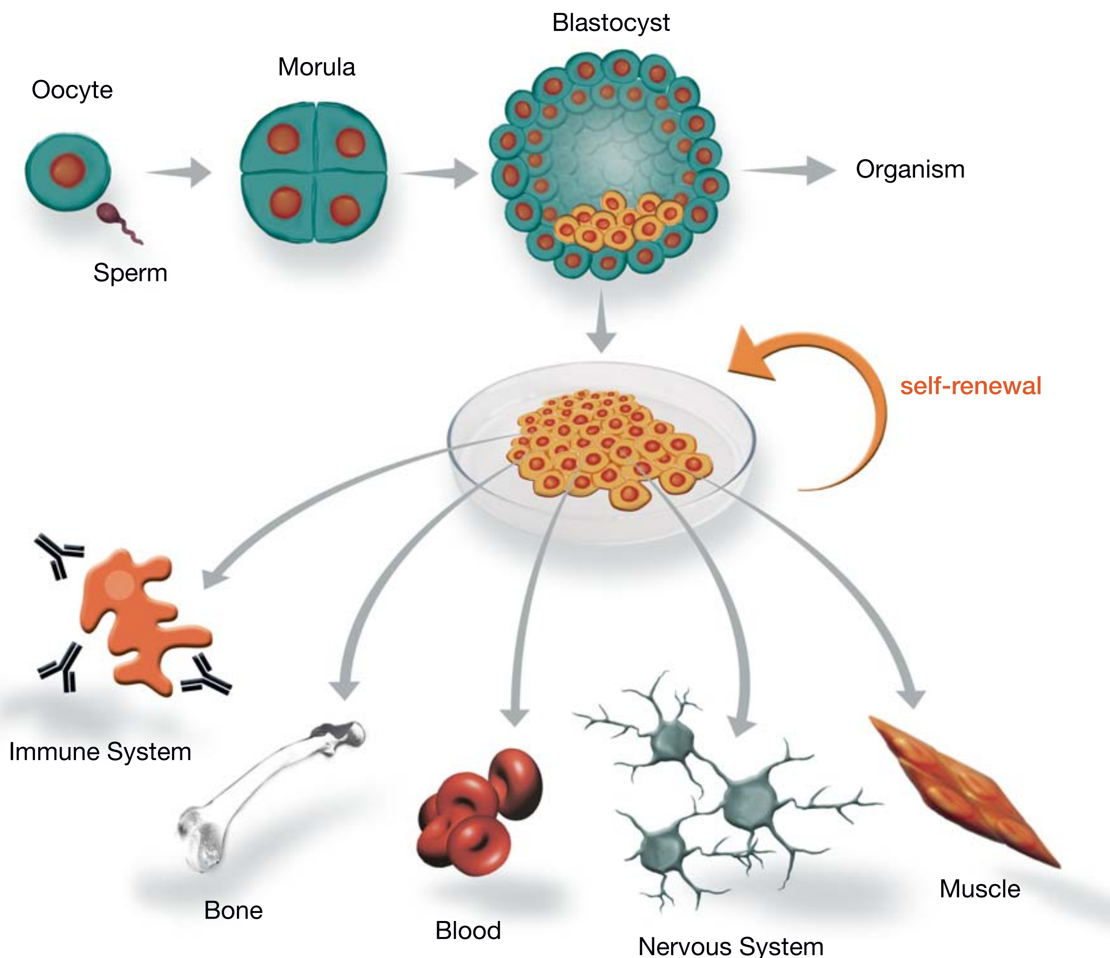
In some cases, stem cell therapy works very well and is already routinely applied in hospitals. Bone marrow transplants are an example. In other cases, however, scientists have not yet figured out how to make best use of the powerful potential of stem cells and how to overcome some of the involved risks and limitations. One such limitation is the high chance that the immune system of a patient rejects stem cells taken from another individual.

A promising new alternative that circumvents this problem is offered by induced-pluripotent stem cells (IPSC). In 2006 a group of scientists led by Shinya Yamanaka at the University of Kyoto, Japan, found a way to genetically reprogram an ordinary mouse skin cell to revert to the virtual equivalent of its embryonic state. All it took to persuade a differentiated adult cell to regress was the induction of 4 genes; four genes that might give doctors the ability to replace cells destroyed by disease or injury with a patient's own cells without fear of immune-system rejection.

"What we shouldn't forget, however, is that these cells now have four genes that don't belong there and we don't know yet what they do to the cell. A lot more research will be necessary before IPSCs can be considered for therapeutic purposes," Nadia says, sounding a note of caution.

But therapies based on natural stem cells without genetic alterations also have their caveats. It will be challenging to find ways to restrict a stem cell's differentiation to only one or a few cell types to make sure that when injected into the brain a stem cell turns into a neuron and does not form to a tooth, for example. Another risk that needs to be addressed before stem cells can be safely applied as therapeutics is their involvement in cancer. One mutation can be enough to turn a healthy stem cell into a cancer stem cell. Cancer stem cells divide uncontrollably, produce dysfunctional daughter cells and outcompete normal stem cells. They form proliferating tumours that displace healthy, functional tissue.

Claus Nerlov, group leader at EMBL Monterotondo, studies cancer stem cells in the haematopoietic system of mice.



Embryonic stem cells taken from embryos at the blastocyst stage are pluripotent. When cultured in a dish, they self-renew and can develop into any cell type found in the body.

Such blood cancer stem cells cause leukaemia by giving rise to large numbers of defective blood cells that displace the healthy blood and impair the transportation of oxygen around the body.

Claus has recently discovered that, unlike previously assumed, mutated haematopoietic stem cells are not the only possible culprits to cause leukaemia. Differentiated or partially differentiated cells that have lost the capacity to self-renew can also be turned into cancer stem cells by mutation. For example, partially differentiated white blood cells of the myeloid lineage become malignant when they acquire a mutation in a gene called C/EBPα. The consequence is a condition called acute myeloid leukaemia. The researchers are now aiming to discover the underlying molecular mechanisms. "Knowing the molecules that allow a differentiated cell to self-renew will open up new avenues for treating leukaemia and other cancers," says Claus. He also pursues this goal on a bigger scale as the coordinator of EuroCSC, a European Union-funded consortium of five research institutes and a biotech company dedicated to identifying drug targets in cancer stem cells.

Nadia, Mathias and Claus are not the only EMBL scientists working with stem cells, but their projects suffice to convey an idea of the diversity of the field. Not all stem cells are the same and raise the same moral concerns and not all claims made about their therapeutic potential are hype or fraud. And even if stem cells do not turn out to be the omnipotent miracle cure everybody is hoping for, they are and will remain one of biology's most fascinating subjects. Carried out in an ethically justifiable manner and by scientists acting in all conscience, stem cell research could undoubtedly grant us remarkable new insights into the biology of development, health and disease.

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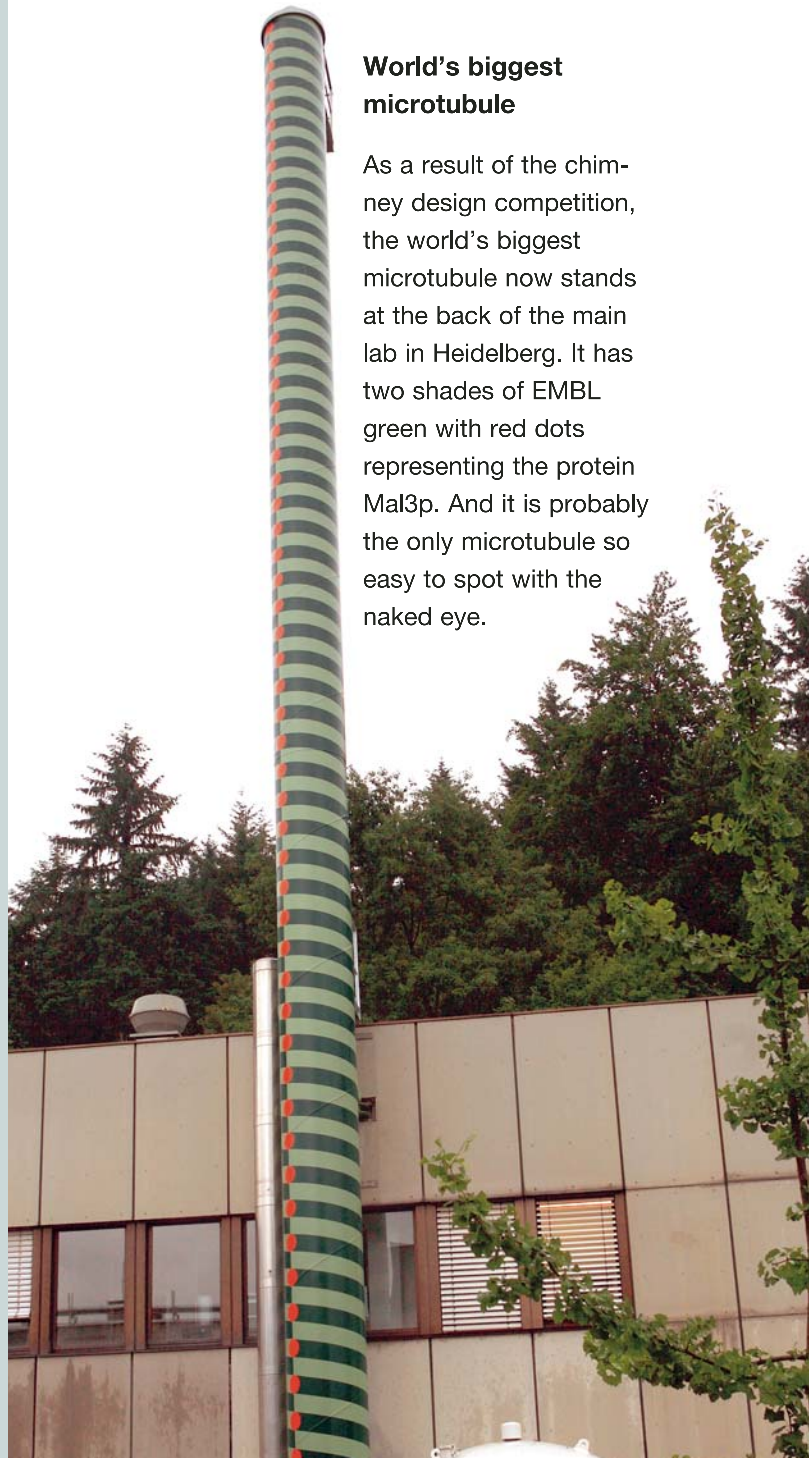
A Year in the Life of EMBL

May

Breaking through the glass ceiling

Things are getting better, but gender imbalance is still a major problem in science, especially in top-level positions. What can be done? This question was the focus of a two-and-a-half day conference, 'Women in Science: The Way Forward', held at EMBL Heidelberg on 9-11 May. More than 270 participants attended the event with contributions from scientists, psychologists, representatives of renowned institutions and grant agencies. The conference was organised as part of the EU project SET-Routes, a collaboration between EMBL, CERN and EMBO to encourage more girls to pursue a career in science, engineering and technology (SET).



World's biggest microtubule

As a result of the chimney design competition, the world's biggest microtubule now stands at the back of the main lab in Heidelberg. It has two shades of EMBL green with red dots representing the protein Mal3p. And it is probably the only microtubule so easy to spot with the naked eye.

2007

June

EMBL Hamburg in the EU spotlight

EMBL Hamburg was among the hosts of the EU's annual European Conference on Research Infrastructures (ECRI 2007) on 5-6 June. Several ministers, including EU commissioner Janez Potočnik, heard talks by EMBL DG Iain Mattaj and EMBL-EBI Director Janet Thornton at the meeting which took place on the DESY campus. The ECRI meeting aims to provide valuable feedback for FP7 and Europe's 'roadmap' of research infrastructures.

The origins of speech behaviour

The first ever EMBL Science and Society Symposium organised jointly with EMBL-EBI, 'Biology and Language', brought geneticists and cognitive scientists together for some unusual and thought-provoking talks. The afternoon-long symposium was held at Robinson College in Cambridge, UK, and focused on the evolution of language, the brain processes underlying language, and biolinguistics, among other topics. The attendees particularly praised the way the various fields were presented coherently to a mixed audience of scientists and non-scientists.

Scientific cross-over

The first call for applications for EMBL's new Interdisciplinary Postdoc (EIPOD) positions was issued in June. EIPOD projects bring together scientific fields that are usually separate or that transfer techniques to a novel context. After the 2007 selection round, which attracted 124 applicants, EMBL selected 15 EIPODs supported by at least two labs from different units. With the new initiative, EMBL underlines its strong commitment to promoting interdisciplinary research.

July

EMBL advertises itself

EMBL presented its research profile to the international gathering of scientists, policy makers, journalists and members of the public at the 32nd annual Federation of European Biochemical Societies (FEBS) congress, held in Vienna on 7-12 July. The FEBS congress was a great opportunity to deepen the knowledge of congress visitors about EMBL, to recruit scientists and to seek new collaborations. Scientists from Europe, the USA and Asia stopped by at the EMBL stand to find out more about open positions, scientific training possibilities and the visitors programme.

EMBL expands to Australia

In July, council delegates representing EMBL offered Australia associate membership in its international community. Through its associate membership, Australia will contribute to the diverse activities at EMBL by sending early-career scientists to join EMBL as faculty and postdoctoral and predoctoral fellows, while EMBL will share with Australian institutions its renowned expertise in research, training and research infrastructure development. The membership officially started in March 2008 and will initially last for seven years.

EMBL Summer Party

Scorching temperatures didn't keep the guests away from the Staff Association Summer Party 2007 at EMBL Heidelberg. Around one thousand party attendees enjoyed the delicious food, cocktails and rich entertainment programme including attractions for children, live bands and DJ music until the small hours. A record sum of 3276 euros was raised during the tombola for the Waldpiraten camp – a nearby facility for children suffering from cancer.

2007

August

New training facilities

The brand new IT training suite at EMBL-EBI, with 40 permanent workstations and the option to double the room's capacity for an additional 40 laptop-based users, was inaugurated in August. The emphasis of the training provided at EMBL-EBI is to equip researchers with the bioinformatics knowledge they need to identify the relevant resources for their areas of work. The training programme started in September with an average of one course per month.

EMBL busy at the BSR meeting

Exhibiting at the 9th International Conference on Biology and Synchrotron Radiation on 13-17 August in Manchester, EMBL had the biggest, busiest stand ever with extra sections devoted to the EU projects Saxier and BIOXHIT, as well as the general display. Members of the structural biology community from all over the world dropped by to update themselves on, among other things, the PETRA III project at EMBL Hamburg.

September

Science and socialising

Barcelona, the beautiful Catalonian capital, was the predocs' choice for their annual retreat this year. The three days in September were filled with sightseeing, socialising and, above all, science. As always, the purpose of the retreat for the 50 attending students was to exchange ideas, collect feedback on their work and identify overlapping interests and ways to collaborate.

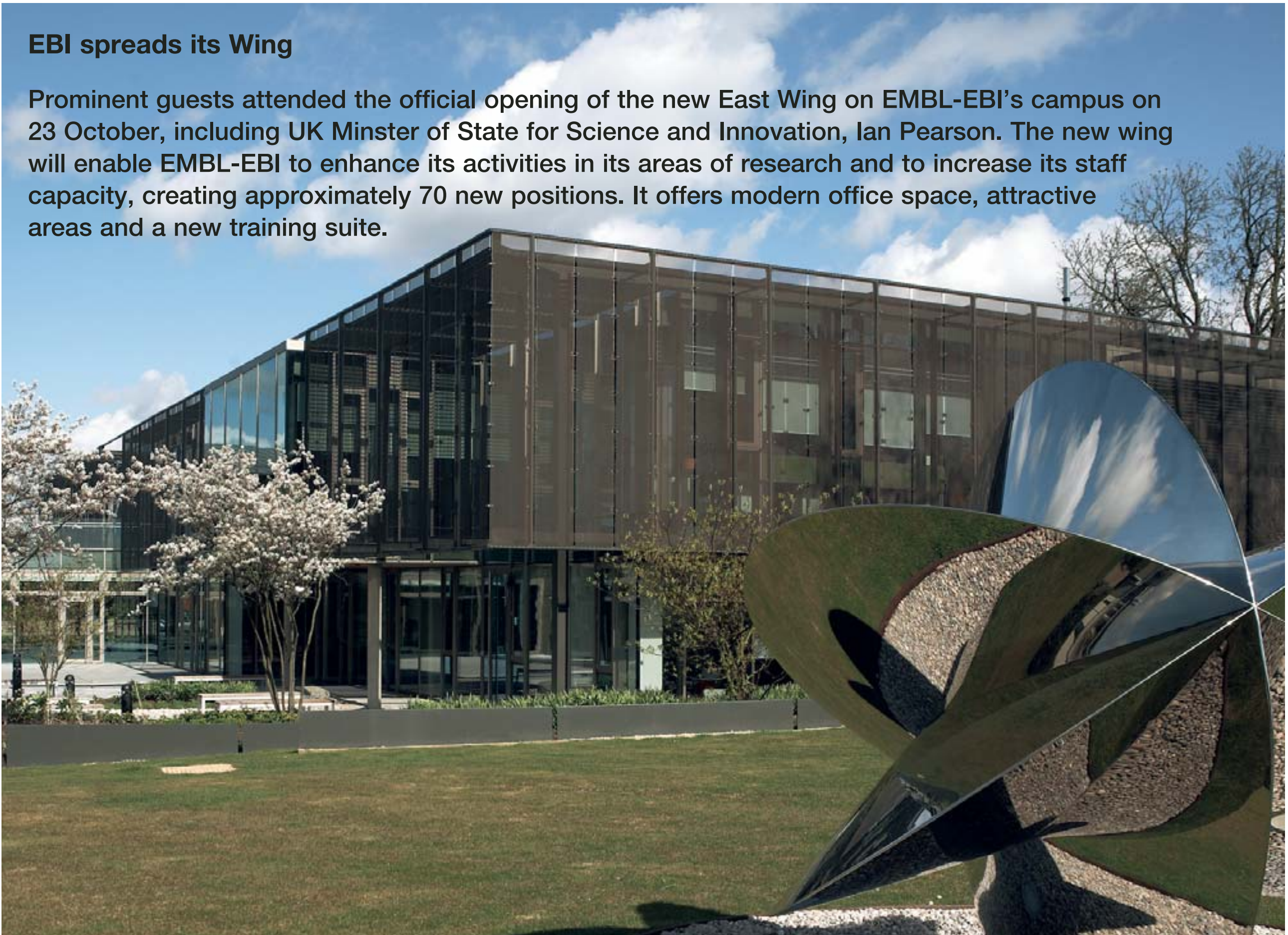
EMBL's old and new faces in Dresden

EMBL presented its activities with a stand at the 2007 ELSO meeting in Dresden. Visitors to the EMBL stand were interested mainly in job opportunities and information about its visitors' programme. The meeting was also an occasion for a get-together of EMBL staff and alumni. The event was very well attended, attracting alumni from all ranks, including former EMBL DG Lennart Philipson.

Faculty retreat in Tivoli

This year, faculty members old and new chose sunny Italy for their annual retreat to exchange ideas and hear about each other's activities. The meeting took place in the Grand Hotel Duca D'Este in Tivoli, near Rome, on 19-20 September. The ten new group and team leaders who had joined the five EMBL sites just prior to the retreat also used the meeting to present their projects and mingle with the old hands.

October



EBI spreads its Wing

Prominent guests attended the official opening of the new East Wing on EMBL-EBI's campus on 23 October, including UK Minster of State for Science and Innovation, Ian Pearson. The new wing will enable EMBL-EBI to enhance its activities in its areas of research and to increase its staff capacity, creating approximately 70 new positions. It offers modern office space, attractive areas and a new training suite.

First self-run ELLS event in Monterotondo

More than 60 teachers from all over Italy applied to take part in the LearningLAB 'Benefici e rischi delle nuove tecnologie applicate al DNA', the first course organised by the European Learning Laboratory for the Life Sciences (ELLS) in Monterotondo. During the three-day course, 23 selected participants got an overview of the newest molecular biology techniques and some hands-on experience with isolating, modifying and expressing genes. The course was a promising debut for ELLS activities in Monterotondo, with huge enthusiasm from both the attending teachers and the speakers.

Predocs' choice

Repetitive biological phenomena from the molecular to the macroscopic scale were the focus of the 9th International EMBL PhD Student Symposium, 'Patterns in Biology: Organisation of Life in Space and Time' on 25-27 October at EMBL Heidelberg. During the symposium, 18 leading scientists offered insights into the 'organisation of life in space and time' to participants from over 40 countries, among them chemists, physicists and physicians.

Attracting young talent

As it does every year, EMBL's ELLS participated in Germany's biggest science festival, Science Days 2007. Twenty thousand visitors flocked to the event at the Europa-Park in Rust, near Freiburg. With the help of six pupils from the Bertha-von-Suttner school in Ettlingen, ELLS ran a variety of hands-on activities about DNA and protein. Hoards of young visitors jumped at the chance to be like real scientists, extracting DNA from bananas or protein from nuts.

2007

November

EMBL doubles its initial size

Accepted by EMBL's council and ratified by the parliament of Luxembourg, the Grand-Duchy has officially joined EMBL as the 20th member state in November 2007. As a member state, Luxembourg will benefit from the various services and training programmes that EMBL offers and will have access to research facilities at the five EMBL outstations. By accepting Luxembourg, EMBL doubled its size from its initial membership of ten states in 1974.

PETRA III gets her top on

On a freezing 26 November, DESY, EMBL Hamburg and a select group of VIPs braved the cold to witness the 'topping out' of the experimental hall of PETRA III, Hamburg's new source of brilliant X-ray light. The traditional Richtfest welcomed German Research Minister Dr Annette Schavan and Hamburg's First Mayor Ole von Beust to the DESY campus. The spectacular synchrotron radiation facility opens in 2010/2011.

A night full of experiments

Isolating DNA from bananas and folding coloured paper into an origami double helix were only two activities that EMBL offered on its stand during the first Long Night of Sciences in Heidelberg on 10 November. The response was overwhelming. Several hundreds of people stopped at the EMBL stand to puree the banana, assemble colourful bracelets and ask countless questions about the function of DNA, amino acids and the transcription process. The experiments went on until almost 2 am.

January

Putting out feelers

In a trip to Turkey at the beginning of the year, EMBL DG Iain Mattaj came a step closer to securing the country's official involvement with EMBL. His visit to Izmir included meetings with representatives from Dokuz Eylül University in Izmir and the Sabanci and Bogaziçi universities in Istanbul. The group discussed potential research and training collaborations between Turkish scientists and EMBL.

Physics meets biology

A workshop on Physical Biology on 17-18 January was the result of EMBL teaming up with a group of European laboratories to promote the interface between physics and biology. Scientists from Dresden and Paris got together with EMBL's François Nédélec at the Physical Biology Circle to organise the informal two-day event, which encouraged students to present and discuss their work with talks and posters, with minimum involvement from senior scientists.

2008

February

Haggis, whisky and more

Guests at the 2008 Burns Night celebration on 16 February had a good time with traditional Scottish neeps, tatties and haggis. George Reid and Joe Lewis (pictured) performed the address to the Haggis and fellow Scot Iain Mattaj donned his nightshirt for a rendition of ‘Holy Wullie’s Prayer’, before the assembled throng was treated to some rousing numbers by the Heidelberg & District Pipes and Drums bagpipe band. After dinner, everyone got up for a spin around the canteen in the ceilidh.

EMBL at the Olympics of science conferences

The EMBL stand attracted many visitors at the annual meeting of the American Association for the Advancement of Science (AAAS) on 14-18 February. It was also situated directly opposite the European Commission’s booth and received a lot of compliments from the European Commissioner for Science and Research, Janez Potočnik, on its presentation. EMBL has also attended several careers fairs in a continuing drive to make itself better known in Europe.

March

EMBL-EBI opens its doors

EMBL-EBI held its annual Masters' Open Day on 3 March in Hinxton. Open Days are designed for bioinformatics-related Masters students from all over Europe to come and learn more about the institute and sample some of its services and research. The day consisted of a series of lectures providing the 83 visitors with an overview of our activities, a brief summary of the EMBL International PhD Programme and presentations focusing on the various research areas. In future, the Masters' Open day will be held in November to synchronise more closely with applications to the EMBL International PhD Programme.

2008

April

Prokofiev instead of PowerPoint

In a second cooperation with the International Music Festival, Heidelberger Frühling, EMBL Heidelberg hosted the violinist Arabella Steinbacher and pianist Robert Kulek on 9 April with a recital comprising works by Ravel, Prokofiev, Fauré and Poulenc. The concert was initiated by EMBL's Klaus Scheffzek. More than 200 music lovers came to EMBL's Operon to listen to the prize-winning young artist.

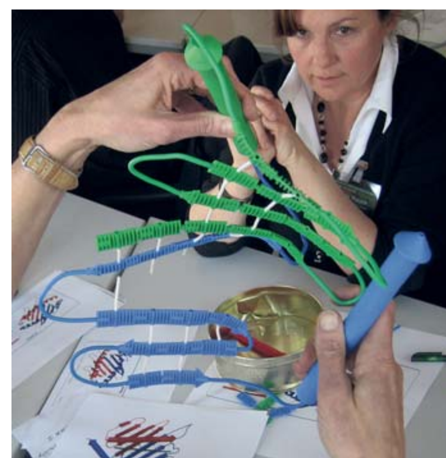
City council inspects ATC

Heidelberg's Oberbürgermeister (Mayor), Dr Eckart Würzner, and some representatives of the city council visited EMBL on 27 March to learn about the lab and the progress at the Advanced Training Centre (ATC). Iain Mattaj outlined the current status of EMBL, and the city council was given a tour of the institute.

Girls take the initiative

Twenty girls and one boy between the ages of 10 and 16 came to EMBL Heidelberg on 24 April to get an insight into what it is like to work in a scientific institute. The young visitors spent time in different EMBL sections, where they helped with experiments in the labs, processed pictures in the Photo Lab, or prepared salads in the canteen. The initiative was part of Girls' Day, a Germany-wide action to discover professions in which women are currently underrepresented.

Train the teacher



An enthusiastic group of 17 high-school teachers from Austria, Belgium, Germany and Sweden took part in the first ELLS LearningLAB ever held in Hamburg. The course, entitled 'Structural Biology: Deciphering the Chemistry of Life', included lectures by EMBL structural biologists and a scientist from Bayer-Schering Pharma who gave a seminar on the use of structural biology in the drug-design process.

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Annual Report 2007-2008

EMBL

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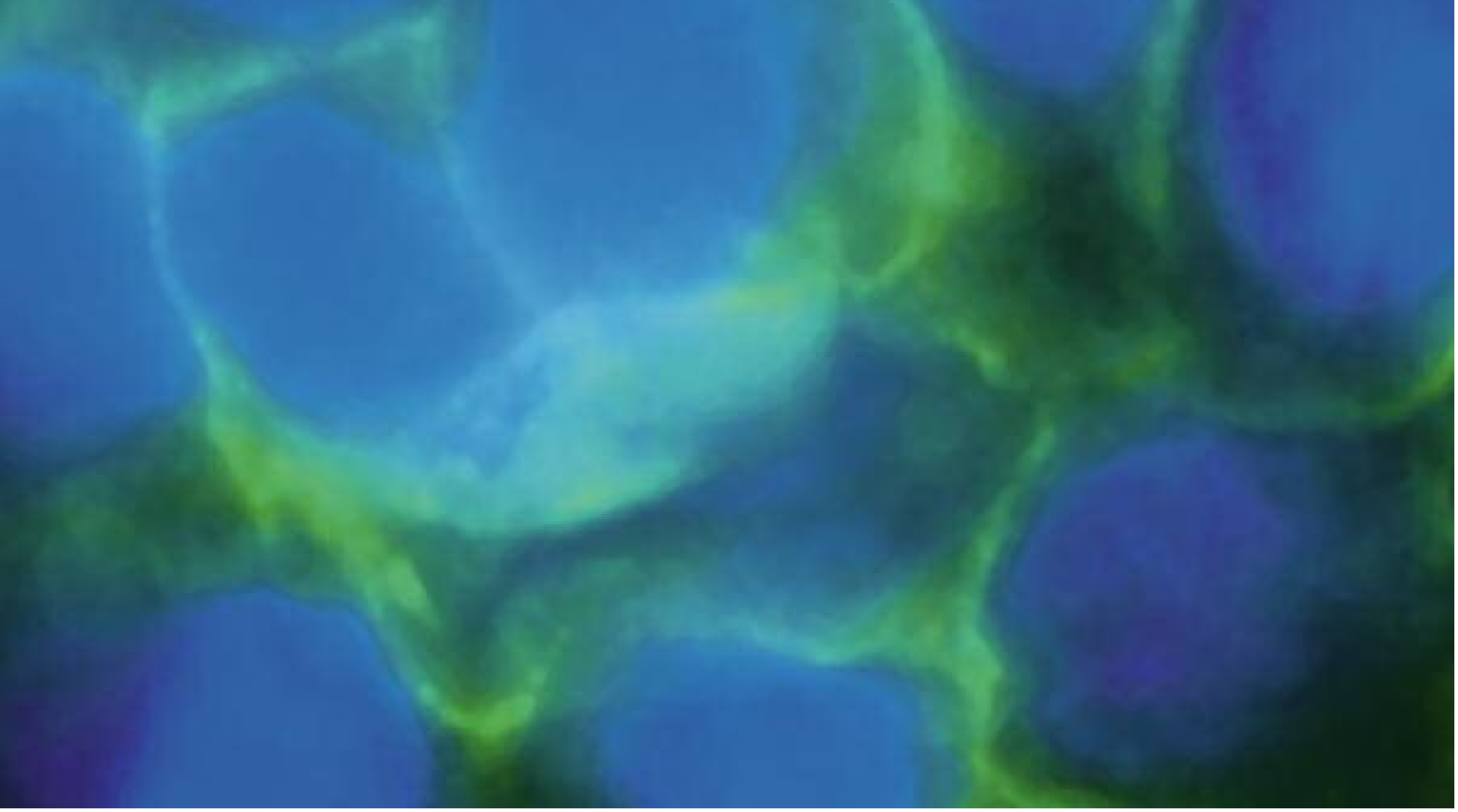

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