

Annual Report 2006-2007





Annual Report 2006-2007

European Molecular Biology Laboratory

Contents

The Director General's Report iv

Foreword
Implementation of EMBL Scientific Programme 2007-2011
State of the Laboratory
Integration of European research
Personnel and financial statistics
Reviews of EMBL Scientific Units

Scientific Report

Networking at all levels	2
The big picture	4
Reading the omens	9
Forging new paths	10
The EB-eye's guide to life	14
Word miners strike gold	17
At the core	18
Gaps in the record	20
Putting science under the microscope	26
Scientists and ethicists minding the brain	30
Bridging the scales	34
A guided tour of systems biology	36
Beauty in symmetry	40
The house guest that stayed	44
Joining the dots	50
Pump it up	54
At the tips of the tree	58
Doing the twist	59

A window on the cell	60
Views on moving molecules	62
A living screen success	65
A wandering eye	66
Pay attention at the back	71
Ingenious cell dynamics	72
Roadworks on the motorways of the cell	73
The great migration	74
An architectural plan of the cell	79
Close encounters	80
An early mover	84
A switch between life and death	85
A delicate balancing act	86
Structuring the bigger picture	90
All for one	92
Locking the cradle	96
Fighting an old enemy	100
It takes two	104
Putting a virus under stress	105
In the land of the blind	106
Setting a trap	111
Shaping up for the future	112
On the fringe	
Caught in the act	116
	116 119
Corsairs of the cell	116 119 120
Corsairs of the cell	116 119 120 124

A year in the life of EMBL 128

Index	141
Acknowledgments	142



Y SECOND YEAR AS DIRECTOR GENERAL was, for EMBL, a very eventful and successful one. After many discussions and negotiations throughout the year the Indicative (Funding) Scheme for 2007-2011 was approved by the member states at the Winter Council meeting in November 2006.

By the end of its five-year cycle the Indicative Scheme will provide a 30% increase in support to EMBL. At the same time we obtained additional support for developing three major building projects: the Advanced Training Centre in Heidelberg, the PETRA-III beamlines in Hamburg and the EMBL-EBI extension in Hinxton. I consider this as a measure of both the past success of the Laboratory and the very positive attitude within the member states towards its new programme.

I wish to express my gratitude to the member states for this vote of confidence in EMBL and to the many people within EMBL who supported me throughout the year for the benefit of the Laboratory as a whole. I am convinced that EMBL will repay this confidence with continued excellent performance.

At the end of the year came the news that Frank Gannon, Executive Director of EMBO, had decided to leave EMBO during 2007. Frank not only was an exemplary and energetic leader of EMBO during his term, but also served as Scientific Coordinator of EMBL during his first years in Heidelberg and ran a very successful laboratory that was loosely associated with the Gene Expression Unit. Throughout his time at EMBO he served on the EMBL Senior Scientists Committee. I want to take this opportunity to thank Frank for the major contribution he made to the life of the Laboratory, and to wish him well in his new position.

What will be the main focus of our research in the coming years?

We will continue to work in areas in which EMBL has traditionally been successful while adding other disciplines to move into the growing field of systems biology. All five EMBL sites put considerable emphasis on interdisciplinarity and collaboration. Together with our long-standing record in training, we feel we are strongly positioned for the future. To find out more about the highlights of last year's research activities at EMBL, you are welcome to read this report.

EMBL is a major research infrastructure for the life sciences in Europe. A large scientific community uses our services in bioinformatics and structural biology every year. Improving the support mechanisms for the European Bioinformatics Institute is a top priority. New beamlines at our structural biology facilities in Hamburg will provide our user community with state-of-the-art synchrotron radiation in 2010.

You will find more detail on many of the topics touched on here in the remainder of this report, together with a summary of many of the EMBL activities over the past year. You will see that the Laboratory is as interesting and productive as ever.

Enjoy!

Jan What

Iain W. Mattaj Director General

Implementation of EMBL Scientific Programme 2007-2011

Over the next five years, we will build on our existing strength in the traditional areas of molecular biology to move into the growing field of systems biology. This will require increasing our scientific scope to incorporate new disciplines and interdisciplinary approaches, for which we will need to hire more physicists, mathematicians and chemists and provide substantially improved IT support. EMBL's strong tradition of collaboration and interdisciplinarity means that it is in a very good position to increase its activity in this area.

We recently reviewed the EMBL Centres, which were set up to increase collaboration between units. They have been a great success in bringing people with common interests together from many parts of EMBL and we will continue to support these networking and training initiatives. But fostering collaboration across the Laboratory will not be limited to specific centres. We will encourage pre- and postdoctoral fellows to work on inter-unit projects, preferably across different EMBL sites.

Many basic research projects now have links to human health and over the next few years, we will continue to see molecular biology grow ever closer to medicine. The Centre for Disease Mechanisms will promote links between EMBL and clinical research centres, as exemplified by the collaboration with the Magdi Yacoub Institute in Harefield, UK, and the newly established Nordic EMBL Partnership for Molecular Medicine. This will help us to maximise the impact of our groundbreaking results in basic research in this vital area.

The European Bioinformatics Institute (EMBL-EBI) is the main provider of bioinformatics services in Europe and has been recognised as an essential research infrastruc-

EMBL Scientific Publications and Collaborations 2006

- Total number of peer-reviewed publications: 437
- Internal collaborations: publications co-authored by more than one EMBL Group leader: 50
- External collaborations: **734** in total of which **75** resulted in a publication

ture. Its funding, however, has always been difficult: only half of its budget currently comes from the EMBL member states, with the other half from external grants. The EMBL Council has recognised the importance of EMBL-EBI and, over the next five years, most of the additional funding that EMBL receives from its member states will be used to expand EMBL-EBI's staff from its current total of 300 to 400 by 2011 and to substantially improve the computational infrastructure on which EMBL-EBI services depend. Over the same period, the proportion of funding supplied to EMBL-EBI by the EMBL member states will be increased from 50% to more than 60% to provide more stability. Growth at EMBL-EBI is needed because of the exponential increase in the quantity of data being generated in many fields of the molecular life sciences, the emergence of new types of data and an evergrowing need for data integration.

Structural biology services provided by the Outstations in Grenoble and Hamburg are the second main area of service provision by EMBL. Together with our local partners, the Deutsches Elektronen-Synchrotron (DESY) in Hamburg and the European Synchrotron Radiation Facility (ESRF) in Grenoble, we are the main providers of synchrotron radiation in Europe used to solve biomolecular structures at high resolution. The construction of new beamlines at the PETRA-III source in Hamburg is the largest single project in this area for the next five years.

EMBL has a long-standing tradition of organising excellent courses, conferences and workshops for the scientific communities at all of its sites, and each year sees an increasing number of applicants. Heidelberg has always been the main site for these activities, but our facilities are no longer appropriate for the number and size of events that we need to organise to remain a leader in this field in Europe. The new Advanced Training Centre (ATC) is made possible thanks to funding from the German government and the Klaus Tschira Foundation and will address this issue and allow EMBL to offer state-of-the-art conference facilities on the Heidelberg campus.

State of the Laboratory

EMBL Heidelberg

Two new Unit coordinators have been appointed: Jan Ellenberg to the Gene Expression Unit and Christoph Müller to the Structural and Computational Biology (SCB) Unit.

Jan Ellenberg has been a Group leader in the Gene Expression Unit since 1999 and holds a joint appointment with the Cell Biology and Biophysics Unit. Jan replaces Elisa Izaurralde, who left EMBL last year to become a Director at the Max-Planck Institute for Developmental Biology in Tübingen, after a period as interim coordinator.

Christoph Müller has been the deputy head of EMBL Grenoble since September 2005. He will share the coordination of the SCB Unit with Peer Bork. Christoph's appointment will help the three sites involved in structural biology – Hamburg, Grenoble and Heidelberg – to work together even more closely. Following advice from the last Scientific Advisory Committee (SAC) review we are planning to acquire a new High Field (800 MHz) NMR spectrometer.

The group of the Associate Director, Matthias Hentze, has moved from the Gene Expression Unit to the Directors' Research Unit.

The coordinator of the Developmental Biology Unit, Stephen Cohen, will leave EMBL later this year and we are in the process of recruiting a new Unit coordinator.

The Chemical Biology Core Facility, which was established in 2004 as a joint venture with the German Cancer Research Centre (DKFZ), has now formed a partnership with the University of Heidelberg. This will allow the core facility to expand its library of compounds and gain access to the University's synthetic chemistry facilities, which is necessary for further development of small molecules as molecular tools or drug precursors.

Advanced Training Centre (ATC)

A groundbreaking ceremony to formally begin the construction of the ATC took place on 6 October in the presence of the German Minister Dr. Annette Schavan, Dr. h.c. Klaus Tschira, the Baden-Württemberg Minister Dr. Peter Frankenberg and Prof. Eero Vuorio, the EMBL Council chair. Thanks in part to the mild winter, the construction site preparations are proceeding well. We have identified a company for general building works and they have started building the ATC foundations. Provided everything goes according to plan, the building will be completed in summer 2009.

Administration

The EMBL administration is preparing for the introduction of a new electronic personnel management system, the SAP HR system, which is due to go live in 2008. It is designed to keep pace with changing requirements in accounting and personnel management practice.



The EMBL Council has agreed to support vocational training for all staff at EMBL, a very important goal for an organisation with our limited job-tenure system. The EMBL International Centre for Advanced Training (EICAT) will take on the responsibility for scientific career development and also work closely with the EMBL Administration to organise non-scientific vocational training courses.

A quarter of EMBL's annual income is raised through grants from European and national funding agencies, about half of which comes from the EU Framework programme. EMBL scientists in general have been extremely



successful in a pplying for external funding and we are currently participating in more than 90 EU-funded projects, of which 17 a re coordinated by EMBL s cientists. This is a major success that allows us not only to do more research and develop our services, but also to collaborate with many scientists across Europe. We have started to reorganise our grants office so that it will be able to offer more support to scientists making applications and better assist them with project management.

European Bioinformatics Institute (EMBL-EBI)

In 2006 the EMBL-EBI's core data resources all grew significantly. For example, the EBI received more than 18 million EMBL-Bank (DNA sequence) entries, including more than 200 new genomes and has, on average, served 293,000 unique hosts per month.

The EMBL-EBI extension building is nearing completion and will be officially inaugurated in autumn this year. It will allow the EBI st aff complement to grow to 400 and will house computer teaching facilities that are currently unavailable on the Wellcome Trust Genome Campus. Late last year, EMBL-EBI re-launched its website. It added a large number of new features to make its services more accessible and user-friendly, including a 'Google-style' search engine, dubbed EB-eye. Also this year, CiteXplore, the EMBL-EBI's portal to its literature resources, was launched. This is a new tool that combines literature searching with text mining tools for biology. As well as increasing the accessibility to its data resources, EMBL-EBI continues to expand its training activities. In 2006, these totalled 118 training events with more than 3100 participants.

The Bioinformatics Advisory Committee (BAC) had its annual meeting in early 2007 to give strategic advice to EMBL-EBI management. One significant development in 2006 came when the European Strategy Forum on Research Infrastructures (ESFRI), an independent advisory body to the European Commission, gave a high priority to bioinformatics – and to EMBL-EBI in particular – as a ma jor biological infrastructure that should be supported in Europe. We are working to convert this positive endorsement into long-term rolling support.

EMBL Hamburg

EMBL Hamburg has a long tradition of high-quality service provision and an excellent reputation in the user community. The construction of new beamlines at PETRA-III, the new synchrotron facility at DESY, will help to maintain, expand and better serve this user community in the future. This represents one of the largest projects at EMBL over the coming years and most of this effort is funded directly by the Outstation's host country, Germany. The project team has now been hired and work has started on the design of the new beamlines. Our goal is to build an integrated centre for structural biology ranging from high-throughput protein expression and crystallisation to automatic data collection and processing. This centre will become the heart of EMBL Hamburg services.

EMBL Grenoble

In Grenoble, we will continue to build upon the integrated approach to high-throughput technology for structural studies pioneered by the Partnership for Structural Biology (PSB). Integration across the diverse research organisations with an interest in structural biology in Grenoble is working extremely well, as evidenced by the many joint publications produced by partners within the PSB and the Institut de Virologie Moléculaire et Structurale (IVMS). Following a recommendation by the SAC during its last review of EMBL Grenoble in April 2005, we have established a mixed international research unit with the Université Joseph-Fourier de Grenoble and the French public research organisation, the CNRS, which completely integrates the IVMS in to the new Unit of Virus Host Cell Interactions (UVHCI).



Publications in 2006 acknowledging

EMBL Hamburg beamlines

Publications quoting use of EMBL Grenoble beamlines in 2006							
ID14-1	14-2	14-3	14-4	23-1	23-2	29	BM14
69	68	28	72	47	15	75	62
Total 436							

Beamline Users Hamburg and Grenoble 2006





EMBL Monterotondo

Working alongside other national and international institutions, the Mouse Biology Unit at EMBL Monterotondo has grown to become a European hub for research, training and service activities in ma mmalian biology and biomedicine. The Outstation's research activities are supported by its superbly equipped animal facilities that provide a full range of mouse transgenic and gene knockout production services, complemented by state-of-the-art behavioural and cardiovascular phenotyping capabilities. Interactions with clinical groups in Rome and throughout Europe through the EMBL C entre for Disease Mechanisms provide exciting opportunities for application of basic research to advanced disease diagnosis and treatment. An active research collaboration with Imperial College London has le d to exciting developments through molecular analyses of cardiovascular disease and treatment in he art-failure patients. During the next EMBL Programme, the resources of the Outstation will be expanded to include two new faculty members, increasing the number of research groups to eight. In addition, a representative of the European Learning Laboratory for the Life Sciences (ELLS) has been placed in Monterotondo to set up local education activities in Italy.

EMBL's International Centre for Advanced Training (EICAT)

EMBL's numerous training activities are coordinated by the EMBL International Centre for Advanced Training (EICAT). Advanced training within EICAT includes the EMBL International PhD P rogramme, the postdoctoral programme, courses and conferences, and the visitors' programme. The PhD programme, with its student body of 180 from 30 countries, has an outstanding reputation and is a focal point of EICAT activities. Currently, students obtain a joint degree with one of 26 partner universities in 18 countries. Beyond the core introductory course the programme adds value with other activities such as training in transferable skills, including scientific writing.

The goal of the postdoctoral programme is to foster networking within the international group of 200 young scientists with a diverse range of skills and interests. Retreats are now organised regularly by the Postdoctoral Association with support from the Postdoctoral Programme. These have been a great success and will be continued and expanded in the future. Through the new EMBL Centres, we will for the first time be able to hire postdoctoral fellows in a mo re centralised manner. This will hel p to increase the visibility of opportunities for postdocs at EMBL and hopefully make the pool of excellent applicants even larger.

Technology transfer

Technology transfer at EMBL is carried out by its affiliate, EMBL Enterprise Management Technology Transfer GmbH (EMBLEM). As a service, EMBLEM is responsible for the portfolio management of EMBL's intellectual property. EMBL's research results are commercialised profitably and, in addition to the commercial aspects, EMBL scientists are supported in setting up inter-institutional agreements and consortium agreements.

In 2006 two spin-out companies were founded: Elara Pharmaceuticals GmbH in Germany; and together with the University of Galway, Ireland, and the University of Groningen, the Netherlands – Triskel Pharmaceuticals Ltd., located in Ireland. Both are working on drug development in the field of oncology.

Outreach activities

Scientific discoveries at EMBL make headlines around the world. We have to make sure that our research findings, training activities and services are also well known in the non-specialist community. Engaging the general public in a dialogue about our science and its role in society is likewise vital.

The Office of Information and Public Affairs (OIPA) has been particularly successful in its media relations and maintains close contacts with journalists from the most renowned newspapers and magazines. Last year we published 30 press releases which were covered in most European media. OIPA maintains and updates five EMBL websites daily. One of these alone, www.embl.de, is visited by 6000 users every day.

Many scientists have been involved in outreach activities. Such activities include courses organised for teachers by ELLS and regular visits by school classes, of which we hosted 14 in the last year. More than 500 teachers have attended ELLS courses, which offer a view of cutting-edge science as well as training in practical activities that the teachers can take back to their schools. A new ambassadors' programme for schools and universities will promote women in science at EMBL and across Europe.

The Science and Society Programme provides a forum for promoting a better and broader understanding of the growing social and cultural relevance of the life sciences. Our programme organises a variety of activities and events where members of our scientific community, scholars from other disciplines and members of the public can meet to engage in dialogue. In 2006, the 7th annual EMBL/EMBO joint conference focused on the theme 'Genes, Brain/Mind and Behaviour'. Running over two days, this event attracted more than 300 people from multiple disciplines to participate in lively and inspiring debates.

Integration of European research

EMBL is in a unique position to integrate research activities across Europe. In addition to its five sites, EMBL can work through partnerships with research institutions in its member states, a large network of close to 4000 alumni, many of whom keep links to EMBL throughout their careers, participate in a large number of externally funded research projects and provide services to thousands of scientists.

Since its foundation in 1999, our Alumni Association has grown rapidly. By early 2006, the number of registrants had topped 1000, helping to create this important network. The Association had a very active year in 2006: it organised three local chapter meetings in Austria, Spain and the USA, initiated the John Kendrew Award for young scientists and saw a large number of EMBL Alumni register to access the shared applicant pool of the International PhD programme.

The EU was expanded and now has 27 member states. We are hoping that many of the new countries will join EMBL. Croatia, which is not yet a member of the EU, joined EMBL last year and we are in discussion with several Eastern European countries. Many of our best PhD students have come from these countries, often with a background in physics, mathematics or IT. In return, EMBL can provide an infrastructure that helps Eastern European scientific communities interact with those of our existing member states and offers a place for some of the best young talents to develop.

But as well as growing within Europe, it is also important to look further afield and pursue the possibility of bringing in associate member states from Asia and Australasia. It would be mutually beneficial to have more EMBLmediated European interaction with countries that traditionally look to the USA for scientific collaboration.

The EMBL partnership scheme continues to develop and we are in the process of establishing the Nordic EMBL Partnership for Molecular Medicine in Finland, Sweden and Norway. Three nodes have been established: the Institute for Molecular Medicine Finland at the University of Helsinki, the Laboratory for Molecular Infection Medicine at the University of Umeå and the Institute for Molecular Medicine Norway at the University of Oslo. All three nodes have been able to raise significant national funding. A steering board will be established to manage the collaborations among the nodes and with EMBL.

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 November 2006 all seven Director Generals signed a statement of support for the Researchers' Charter and Code of Conduct that was published by the European Commission. The EIROforum statement is based on a detailed analysis of the working environment at all seven organisations, and concludes that most of the requirements laid out in the Charter and Code are already met.

The most successful joint EIROforum project to date is the European Science Teachers Initiative which includes two activities: 'Science on Stage', a festival for science teachers from more than 27 countries, and *Science in School*, a quarterly journal run by EMBL for science teachers in Europe. The second 'Science on Stage' international festival took place in Grenoble in April 2007 and was hosted by the ESRF, ILL and EMBL Grenoble. The participating scientists and school teachers thoroughly enjoyed this inspiring event. Their motivation bodes well for the future of European science.

Personnel statistics

On 31 December 2006, 1373 people from more than 60 nations were employed by EMBL. 79% were from EMBL Member States and 43% were female.

800 Total 1373 724 700 600 500 400 300 245 202 200 96 100 68 38 0 Staff Members Ancillaries Visitors Predoctoral Postdoctoral Diploma and fellows fellows students and supernumeraries trainees

Personnel on 31 December 2006

Visits to EMBL Research Units during 2006





Visitor Types during 2006



Financial report

External Funding (Grants)

		2006		2005
	k Euro	%	k Euro	%
EU	15,648	50.5	12,801	47.0
NIH	2,981	9.6	3,773	13.9
Wellcome Trust	2,717	8.8	1,766	6.5
DFG	1,619	5.2	1,947	7.2
Swissprot	1,343	4.3	867	3.2
BMBF	1,241	4.0	1,496	5.5
BBSRC	1,210	3.9	913	3.4
HFSPO	406	1.3	362	1.3
VW Foundation	316	1.0	393	1.4
MRC	142	0.5	371	1.4
Other	3,383	10.9	2,523	9.3
TOTAL	31,006	100	27,212	100

Income/expenditure statement

INCOME	2006	2005
	k Euro	k Euro
Member States Contributions	68,055	65,309
Internal Tax	16,972	16,824
External Funding	31,543	27,212
Other Receipts	10,861	9,021
Total Income Excluding pension contributions	127,431	118,366

EXPENDITURE	2006	2005
	k Euro	k Euro
Staff Costs	75,591	71,031
Operating Costs	36,320	29,826
Capital Expenditure	15,510	11,326
Special Capital Investment		3,836
Total Costs	127,421	116,019
Surplus transferred to reserves	10	2,347

Member States Contributions

	Ordinary and one-off contributions				Pension contribution	
	Euro	2006 %	Euro	2005 %	2006 Euro	2005 <i>Euro</i>
Austria	1,425,475	2.22	1,408,712	2.22	2,583,643	25,463
Belgium	1,746,528	2.72	1,725,990	2.72	3,165,544	31,198
Denmark	1,110,843	1.73	1,097,780	1.73	2,013,379	19,843
Finland	879,685	1.37	869,341	1.37	1,594,410	15,714
France	10,228,746	15.93	10,108,463	15.93	18,539,382	182,717
Germany	14,087,801	21.94	13,922,139	21.94	25,533,838	251,652
Greece	943,896	1.47	932,796	1.47	1,710,790	16,861
Israel	783,369	1.22	774,157	1.22	1,419,840	13,993
Italy	8,347,376	13.00	8,249,217	13.00	15,129,439	149,110
Netherlands	2,806,002	4.37	2,773,006	4.37	5,085,819	50,124
Norway	1,277,791	1.99	1,262,765	1.99	2,315,968	22,825
Portugal	744,843	1.16	736,084	1.16	1,350,011	13,305
Spain	4,481,899	6.98	4,429,195	6.98	8,123,345	80,061
Sweden	1,708,001	2.66	1,687,917	2.66	3,095,716	30,510
Switzerland	2,099,686	3.27	2,074,995	3.27	3,805,636	37,507
United Kingdom	11,538,642	17.97	11,402,956	17.97	20,913,540	206,116
SUB TOTAL	64,210,582	100.00	63,455,512	100.00	116,380,300	1,147,000
Ireland	462,776		455,488		8,379	8,258
Special Contribution Ireland	146,394		146,394			
Iceland	40,760		40,118		732	721
Special Contribution Iceland	16,396		16,396			
Croatia	61,492				1,114	
Special Contribution Croatia	23,269					
TOTAL CONTRIBUTIONS	64,961,669		64,113,908		116,390,525	1,155,979

In addition, one-off contributions from Germany were received in 2006 as follows:

	2006	2005
	Euro	Euro
Contribution to building of a new parking garage	0	501,000
Contribution to building of a multi-purpose building	3,093,093	693,564
TOTAL	3,093,093	1,194,564

2006/2007 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 EMBL Structural and Computational Biology Unit Review Report, Heidelberg, 10 and 11 May 2006

- I begin by expressing my gratitude to the entire review panel and in particular its chair, Søren Brunak, for their detailed, expert assessment of the Structural and Computational Biology Unit. EMBL very much appreciates their evaluation of the past and present performance of the Unit and their advice on its future structure and organisation.
- 2. The panel strongly endorsed the vision and leadership of the Unit Coordinator and Associate Coordinator, Luis Serrano and Peer Bork. They expressed the opinion that the reorientation of the Unit towards integrating structural biology with cellular organisation is not only innovative, but also useful in favourably positioning EMBL as a whole within the context of the emerging discipline of Systems Biology, the focus of the EMBL Programme, 2007–2011.
- 3. The panel felt that the joint leadership of the Unit had been highly successful and recommended to maintain a similar structure in future by appointing a new Joint Coordinator to complement Peer Bork on Luis Serrano's departure. The panel recommended appointing an experimentalist, but suggested that the specific discipline of the appointee will be less important than other qualities such as leadership, vision and commitment to the EMBL model.

- 4. The panel recommended to maintain a breadth of expertise within the Unit, and not to adopt a narrower focus as a result of the upcoming turnover. They felt it will be valuable to search for excellent young scientists who combine different approaches to solve biological problems. They suggested to continue building strength in high-resolution electron microscopy, to retain strong activity in computational biology and X-ray crystallography, and to ensure that groups within the Unit retain access to NMR. We will attempt to follow this advice within the constraints provided by the availability of suitable candidates and funding.
- 5. The panel felt the addition of an activity in chemical biology that is well integrated into the future plans of the Unit had been a good choice. They recommended strengthening chemical biology in other parts of EMBL and integrating these activities across the Units of EMBL Heidelberg. We will follow this advice.

lain W. Mattaj Director General Director General's Response to the EMBL Hamburg Outstation Review Report, Hamburg, 12 and 13 February 2007

- This is a critical time for the Hamburg Outstation, engaged as it is in refocusing on the ambitious and important programme of PETRA-III beamline design and construction. My sincere thanks go to the panel that informed itself about the current and planned activities of EMBL Hamburg in considerable detail. This, in turn, allowed the provision of excellent advice that has both breadth and depth. I consider this report as being particularly useful to EMBL.
- 2. I appreciate and share the panel's opinion that three of the senior staff of the Outstation, the Head of Outstation Matthias Wilmanns, Deputy Head of Outstation Victor Lamzin and Group leader in charge of small-angle X-ray scattering (SAXS) activities Dmitri Svergun, have carried out outstanding research in the areas of macromolecular crystallography, SAXS and/or software development during the review period.
- **3.** I am pleased to acknowledge the praise of the panel for EMBL Hamburg's dedication to high-quality service provision and user support, as well as to high-quality research and development. I am grateful to the Outstation leadership, and in particular to Matthias Wilmanns, the Head of Outstation, for engendering and encouraging the team spirit required for the Outstation to perform well in these diverse contexts.
- 4. I note the panel's evaluation that there is an essential requirement for continued community access, during the PETRA-III construction phase, to the DORIS beamlines, in spite of their agerelated limitations and the increasing provision of synchrotron access to a subset of national structural biology communities through local sources.
- 5. The panel positively evaluated the efforts of the Outstation staff and leadership in building towards the provision of an integrated user facility for external structural biologists that

includes protein production, characterisation, high-capacity automated crystallisation and automated data capture, evaluation and analysis. I note the panel's concern that maintaining these facilities during the construction of the PETRA-III beamlines, and in particular during the ninemonth period in 2008 when no beamtime will be available, will require special effort, including the diversion of excess demand from Grenoble.

- 6. The panel recommends the integration, where appropriate, of beamline design and construction staff from Grenoble into the PETRA-III project. I am happy to report that concrete plans have been made for this to happen.
- 7. I am happy to acknowledge the panel's judgement that EMBL Hamburg has successfully recruited a very strong team of beamline scientists and note that this recruitment is not yet complete. We will investigate whether this, or a requirement for consultation with others on the shared beamline on sector 8, justifies delaying the construction of this beamline.
- 8. I note the advice from the panel that the three beamlines to be designed and built by EMBL staff should be constructed in a staggered sequence. We will act on this advice. We will announce, as recommended, that the delivery of the three EMBL beamlines will occur in 2011 rather than 2010. I also note and agree with the panel's judgement that the PETRA-III project must take priority over all other Outstation activities in the coming period.
- 9. I appreciate the panel's support for EMBL's decision not to provide X-ray Absorption Spectroscopy (XAS) activities on PETRA-III and their agreement with our evaluation of the reasons for not doing so.
- **10.** I note the advice of the panel to form a beamline project technical management group, consisting

of T. Schneider, S. Fiedler and C. Hermes, to operate closely together with the Outstation leadership. This has been done.

- 11. The panel recommends providing a course, open to all EMBL PhD students, on practical aspects of macromolecular crystallography. A related course was offered to PhD students in Grenoble this year, and will be duplicated in appropriately modified form in Hamburg and Heidelberg.
- **12.** The short initial contract period for postdoctoral fellows was criticised by the panel. The Head of Outstation will recommend to the group and team leaders that they extend this period.
- **13.** I acknowledge the observation of the panel that the group and team leaders have been outstandingly successful in obtaining external funding during the period under review. This funding has been deployed such as to enable further integration of the Outstation with other structural biology activities throughout both EMBL and, in a broader context, Europe.
- 14. I appreciate the panel's advice that, looking to the future, EMBL should investigate ways in which it can interact with other local, national and international organisations to ensure optimal onsite utilisation of the new PETRA-III beamlines and investigate the potential for structural biology of the X-ray free-electron laser that will be constructed by DESY. As the panel suggests, this might involve the participation of EMBL in a new centre for structural biology research on the DESY site.

Iain W. Mattaj Director General

Scientific Report



Networking at all levels

"I DO NOT KNOW what I may appear to the world, but to myself I seem to have been only like a boy playing on the sea-shore, and diverting myself in now and then finding a smoother pebble or a prettier shell than ordinary, whilst the great ocean of truth lay all undiscovered before me." This quotation, from the great 17thcentury mathematician Isaac Newton, sums up how many biologists feel when confronted with the enormity of the living world. One can study individual molecules, cells, animals and even ecosystems, but how does it all fit together? How can we rise above the pebbles to see the beach or the ocean as a whole?

Modern-day mathematicians are still seeking an underlying, unifying theory to let them see the 'great ocean of truth' in mathematics. But even as they strive to do so, a related field, computational science, is already helping to unite biology and bring the big picture together for life scientists. This unifying force, bioinformatics, is linking biological systems together at many different levels, from molecules, to cells to whole organisms. But to get the biggest perspective of all, you need to link these systems together into networks.

Networks can take many forms: connections between neurons in the brain, between genes or proteins within a cell, or even social networks in science. All offer to help us understand how biological systems integrate with each other to produce a bigger whole. Scientists at EMBL are studying myriad networks in the hope of understanding, or even predicting, living behaviour.

At the EBI, for example, teams are studying how protein networks in the cell control gene activity and how such networks can be used to predict what proteins will make good drug targets and even help biologists discover new genes involved in the process of interest. Meanwhile, researchers at EMBL Heidelberg and Monterotondo are studying how intricate networks of neurons form, through evolution or in development, to result in complex brains and behaviour.

But networks are not limited to cells and molecules. Many EMBL researchers take part in international networks of scientists, all collaborating to collect or interpret data; the network is able to produce results and insights that no one researcher could achieve alone. And an intriguing study of Group leaders at EMBL has revealed that interaction with networks of talented colleagues is a key driving force in scientific creativity. Having friends with you to play on the shore, it seems, makes picking up pebbles a whole lot easier.

The big picture

The blue marble – Ewan Birney, Nicolas Le Novère and Nicholas Luscombe T'S 3:30 PM ON A FRIDAY AFTERNOON and you're fed up with work. Sigh. What to do? Your eyes alight on the computer desktop icon of Google Earth, a program that lets you peruse high-resolution satellite images of the Earth's surface. Aha! That's it. You decide to find your house.

Starting with an image of the whole Earth, the program gradually zooms in on the target you choose: first, the continent, then the country, followed by the city, district, street and finally, there it is. Your house, with the driveway empty of your car. The satellite must have passed overhead while you were out at work.

Just as you're having a good old nose around your colleagues' houses, you feel a tap on the shoulder. It's your boss. What to say? How about: "I'm exploring Google Earth as a metaphor for the exciting new field of systems biology." It could get you off the hook and you wouldn't even be lying. Here's why.



Google Earth - zeroing in

Google Earth allows you to start with the whole picture, the Earth, locate points of interest and then zero in on them. Systems biology, too, aims to produce a big picture – of the entire cell and how all its components function together to produce living behaviour. But as well as helping biologists understand how the cell functions as a whole, systems biology can help them take some useful shortcuts through all that complex biochemistry and zero in on the gene, protein or process that really matters to them. Several researchers at the EBI are working on systems that let biologists take those all-important shortcuts, while others are working on projects that are more like the Google Earth satellites, scanning biological systems for the fine details that biologists need when they focus down. Nicholas Luscombe is one of the Group leaders working on shortcuts and big pictures. His interest began in looking at how the cell controls the activity of its genes using 'transcription factors'. These are proteins that interact with DNA, switching a particular gene on or off. While working as a postdoc at Yale University before joining the EBI, he and his colleagues constructed diagrams, or networks, that charted the interactions between transcription factors in yeast cells. They studied how these network connections change with alterations in the cell's environment or when it started dividing and found many unexpected changes that had not been predicted from experimental observations.

Now at the EBI, Nicholas is looking at a range of networks within the cell. "We want to look at general principles that apply across different systems," he explains. "Networks allow us to look at the entire system in one go and let us integrate different systems, such as metabolic pathways, together." Like Google Earth, this gives scientists a global view of a system, but one that you can look at on many different levels. They can also alter the inputs to a computer model of a network, to try and predict the outcome. "You are really moving towards looking at causality and also predictions that are testable in the lab," says Nicholas. One of the projects in his lab is building network models of how bacteria form swarms - a key process in disease. "This is a very complex, multicellular behaviour," he says, "but although it has been studied for 20 years, only a handful of proteins that regulate the process have been found. We hope to change that."

Another EBI Nicholas, Nicolas Le Novère is also working on making predictions, such as predicting which proteins might be good drug targets. His group is building two kinds of model of how proteins and biochemical signalling systems work in neurons and the synapses that transfer information between them.

The first kind is based upon studies of large populations of molecules in the cell and can reveal what happens inside the cell when it receives a signal from the synapse. Such models can be used to predict what effect a drug targeted at a particular molecule might have, or can even help researchers decide what protein to target in the first place. "It may not be obvious using common sense," says Nicolas. For example, his team recently modelled the behaviour of a protein called DARPP-32, which is involved in the processing of the neurotransmitter dopamine. Dopamine is a key player in the neurodegenerative condition Parkinson's disease and DARPP-32 seems like an obvious drug target. But Nicolas's team showed that the protein's behaviour is very robust, and so likely to be difficult to change with drugs. Other proteins involved in the same process would make better targets.



The second kind of model focuses much more on individual molecules, such as the huge, complex protein machines that sit, rather like TV satellite dishes, in the membranes of the synapses and receive signals from neurotransmitters. It allows researchers to model how the numbers and shapes of the proteins change – all factors that are involved in the synapse's 'plasticity', or ability to change. "This is important for understanding the molecular basis of memory," explains Nicolas.

As well as working on the basic science, Nicolas is developing computer resources to help systems biologists exchange models and data. These include developing a formal programming language, SBML, to standardise how reactions and networks are represented; developing systems biology 'ontologies', a standard vocabulary for describing the components of models; and developing an EBI database, the BioModels Database, so that biologists can find any models that have been published. It's all about getting everything to integrate seamlessly, explains Nicolas. "If you don't develop these three things, you will never get true systems biology," he adds.

If you want to build a global picture, though, you need details to zero in on. This is where Ewan Birney and the EBI teams he coordinates come in. The teams' work focuses on understanding how genomes function; how the string of DNA letters in a genome actually produces biology. At the moment, biologists have a sketchy outline of how the human genome works and are keen to assign function to its sequences, a process known as annotation. Ewan and his colleagues are part of a large international consortium that aims to fill in some of these details. The project, dubbed ENCODE - for Encyclopedia of Genome Elements - is sponsored by the US National Institutes of Health and is currently focusing an intensive set of experiments on one percent of the human genome. "The human genome is like War and Peace, but it's written in Martian," says Ewan. "We would like to get to the next level of interpretation of what's going on."

To that end, the consortium is scrutinising scattered sections of the genome to see what proteins they encode and what RNA molecules they produce – these are the 'words' of the genome. The researchers are also looking at the 'punctuation' of the genome: DNA sequences and chemical modifications to the DNA that regulate how it should be read, or even if it should be read at all. ENCODE is currently in its pilot phase, and will eventually be rolled out to cover the entire genome. But even this early stage has revealed some surprises, says Ewan. "The genome is a lot more complicated than we thought." For example, it seems to be producing far more RNA molecules than expected. "It's shifting people's perspective of how these different elements of the genome work," he says. ENCODE's data will be made publicly available and will be accessible on Ensembl, a genomics resource jointly run by the EBI and the Wellcome Trust Sanger Institute.

Making sure all these pieces of the puzzle fit together to build a big picture is key, and something in which Janet Thornton, director of the EBI, takes a keen interest. She coordinates BioSapiens, a European network of excellence whose aim is to develop new annotation methods, delivered in one place, by labs distributed all over Europe. The idea is that the annotation data should be spread over different sites, rather than on single databases. A software system called the Distributed Annotation System, or DAS, allows the data to be spread out in this way. Each lab can add its annotation to a particular gene, but a user can see all the annotations for that gene at once. "It also allows bioinformaticians to compare different research annotations," says Janet.

Janet's research focus is on applying bioinformatics to structural biology, and her team is using information about protein structures to help annotate genomes and so sharpen the focus if the details within the big picture. As part of the ENCODE consortium, she has been using studies of protein folding to work out which RNA molecules in the mysterious glut produced by the genome actually end up being converted into functional proteins. "In collaboration with teams at Oxford University and in the Sanger Institute, the BioSapiens network will use all our different expertises to shed light on studies of individual genetic variation and cancer," she adds.

So just as all the parts of the cell work together to create an emerging living system, so the EBI systems biologists and annotators are producing work whose whole promises to be much greater than the sum of its parts. It's something that should impress even the sternest of bosses who catch you messing about with Google Earth.

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Reading the omens

N ANCIENT ROME, the auspex or augur was the man who studied the flight of birds to interpret the will of the gods. So when Cornelius Gross and his team identified two novel proteins by scanning the human genome – rather than deducing the gene from the protein, which is the usual direction of discovery – it seemed natural to name them spexin and augurin, after the diviner, particularly as the Gross group is based at EMBL Monterotondo on the outskirts of Rome.

The main focus of Cornelius' research is gene-environment interactions that modulate anxiety in the mouse, but the discovery of spexin and augurin came about independently of that ongoing project, and perfectly illustrates the cross-fertilisation of ideas that EMBL fosters. In 2002, just before he took up his post at EMBL, Cornelius was invited to attend a Group leader retreat, where he heard a talk by Ewan Birney from the EBI. "Ewan was talking about trying to find genes from the genome sequence alone," says Cornelius,

who recalls thinking that this might be one way to find novel members of a particularly elusive class of proteins – small peptide hormones.

These hormones are secreted by the cell and bind to cell membrane receptors, activating signalling pathways which are critical in both health and disease. An example is vasopressin, which is important for the regulation of blood pressure and temperature, among other functions. Many of the receptors to which these hormones bind have been identified, but at least 30 of them are 'orphans', meaning that their ligands remain unknown. "Knowing the ligand can help a lot in guessing what sort of drugs will activate or block the receptor," says Cornelius.

The conventional approach to finding new peptide hormones has been biochemical: you take a hormone-rich tissue, the pituitary gland for example, grind it up and use chemical separation techniques to identify its protein components. But this strategy is more likely to find large or abundant proteins than small ones or ones that are present in trace quantities. Since both kinds of protein are similarly represented in the genome, scanning the genome for them is, in theory, a less biased approach which can ultimately be tested in the lab. "This sort of dry/wet cycle is the biology of the future," says Ewan. "Sadly there are still too few biologists exploiting genomic information, mainly because people with the necessary interdisciplinary skills are not present."

The challenge is to develop a computer program that sifts through the genome sequence, looking for sections of interest. With the help of Ewan's bioinformatics postdoc team, Olivier Mirabeau devised an algorithm that could pinpoint stretches of the human genome that shared motifs with the genes of known peptide hormones, and ranked them according to the probability that they encoded novel peptides. Studying this list, the researchers noticed that the top 300 stretches contained not only the genes for most known peptide hormones, but also some novel ones - suggesting they were on the right track.

Of the unknown 'candidate' genes, they selected two – spexin and augurin – for further investigation. "The key thing is to show that they have some physiological activity, which is not so straightforward," says Cornelius. In collaboration with Cinzia Severini and Roberta Possenti of the Institute of Neurobiology and Molecular Medicine in Rome, however, they have been able to show that both proteins are secreted by cultured cells – a promising sign. Though they have not yet found any physiological function for augurin, they have for spexin, which appears to affect the ability of stomach tissue to contract. In principle, it seems, divination works.

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Forging new paths

There's far more to curating biology databases than simply collecting and labelling information. You also need to link it all together, to let biologists freely roam the 'data landscape' and make new discoveries. Janet Thornton, Director of the EBI, and Graham Cameron, Associate Director of the EBI, outline their vision for the next five years.

In terms of the services it provides, what are the key challenges facing the EBI over the next five years?

Graham: Historically, the EBI's work has been about collecting and organising the information flowing out of biology in electronic form. We are a major European centre for this and have been for many years. But what is happening now is that the sheer diversity of data that can be subjected to bioinformatics is growing: it's enormous. A few years ago, genome sequencing was really the only 'high-throughput' science. But now, many kinds of data, such as gene expression data, can be collected this way, and need to be integrated. The buzzword now is 'systems biology', which means trying to understand how biological systems such as cells function as wholes, rather than looking at individual molecules or biochemical reactions. This demands bioinformatics tools that can link everything together. So a main thrust of what we have been doing recently is finding ways of exploring this information space and linking things up.

But there's a further challenge to this too: making the tools accessible to bench scientists who don't have much of a background in computing. Biologists are not patient with being surrounded by computational gobbledegook. They expect to get the information they want easily. This is where projects such as the recently launched 'EB-eye' resource, an accessible web-based interface developed by Rodrigo Lopez and his team at the EBI, prove invaluable.

EMBL ANNUAL REPORT 06.07

Janet: The nature of the data we are getting in now has changed; it has become more complex, and in many cases, more disjointed. Metagenomics is a case in point. This is where, instead of sequencing the entire genome of one particular organism, scientists simply sequence all the microbial genes they can find in a given environmental sample, say a sample of soil or seawater. Although this is great for discovering new genes and proteins from previously unknown organisms that no-one can grow in the lab, it presents something of a headache for curators like us, who have to not only store data, but also make sense of it and help others exploit it. Before, in a family of proteins, we might have a few hundred members to deal with. Now we have thousands, and we have no idea where they came from.

Another key example is the human 'variome'. This is an international project to catalogue individual variations in human genes. Ultimately, scientists want to connect these variations to particular physical characteristics, or pheno-types. Many of these phenotypes will be medically-orientated, such as a person's propensity for developing a particular kind of cancer. Biomedical research is going from being generic to becoming more personal.

Graham: This all means that as well as making sure our internal databases here at the EBI integrate well together, we also have to ensure they can link up with external resources at other institutions. This includes specialist collections, for example on enzymes, where the challenges tend to be purely technical. However, more substantial challenges arise from the need to exploit our obvious relevance to medicine. Linking genotype information to individuals' medical history could yield valuable insights, but requires extremely careful attention to ethical and confidentiality issues.

Janet: One of the external links will involve metagenomics. Another will be the chemical world, for example, linking to databases of 'small molecules'. These, as the name suggests, are chemicals that don't belong to the class of big biological molecules, such as proteins and nucleic acids, and can include drugs and metabolites, chemicals produced by the cell's metabolism. There are projects underway to catalogue all these metabolites, dubbed the 'metabolome'. This is something we also want to get involved with.

These are pretty hefty challenges. How are you going to meet them?

Janet: We already have three databases under development to meet these challenges internally. ChEBI, or Chemical Entities of Biological Interest, is a database of small molecules. PRIDE, the PRoteomics IDEntifications database, is a collaboration with the University of Ghent. It allows researchers working on proteomics, the largescale study of protein structure and function, to share data with each other more easily. Then there is the Biomodels Database. This captures mathematical models being published in the field of systems biology. Looking further forward, we have just started to develop another resource called the EGA, or European Genotyping Archive, to tackle the human variome data.

How do you plan to allocate your funding over the next five years?

Janet: We are glad that the member states recognised the great potential and the growing need for bioinformatics infrastructure with an increase in funding. To meet the challenges, a third of the increment we receive from EMBL will be devoted to consolidating the databases we already have. Another third will be used to grow and change the projects already underway, such as the metagenomics project. The last third will be devoted to developing completely new resources, such as the human variome resource.

As well as providing bioinformatics and data services, part of the EBI's mission is to undertake research and offer training. How will these grow over the next five years?

Janet: On the research side, as the EBI grows, the research will grow in parallel, but remain at its current percentage. We see investment being in areas that are currently new, for example, complex genetics and human variation. Another area is in chemistry. Going forward, efforts to develop better literature resources will grow, because these are based very much in the theme of data integration, which is a major drive for us.





Graham: There will be a strong axis in functional genomics and proteomics, but to complement this global view of biology, I think we still need to keep recruiting in the area of basic sequence comparison and analysis. Almost everything we do depends upon that technology, so any improvement there immediately impacts our other areas.

Janet: It all comes down to making sense of the data. Increasingly, the discoveries in biology will be led from analysis of data as well as from experiments; the data will be used to drive the formation of new hypotheses. Just as physics turned from an experimental science to include more theoretical considerations, so will biology. This is why the EBI needs more scientists who can work across different sorts of biological data and datasets.

What about training?

Janet: We want to train scientists to get the most out of our databases. The EBI already does a lot of outreach training for biologists and students. We are thinking about trying to broaden access to our training by providing more 'e-learning'. This is tuition that can be supplied over the internet, which means that researchers don't have to travel to the EBI to do all their training.

Graham: We are committed to serving industry well – many of the benefits of our work will depend on commer-

cial exploitation. All scientists, including those from industry, have access to our services at no cost. However, major bioinformatics-using industries support us through subscriptions to an 'Industry Club'. In return we provide discussion forums and training. Smaller companies are harder to serve; they often simply cannot spare the time to attend EBI events. We are experimenting with 'e-learning' as a way of helping them.

Although it is supported by EMBL, the EBI has to find 40 % of its funding from external sources. How are you planning to do this?

Graham: We are in constant discussion with all European stakeholders to ensure that the EBI receives the necessary infrastructural support. We have recently secured infrastructure funding from the European Commission for FELICS, or Free European Life-Science Information and Computational Services, a project that teams the EBI with three other European institutions to supply biomolecular information services to European life scientists.

Janet: We are now developing a proposal called ELIXIR for EU funding, to construct an infrastructure for biological information across Europe. ESFRI, the European Strategic Forum on Research Infrastructures, identified that this is one of Europe's top priorities.



Can you read me? Rodrigo Lopez, Head of External Services at EMBL-EBI

The EB-eye's guide to life

THE BABEL FISH is small, yellow, leech-like and probably the oddest thing in the Universe. At least, this is how author Douglas Adams describes his fictional creation in the sci-fi comedy classic, *The Hitchhiker's Guide to the Galaxy*. This fish lived happily in its host's ear, feeding on the brainwaves of the people surrounding it, excreting a telepathic signal into its host's brain. The upside of all this is that anyone having a Babel fish in their ear would immediately understand anything said to them in any form of language.

Graham Cameron, Associate Director of the EBI, and Rodrigo Lopez, Head of External Services at the EBI, have developed their own kind of Babel fish for bioinformatics. The new software framework, called 'EB-eye', takes the form of a web interface that allows scientists from all over the world to access the extensive biological data available from the EBI through a single portal. EB-eye embraces many of the different types of information and software systems that have sprung up in bioinformatics, and, like the Babel fish, translates them into a common format and language that everyday research scientists can easily use.

Launched on 11 December 2006, EB-eye looks like a small addition to the EBI's website, taking the form of a search box sitting unobtrusively at the top of the page (www.ebi.ac.uk). But its looks are deceptive: it is a powerful, exhaustive search engine linking vast amounts of data.

Until EB-eye came along, users depended on specialist portals to different EBI databases, covering many aspects of biology, from small molecules to entire genomes, and from individual proteins to taxonomies. This worked for

EMBL ANNUAL REPORT 06.07

experts, but visitors to the EBI whose interests spanned several databases had to learn how to use each one individually. A user survey undertaken in 2005 confirmed this impression: navigating the sites was not a problem, different warehouses using different semantics was. "It didn't present a cohesive view to the user," explains Graham. "That needed to be addressed."

The issue was set to become even more important as the use of biological databases swelled in the wake of numerous genomics and other 'big biology' projects and as the current emphasis on systems biology required an ability to search the entire information space spanning many databases. Crucially, the EBI's new portal needed to be easily accessible to bench scientists with little background in computing.

The idea behind EB-eye is not to replace the existing databases, but to create a common framework for accessing them. The aim of the first phase of the project was to encourage the service groups at the EBI to work together to provide a homogeneous view of the data derived from the independent databases.

Having addressed these concerns, phase two involved working with the groups to agree on a set of standard methods and protocols. "Our task was to make them interoperate," says Rodrigo. To do this, Rodrigo asked each group to produce a digest document describing biological facts and cross-references from the data objects they had on their systems. "This introduced a new semantics," explains Rodrigo. "These documents provided the



foundation for applying EB-eye's method of indexing and linking pieces of information across databases."

This allowed the production of a web interface that unites the databases, allowing users to surf all the information through one site. "You can see all the databases in the context of all the other databases," explains Rodrigo. It is now possible, for example, to search Ensembl, a genome browser developed jointly by the EBI and the neighbouring Wellcome Trust Sanger Institute, in relation to data from other sources, such as microarray data from the EBI's ArrayExpress database, and structural biology data from the EBI's Macromolecular Structure Database. EBeye also links to external databases, such as Medline, allowing users to view relevant literature entries and patents.

It is also extremely user-friendly. Simply type in a search term, such as 'collagen', into the EB-eye search box, and up pops a page listing everything you would want to know, from genome and protein sequence information to reactions and molecular interactions. Users can simply click through these lists to find whatever they need, without having to leave the site. They can also refine searches after they've performed them, and drill right down to search individual fields in a specific database.

The thing that makes EB-eye unique among bioinformatics search engines is an indexing system that ties all the databases together. "It is the best springboard for a scientist to use at the moment to jump between different kinds of information," adds Graham.

The Hitchhiker's Guide describes the Babel fish as being "mindbogglingly useful", an epithet that EB-eye seems destined to share. Although emulating the fish's other fate – proving the non-existence of God – may have to wait for a system upgrade.

www.ebi.ac.uk


Word miners strike gold

NEW LITERATURE-SEARCHING RESOURCE at the EBI is linking papers to scientific databases, and even pointing database scientists to new research avenues. Only 15 years ago, searching through the literature for information on a topic often meant a tiresome day in the library, wearily thumbing through hundreds of index cards and chasing red herrings. Then online literature databases came along and changed biologists' lives by letting them quickly search through abstracts of scientific papers electronically to find the ones they wanted. Now, Peter Stoehr and his team at the EBI have brought a whole new dimension to literature searching, by integrating abstracts with genomic, proteomic and other molecular biology databases. As well as changing the way biologists find papers and perhaps even revealing new research avenues for them to follow, the new literature-searching interface, called CiteXplore, is destined to help those outside the lab, such as patent lawyers and grant funding bodies, to extract more information from the world of biological knowledge. "Here at the EBI, we have all kinds of biological databases that we manage, but the literature has always been outside that domain," explains Peter. "We are now trying to connect it with our databases." CiteXplore was launched in November 2006 and is based on the idea of text mining: designing software that can derive new information from text, such as its relevance to other terms, its importance or linguistic characteristics. CiteXplore's text mining algorithms, 'Whatizit', were developed at the EBI by Dietrich Rebholz-Schuhmann and his team. These algorithms can find words in journal text and link them to relevant parts of other databases: for example, allowing a user to click on a gene name in a text and be routed to a genomics database containing information on that gene. Conversely, users can easily look up literature relating to database entries. Text mining can

also help EBI scientists keep an eye out for emerging new trends in the biosciences, by monitoring the presence or frequency of particular words in the literature, or by exploiting author and citation networks. "We are not interested in being a dusty old library," says Peter. "We want to be exploratory as well." Peter and Dietrich have designed CiteXplore, and the associated Whatizit, EBIMed and ProteinCorral tools, with two main usergroups in mind: the first is lay users who want to find papers and links from them to molecular biology databases. The second is specialist database annotators, who assign information about biological function to data, such as gene sequences, in a database. They are presented with key literature information in a form that can be easily assimilated into the relevant database, thus making the annotators' work more efficient. It could also help them find things, such as candidate protein - protein interactions that they might otherwise have missed. Although CiteXplore uses the data records of the US-based literature resource PubMed, Peter is keen to ensure that the two complement, rather than duplicate, each other. CiteXplore is geared more towards molecular biologists, although it still maintains a general scope like PubMed. As well as being more tightly integrated into the bioscience databases, CiteXplore covers a few bases that PubMed does not, such as incorporating patents and abstracts from Chinese-language journals. In March 2007, the team made searching even more flexible by introducing a web interface, which allows computer programs to perform automatic searches for users. So forget libraries, index cards or even bothering to type your own searches into a computer - the smart way to do your literature searches could simply involve putting your feet up in the tearoom.

www.ebi.ac.uk/citexplore

At the core...

The Chemical Biology Core Facility

Advanced Light Microscopy Facility (ALMF)

Rainer Pepperkok and the ALMF help characterise the visible effects that small molecules have on cells, which are often used as a secondary method to identify the cellular phenotypes caused by compounds.

The Cohen Lab

Steve Cohen and his lab work on protein interactions that regulate metabolism. With the help of the CBCF they screen for biotools to shed light on this process, which is involved in various diseases, such as diabetes, obesity and cancer.

The German Cancer Research Centre

The CBCF is run as a joint facility of EMBL and the German Cancer Research Centre (DKFZ). While EMBL scientists use it mostly for basic research, groups from the DKFZ look for compounds with potential clinical relevance.

The lab of Michael Pawlita at the DKFZ used the CBCF to find compounds that disrupt interactions between the human papilloma virus and human host proteins, which normally result in cancer. These compounds are now being chemically optimised to develop them into anti-cancer drugs. In the case of Ingrid Hoffmann's lab, inhibitors for a central enzyme in cell division were identified by the Facility, and these are now being used as biotools to dissect the role of this factor in the cell cycle. They may also provide good starting points for further development towards therapeutic drugs.

Genomics Core Facility

For technology and expertise that cross Facility boundaries, such as automatic liquid handling and IT support, the CBCF regularly collaborates with Vladimir Benes and his Genomics Core Facility. In addition, potential synergies between chemistry and genomics are being exploited to determine compounds' mechanism of action.

Protein Expression and Purification Core Facility

All biochemical screening assays require certain amounts of target protein. These proteins are often provided by Hüseyin Besir and his Core Facility, who express and purify them. The proteins can then be characterised for activity in the CBCF.

The Müller Lab

Together with Christoph Müller's lab in EMBL Grenoble the CBCF is searching for compounds that block the infectious cycle of Epstein-Barr virus, which causes infectious mononucleosis, the so-called kissing disease.

Flow Cytometry Core Facility

Andrew Riddell uses flow cytometry to characterise the effects of compounds on the expression of genetic markers and the distribution of cells.

The Chemical Biology Core Facility

Established in 2004 the CBCF is one of the newer additions to EMBL's Core Facilities, but it quickly turned into an important node in EMBL's research network.

The Facility develops and carries out screens to identify small molecules that modulate biological pathways and enzymatic activities and can serve as biotools to help dissect cellular processes. If compounds disrupt processes involved in disease they can also serve as a starting point for drug development.

The CBCF comprises all infrastructure needed for chemical screening. It provides state-of-the-art technology, an extensive diversity-oriented compound library and the expertise of six highly trained staff to guide researchers through the challenges of identifying and characterising small molecules.

Monoclonal Antibody Core Facility

The technology and expertise underpinning chemical screening could, in future, be applied to screens for antibodies. The overlap might lead to collaborations between the CBCF and Alan Sawyer's Monoclonal Antibody Facility in EMBL Monterotondo. Recent advances in technology may allow antibodies to enter the cell more efficiently. Offering critical infrastructure, vital services and specialist expertise, Core Facilities form central hubs in EMBL's research networks. How they interlink with the diverse projects inside and outside EMBL is exemplified by the Chemical Biology Core Facility (CBCF) in the schema below. The CBCF is a prime example of how molecular biology can benefit from chemistry. During the past few years several breakthroughs have been achieved thanks to small molecule 'biotools' identified by the CBCF. Some of the discovered compounds may even be developed into new drugs in future.

The Conti Lab

Elena Conti's group identified a novel way to inhibit the kinase activity of Aurora A, an enzyme that plays a central role in cell division. The CBCF found small molecules that block the enzyme Aurora kinase acting through an allosteric site. The biotools are helping dissect the function of this important cancer target in the cell cycle and may provide the starting point for the development of novel anti-cancer drugs.

"We created the CBCF to complement EMBL's excellence in biology with chemistry of the same standard and to create synergies," says Joe Lewis, head of the CBCF. He has great plans for the future of the facility: an extension of the compound library, infrastructure for cheminformatics and a new platform for medicinal chemistry to chemically optimise the efficiency of a compound. Joe's long-term vision is to scale up the CBCF and implement complementary high-throughput technologies and medicinal chemistry, which is essential to realise the full potential of the Facility regarding the development of biotools or novel drugs. "One of the ideas is to centralise screening infrastructure and make it available to Europe's scientific community in a European Centre of Excellence for screening. This would speed up the development of biotools and allow projects on a scale normally only accessible to large pharmaceutical companies. The CBCF is ideally placed to play a central role in such a pan-European set-up."

The Surrey Lab

The CBCF found inhibitors for motor proteins of the parasite *Leishmania*, the causative agent of sleeping sickness. Thomas Surrey and his lab use them to decipher the precise roles these proteins play in cell division. In future the compounds could form the basis for anti-parasitic drugs.

ELARA

Together with EMBL scientists George Reid and Frank Gannon and with support from EMBL Ventures and EMBL's commercial affiliate EMBLEM, Joe Lewis founded the drug development company ELARA in July 2006. The company aims to bridge the gap between lab and clinic. ELARA is developing promising leads identified by the CBCF to further optimise them into anti-cancer drugs with novel mechanisms of action. The goal is to develop drugs with better efficacy and fewer side-effects than current therapies especially in cancers with large unmet medical need.

In October 2006 the GoBio initiative of the German Ministry for Education and Research awarded 3.4 millon Euros to George Reid and Joe Lewis to help translate basic research findings from bench to bedside and develop small molecules into more effective anti-cancer drugs.

The Gannon Lab

Biotools identified by the CBCF proved crucial for dissecting the complex molecular basis of the estrogen receptor signalling pathway that is studied by Frank Gannon and his lab. Screens have revealed small molecules that disrupt the pathway, which is involved in breast cancer. The compounds identified in the screen are helping to provide novel insights into the molecular mechanisms of estrogen receptor mediated transcription. Some of them some are undergoing medicinal chemistry optimisation to develop them further into new therapeutics against breast cancer.

Proteomics Core Facility

To ensure that the CBCF is working with the correct proteins in their assays, the Proteomics Core Facility, headed by Thomas Franz, checks their weight and provides quality control.

EMBL ANNUAL REPORT 06.07

Detlev Arendt, Gáspár Jékely and Alexandru Denes

Gaps in the record





S WIMMING IN THE GULF OF NAPLES last summer, Detlev Arendt came face-to-face with the creature he's used to seeing in Petri dishes in his lab in Heidelberg, a tiny marine ragworm named *Platynereis dumerilii*. The encounter might have pleased the zoologist Anton Dohrn, who in 1873 set up a research station on the shore at Naples, from which visiting scientists could explore the gulf's rich biological diversity. Two years later, Dohrn published his theory that humans and *Platynereis* inherited their nervous systems from a common ancestor.

To explain why *Platynereis* carries its brain and nerve cord on its front side or belly, while in vertebrates these are at the back, Dohrn suggested that vertebrates had turned themselves back-to-front during the course of evolution. He wasn't the first to propose the controversial dorsoventral inversion hypothesis, nor was he the last to see it rejected. In his lifetime, the prevailing view was that the vertebrate and invertebrate nervous systems had developed on opposite sides of their common ancestor's body. Since then, however, support for his theory has grown, and Detlev thinks he now has the evidence that will vindicate Dohrn.

Platynereis lives in tubes stuck to rocks in shallow water, where it is bathed in a nutritious blanket of algae. "We think that it has always lived in this ecological niche," says Detlev, "and that this might resemble the environment of the common ancestor." That ancestor is known as Urbilateria, so-called because it was symmetrical along the axis from head to tail. One reason to study *Platynereis* is that, if it occupies the same niche as the ancestor, it may have preserved ancestral features in its development and nervous system.

In general, Detlev's group is interested in how the nervous system evolved from that of the first metazoan or complex, multicellular organism, to the human brain. "Most people agree that the first metazoan was a ball of cells resembling a sphere," he says. "They also agree that there was a first cell diversification event – probably a separation of reproductive and non-reproductive cells – and that one day the first neuron appeared. One became two, and then they duplicated further. Eventually there were between 100 and 200 types of neuron, and since that's the number in the human brain, the story ends there."

Another feature of nervous-system evolution was centralisation. Early metazoans had a diffuse nervous system, with cells serving both sensory and motor functions distributed throughout the organism. Vertebrates, by contrast, have centralised nervous systems – their nerve cells are agglomerated into a distinct brain and nerve cord. The nervous system of *Platynereis* is centralised too, which led Detlev to wonder if Urbilateria also boasted a centralised nervous system. If so, that would provide evidence for Dohrn's theory.

To answer the question, he set out to show that the nervous systems of *Platynereis* and vertebrates were suffi-

ciently similar to rule out convergent evolution - when a trait arises independently in two unrelated species meaning that they could only have been inherited from a common ancestor. He and his team mapped the molecular fingerprints of neurons in Platynereis and compared them with those of vertebrate neurons. "We wanted to find out what makes a cell specific at the molecular level or, in other words, how a cell type is defined molecularly," he says. One way to trace a molecular fingerprint is to perform microarray analysis of single cells and identify all the genes that are expressed in those cells - but this is timeconsuming. Detlev's strategy is to home in on genes of potential interest, or candidate genes, and to see if the transcription factors that regulate those genes, and the receptors, transmitters and other molecules they in turn control, are active in cells.

Identifying the candidate genes was itself a challenge. "Because we lacked a lot of genomic information for *Platynereis*, most of the crucial genes were simply not available to us," explains Alexandru Denes, a PhD student in the lab. "I cloned them and then I assembled a rough picture of the neuronal patterning and specification. When I did the first stainings for these candidate genes, I was amazed to see how similar the patterns were to those in the vertebrate neural tube." The details of this rough picture were then filled in using a technique devised by postdoc Gáspár Jékely, which involved scanning the wholemount *in situ* hybridisation patterns of more than 30 genes in developing neural tissue that was counterstained with antibodies labelling earlyoutgrowing axons by confocal reflection microscopy. "When Alex and I started using this technique, we often stayed in the confocal room for hours, just to watch these beautiful images," says Gáspár.

What did the beautiful images reveal? In both *Platynereis* and vertebrates, the developmental precursor of the nervous system is the neuroectoderm. This tissue is symmetrical, and between the midline of the tissue and each side it is divided into four domains of progenitor cells. These domains have been known about in vertebrates for some time, but the confocal images showed that the transcription codes that define them are almost identical in the worm. "These are transcription factors that act independently of each other in other developmental contexts, such as eye or muscle development, so we can rule out the possibility that they were recruited together, as a package," says Detlev.

It cannot be coincidence that the same array of transcription factors was co-opted in the two species, he adds: "This was not invented twice, it's the same system." Both vertebrate and invertebrate neural progenitor cells turn



Detlev's favourite pet: the annelid worm *Platynereis dumerilii* The molecular fingerprinting approach has already thrown up some surprises in the Arendt lab. A photoreceptor buried deep in the *Platynereis* brain turns out to be related at the molecular level to the rods and cones in the vertebrate eye – a finding which has important implications for human eye development.

More recently, the group has found that proteins expressed by *Platynereis* brain cells resemble hormones secreted by the vertebrate hypothalamus, throwing doubt on the notion that the hypothalamus was a vertebrate invention, and suggesting instead that it evolved from cells that once had both sensory and neurosecretory functions. The hypothalamus regulates metabolism and reproduction in vertebrates, but the exact function of those neuropeptides in *Platynereis* remains a mystery.

out to be sensitive to a signalling cascade that is known to regulate neural patterning in vertebrates – the Bmp pathway. And the corresponding domains give rise to the same kinds of neurons in the two systems. "I started the project with a healthy dose of scepticism," says Alexandru, "but when, for the third time, a newly cloned transcription fac-

tor fitted seamlessly into our vertebrate-like model, my doubts evaporated."

If Urbilateria's nervous system resembled that of *Platynereis*, then something must have happened during evolution such that the nervous system which is located in the worm's belly came to be found in the vertebrate back. The nervous system need not have shifted relative to the other organ systems; the whole body could simply have

been turned around. Critics of Dohrn's theory have argued that this is impossible, but Detlev believes it isn't hard to imagine a scenario which could have given rise to it. "It wasn't sudden or dramatic," he says. "It probably happened in a marine environment. A bottom-dwelling or burrowing ancestor may have gradually become a filter-feeder, half-buried in the substrate and protected by huge bony plates, until an early vertebrate left the substrate and swam. In this way, inversion could have happened without any change of anatomy."

One puzzle remains, however: if Urbilateria had a centralised nervous system, why do the centralised nervous systems of some of its other descendants, the fruitfly *Drosophila* and the nematode *Caenorhabditis elegans* for example, look so different to that of *Platynereis*? Detlev thinks the answer to this question illustrates an important

It's a big problem with the evolutionary concept so far that loss has been underestimated – functional loss, cell type loss, gene loss, everything.

principle in evolutionary biology that is only now coming to light. "It's a big problem with the evolutionary concept so far that loss has been under-estimated – functional loss, cell type loss, gene loss, everything," he says. In the case of the fly and nematode nervous systems, he believes, many ancestral features were modified beyond recogni-

tion or lost completely, while other features were gained.

Historically, phylogenetic trees have been constructed on the basis of morphological similarities between species, but morphologists could only see what was there, not what wasn't there. "With the molecular fingerprint, we can show that the same cell type may be involved, even if the morphology is different – and that the cell type was inherited

from a common ancestor," says Detlev. This may be why so much of classical phylogeny has turned out to be wrong, he thinks, and why so many of the relationships between species have had to be rethought – because the phylogenetic links between them were hitherto invisible. If he is correct, we could be about to see some major revisions of our family tree.

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Putting science under the microscope

T THE BEGINNING OF THE 20TH CENTURY, calling a scientist a 'creative mind' could have been taken as a serious insult. Creativity and imagination were reserved for artists, musicians and writers. Scientists worked with other tools: reason, rigorous analysis, objective observation; qualities not to be contaminated by something as subjective and vague as creativity. Almost 100 years later, when a Group leader at EMBL dares to pursue the topic of science and creativity, times seem to have changed quite a lot.

Normally, Carl Neumann studies how organs and limbs of zebrafish embryos form during development. "Even if you would not expect it, investigating the development of fish often requires a lot of creativity," Carl explains. "If an experiment does not work out the way you planned it or your results don't match your predictions you have to be very creative sometimes to generate new ideas and models." These experiences led Carl to suspect that creativity might be much more important for science than was assumed for a long time. Progress in science depends on conceptual and technological innovations and these in turn need creativity: such is Carl's hypothesis. And with this idea he finds himself in high-ranking company. Famous physicist Max Planck, for example, claimed that a pioneer scientist must have "a vivid intuitive imagination, for new ideas are not generated by deduction, but by artistically creative imagination."

Rather than relying on support from great minds of the past, Carl approached the question of creativity in science in a more empirical way and carried out systematic inves-

EMBL ANNUAL REPORT 06.07

tigation. He put zebrafish specimens aside for a little while to put subjects of a very different nature under the microscope: his fellow Group leaders. In a series of interviews with 15 colleagues, Carl gathered their ideas about their biggest scientific breakthroughs, the characteristics of a good scientist and the role that creativity played in their work. "Most agreed that creativity is absolutely essential to scientific research," Carl reports. Apart from the traditional qualities of a scientist, such as a rigorous intellect, analytical thinking and the ability to get the job done, most interviewees insisted that to be successful a scientist needs to have creative ideas.

But what exactly is creativity and where does it enter science? "Creativity is coming up with an approach that leads to a new discovery. At the moment of insight we're creative because we make a new connection in our mind – or we see a connection that was already there but hidden," explained one of the interviewed Group leaders, while another one said, "Creative scientists have the ability to step back from what's happening in the lab and look at the big picture and get things in perspective."

So, clearly the synthetic nature of creativity is important in science; it helps to draw together different and even seemingly unrelated aspects of a problem into a new understanding. But according to the most common models of creativity there is more to it than just synthesis. Analysis is equally important. In fact, creativity is often described as a balance between analytical thinking, needed to take apart and investigate the details of a situation,



Science means thinking outside the box.

and synthetic thinking, which puts the parts back together in a new, meaningful way.

"This is precisely what happens in science," Carl says. "You break down a system into individual components that you can study in experiments and then you put them back together to generate a new theory based on your findings. Something very similar happens when an artist paints a picture, but in art this often happens unconsciously. Still, the same creativity appears to be required in arts and in science."

Unlike in the arts, however, in science it is not so much the creativity of the individual that counts, but the collective creativity of an entire community. All of the interviewed Group leaders emphasised that on the way to their biggest scientific breakthroughs, input from colleagues was crucial. "Feedback on a new idea, technical advice on how to solve a problem or simply inspiration by colleagues' enthusiasm or a clever approach they developed – it comes in many shapes and sizes, but interaction with peers is very important to stimulate creativity in science," says Carl. One of his interviewees confirms: "Although the actual idea and the experiments came from myself, I couldn't have done it if I hadn't been in the right lab at the right time, with the right colleagues around."

So, the tale of the lonely genius working days and nights locked away in his lab until he finally has a 'Eureka!' moment and solves a longstanding scientific puzzle, is often an oversimplification. Instead, the tale of science should be one of creativity and culture. Interaction and discussion with colleagues are as important to a scientist as logical thinking, technical skills and scientific expertise. "It's by listening and talking to people that I get my inspired thoughts. As I listen to them, I immediately try to find a way to use their approach for my own question," explaines one of the scientists about the collective creativity that emerges from discussions in the lab. If collective creativity is the way to good science, can we do anything to help stimulate this creativity? The answer lies in the environment that scientists work in, says Carl. In his interviews he tried to determine the best conditions to generate and foster creativity in research. "You need an environment that is not too set in its ways and allows you to explore new ideas," Carl paints the picture of the ideal research institute. "It should also encourage communication between people, for example by organising official meetings, but also by providing informal settings where people can exchange ideas."

An excellent example of how this can work is the various scientific centres at EMBL. These networks of scientists have a thematic focus, such as computational biology or biological imaging, that spans units and disciplines and promotes exchange of ideas through seminars, conferences and training activities and most simply by just encouraging people who would not be in the same lab to meet and talk together.

The organisational structure of an institute also seems critical. Whereas rigid hierarchies often impede open communication, more free-flowing arrangements encourage people to talk across levels and scientific disciplines. The result is what Carl calls 'cross-fertilisation'. "Often the best results come from talking to people with the most different backgrounds or from different cultures. They can offer a completely new perspective on your work," Carl explains. "The more diverse a community, the better it stimulates creativity." This is especially important in areas like systems biology that are based on a combination of several traditional disciplines such as chemistry, computer science, mathematics and biology. To integrate the disciplines you first have to integrate the people and that only works when the environment is right, says Carl.

An interactive environment, free-flowing hierarchy, international and interdisciplinary community – any of that sound familiar? These factors have been a part of EMBL for more than 30 years. Recently, the Howard Hughes Medical Institute has opened a new research institute designed as an experiment to generate ideal conditions for creativity. Strikingly, Janelia Farm, as the institute is called, shares many of its distinctive features with EMBL. Concepts like small research groups, Group leaders that still carry out benchwork in senior positions and regular turnover of staff were newly implemented at Janelia Farm to maximise the creativity of its researchers. These are the same key concepts that informed the original foundation of EMBL.

So, in theory EMBL should be oozing with creativity. And in practice? "We have always tried to leave our scientists a lot of space for creativity," says Director General



The annual labday is a chance for everyone at EMBL to mingle in an informal setting and find out what the colleagues are working on.

Iain Mattaj. "In fact when new Group leaders start at EMBL we don't expect them necessarily to come with a precise plan or to publish quickly. Most arrive with a rough idea and let themselves be inspired by their colleagues and the technical possibilities available. The main role of the institute is to foster creativity and provide the means and the time needed to make creative ideas reality." The concept seems to work. "This place is really inspiring," confirms one of the interviewed Group leaders. "Before I came here, I hadn't looked for other serious influences that would push me in a different direction. Here you are almost forced to change your tune."

The same must have happened to Carl himself when he embarked on a project as different as his survey on creativity in science. When asked how he became interested in creativity, Carl replies: "I have always felt that something about this place is different and I wanted to find out what, but I needed inspiration as to how to do that. The idea itself was sparked off by a chat with Halldór Stefánsson, who heads EMBL's Science and Society programme. With his knowledge of sociology and philosophy of science, he helped me find a way to address the questions I wanted to ask. Here you really see collective creativity at work."

So, it turns out that Carl's creativity study is none other than a creative idea inspired by EMBL's special culture. \blacksquare

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CEOSE IEN COR Genes, brain mind and behaviour

7th EMBL/EMBO joint conference on Science and Society

EMBL HEIDELBERG, GERMANY 3-4 NOVEMBER 2006



Genetic and Neural Control of Drosophila Sexual Behavior

Scientists and ethicists minding the brain

Highlights from an interdisciplinary meeting on behavioural genetics and the neurosciences

OU THINK YOU'D NEVER BE TAKEN IN by a confidence trickster. But what if he had access to a drug that would make you trust him more? Or imagine what you would do if your child had ADHD and you were offered a drug to treat it – a drug which would fundamentally alter his personality forever. Such issues were just some of the subjects arising in 2006's EMBL/EMBO Science and Society conference, 'Genes, Brain/Mind and Behaviour'.

It has been shown that genes don't really cause behaviour on their own, but only partially specify how an individual will react to environmental circumstances. One of the great scientific challenges of the moment is to integrate the results from two different kinds of investigation into the biology of behaviour – that of our genes and that of our brain – to gain a deeper understanding of both.

Behavioural genetics and the different branches of the brain sciences continue to reveal new findings about many of the fundamental determinants of animal and human behaviour. But it's vital to predict the socio-cultural repercussions of new know-how and technologies coming out of the brain sciences and to make sure that new applications are for the benefit of all – individuals as well as societies. Broadly divided into two halves, the Science and Society conference in 2006 looked at the thriving fields of behavioural genetics and neurosciences, and then at the uses of the knowledge and the new technologies that have grown out of these sciences. Finally, it considered the impact that their application is having, or is expected to have, on society. Genes are seen as acting as determinants of behaviour when they condition the assembly of neural circuits necessary for the development and survival of the whole organism. There is a growing body of knowledge about how phenotypic variation relates to, and influences, distinct patterns of behaviour. Researchers recognise the importance of environmental factors in the development of living organisms, but have produced solid evidence showing how genes modulate basic behaviour. At the conference, several speakers reviewed how pleiotropy, epistasis, interactions between genes and the environment, alternative splicing and neuronal integration are all crucial mechanisms contributing to the many and varied aspects of brain-related genes.

But then how does a central nervous system, a brain that we know is the product of evolutionary history embedded within our genome, cause behaviour? The passage from genes to brain is highly complex, but molecular and cellular mechanisms underpinning gene expression and brain function are gradually being elucidated. A vast body of knowledge has accumulated on brain physiology, its structure and function. However, knowledge of how neurons are interconnected and form systems and networks is relatively underdeveloped.

The biggest challenge is to understand how the intricate neural structures of the brain give rise to consciousness and a sense of 'self'. Neuroscientists remain confident that they now have the tools to solve this mystery. The second main research topic that came under scrutiny at the conference was neuronal organisation and cognitive func-



Invited speakers Judy Illes and Adina Roskies engaged in an audience discussion about neuroethics.

tioning of the brain – how basic molecular mechanisms and neural networks give rise to awareness. For instance, Wolf Singer from the MPI for Brain Research in Frankfurt, Germany, explained how neurons encode information in at least two ways: by varying the amplitude of their response, and/or by adjusting the precise timing of electric discharges. He proposed that cortical networks use both coding strategies in parallel, exploiting their respective advantages. Synchronisation of responses across distributed cortical networks or neuronal assemblies seems to allow the fine-tuning of cognitive processing and the emergence of consciousness.

The second group of talks dealt with various applications of science to monitor and map the brain, and to intervene with or influence human behaviour, and the ethical questions that many such applications entail. 'Neurotechnology' refers to the set of tools that have been developed to analyse, cure and enhance the human nervous system, especially the brain. At the leading edge of neurotechnologies are various forms of brain imaging and scanning technologies and neuropharmacology. Thomas Dierks, from the University Hospital for Psychiatry in Bern, Switzerland, explained how efforts to understand the organisation and function of the brain on a systems level have been made possible by the development of powerful methods of non-invasive neuroimaging. These techniques not only have been used for understanding normal brain function, but also provide new insights into the physiological basis of neuropsychiatric disorders. Their future uses may eventually extend to various forensic and commercial purposes such as the mapping of consumer preferences.

Any drug capable of affecting the mind, emotions and behaviour qualifies as being psychotropic. The production of such drugs constitutes a lucrative part of the modern-day pharmaceutical industry. Attention deficit hyperactivity disorder (ADHD) is a vague but widely applied disease category in Europe and the USA. Ilina Singh from BIOS at the London School of Economics, UK, reported at the conference on her investigation of children subjected to Ritalin treatment, including how they assess their own behaviour and self-esteem, which provided a unique insight into the subjective side of engineered mind-altering behaviour.

Degenerative diseases such as Alzheimer's and Parkinson's diseases are among the biggest health problems today. In the view of Lars Sundstrom from Capsant Neurotechnologies LTD in Southampton, UK, in more predictable systems to test whether drugs work are needed, so that important resources aren't wasted on useless clinical trials. A new approach that he advocates, chemical genomics, systematically exposes slices of cultured brain tissue with engineered pathological symptoms to chemicals, looking for those with desirable effects.

But future neurotechnologies will not be limited to medical use alone, as shown by the emerging field of 'neuroeconomics'. The aims of this new area of research are to analyse the neurological determinants of decision-making and their social and economic implications. Michael Kosfeld from the University of Zürich in Switzerland gave an introduction to neuroeconomics in a case study of the neurobiology of trust. Trust in human relations is largely determined by social and institutional conditions, but some economists have developed a keen interest in a possible biological basis - the neurobiological mechanisms that are responsible for the ability to trust. In arguing that the hormone oxytocin permits, facilitates or enhances human trust, he described a study that suggested the positive impact of oxytocin on trust in interpersonal relations. Assuming this substance was effective and found its way into commercial applications, its potential would also cause serious ethical concerns.

The final part of the conference was concerned with ethics. Attention focused on whether recent developments in the brain sciences justify the establishment of a new branch, 'neuroethics', within the field of bioethics. Actually, the term seems to have two meanings. On the one hand it is used to refer to the assumed existence of hard-wired moral dispositions with verifiable loci in the brain. Here it implies the possibility of a scientific study of ethics as a human disposition acquired through evolutionary processes; it might then be able to provide muchneeded foundations for the established field of evolutionary psychology. On the other hand, neuroethics is also



commonly used to refer to ethical concerns about sociocultural repercussions of new knowledge and technologies coming out of the brain sciences. Thomas Metzinger from Johannes Gutenberg University in Mainz, Germany, argued that the brain sciences will eventually enable us to assess moral judgements in a 'value-free' fashion. Neuroethics, or 'consciousness ethics', should aspire to become a scientific form of descriptive ethics. Others have objected to such attempts at naturalising ethics, pointing out that although they may be grounded in a jumble of neural devices connected with the origin of emotion, they are ultimately man-made.

In their talks, Adian Roskies, from Dartmouth College in Hanover, USA, and Judy Illes, from Stanford University, USA, argued for neuroethics as a field within bioethics to deal with issues and concerns over the anticipated uses of neuroscientific technology. In their view, neuroethicists should observe and monitor how the brain sciences develop and introduce new ways of enhancing, controlling and reading the brain. According to them, the stakes may be high. What if new tools become available to allow us to distinguish lies from truth, true versus false memories, the risk of future violent crime, styles of moral reasoning, the inclination to cooperate, and even specific contents of thought? Erik Parens from The Hastings Center in New York, USA, expressed reservations about neuroethical directions in bioethics, arguing that dividing bioethics up into multiple branches, each focusing on separate sets of issues, would mask the underlying commonalities between issues relevant to the uses of science and technology. Finally, Raymond G. de Vries from the University of Michigan in Ann Arbor, USA, suggested that along with its declared objectives, from a sociological perspective neuroethics is just as much about the mindset and interests of it practitioners.

Organised over two days, this multidisciplinary event brought together scientists, philosophers, social scientists, policy makers, consumer associations and members of the public for inspiring debate. The annual Science and Society conferences have become an important forum for breaking down communication barriers between scientists and non-scientists and for promoting mutual interest, understanding and dialogue on subjects that concern everyone.

The 8th annual EMBL/EMBO joint conference on Science and Society, 'The future of our species – Evolution, disease and sustainable development', will take place at EMBL Heidelberg on 2-3 November 2007. For more information, visit http://www.embo.org/scisoc/conferences.html.



Bridging the scales

N THE SPRING OF 1940, the American novelist John Steinbeck and his good friend, marine biologist Ed Ricketts, set sail on an expedition of science and adventure around the Gulf of California. Both were driven by the idea that by taking a holistic view of organisms and how they interacted, one could gain a deeper understanding – see the big picture – of what life is and how it works.

On their travels, the pair collected and studied hundreds of marine creatures. Steinbeck's account of the trip, *The Log from the Sea of Cortez*, documents how these studies strengthened the friends' conviction that wholes in living systems emerged from the cooperation of their individual parts and that these wholes were somehow greater than the sum of their parts.

Today, modern biologists are embarking on a similar adventure, albeit an intellectual one. This time, their quarry is not tide-pool creatures, but molecules and cells; their instruments are laser microscopes and computers, rather than nets and collecting-bottles. But the idea is the same: to see how molecules and networks interact to produce a greater whole, such as a living cell.

The name of this burgeoning field is systems biology, and it aims to link all levels of biological organisation together: to bridge the gaps between different scales, from molecules up to organisms and even ecosystems. This global view will help transform biology from a largely experimental science to a more theoretical one, allowing scientists to make predictions about how systems will behave or where genes and molecules of interest may lie.

Scientists at EMBL are taking the lead in these systems approaches, such as studying the global function of the cell's powerhouses, its mitochondria, and relating this to human disease, or working out how connections between brain cells help us form memories. Other groups are looking at how molecules come together within the cell to form structures like the cytoskeleton, the cell's internal scaffold, and how this can produce cell behaviour.

So, having departed somewhat from the holistic view when scientists discovered molecular biology and rushed to focus in on the details, biology has come full circle, back to looking at the big picture. As Steinbeck noted, while pondering the nature of individuality in the colonial sea-squirts he was studying: "I am much more than the sum of my cells and, for all I know, they are much more than the division of me." There is "no quietism in such acceptance," he concluded, "but rather the basis for a far greater understanding of us and the world."



Eric Karsenti and Peer Bork talking systems biology

A guided tour of systems biology

Peer Bork, joint Head of the Structural and Computational Biology Unit, and Eric Karsenti, Head of the Cell Biology and Biophysics Unit, take us on a guided tour of systems biology at EMBL.

What is systems biology?

Peer: There are many different definitions, but all boil down to achieving a comprehensive understanding of how interrelated components of a system form higher order entities. When such entities emerge they develop new properties. By definition, these so-called emergent properties are not amenable to classical, reductionist analysis, which involves pulling a system apart and studying the different elements in detail.

Eric: Biology has always been about complex systems – that's not new. To understand a system you need first to identify the parts, and then to understand how the parts interact with each other to generate behaviour. Both the reductionist and the systems approaches are therefore necessary.

If biology has always been about systems, why does one get the sense that systems biology is a young field?

Eric: For a long time biologists tried to understand biological organisms without having a clue about their molecular underpinning. Then, in the 20th century, the pendulum swung the other way and molecules became the focus of attention. As a result, biologists lost sight of the bigger picture. Now, with the new tools that are available, we can go back to the fundamental question we are trying to answer which is, what is a living organism? You could see this new emphasis on systems as a backlash to what had become extreme reductionism.

Peer: I think it's also a generation issue. In the last ten to 20 years, tonnes of molecular data have been generated and a new generation of scientists has emerged who identify themselves as data-driven biologists. They are, perhaps, more inclined to think in terms of systems.

What are the tools of systems biology?

Eric: First, the tools of molecular biology - those that have generated large amounts of data on the molecular components of living matter. Bioinformatics has been very important in this context, particularly the organisation and management of large databases that goes on at the EBI, for example, or at the US National Center for Biotechnology Information. And second, the visualisation of dynamic phenomena using high-resolution microscopy. In the cytoskeleton - the cell's scaffold or skeleton - field, for example, people have been looking at dynamic processes at the molecular level for almost 20 years - people with a background in physics or biophysics. When they saw how incredibly dynamic the inside of a cell is, they realised that it would be necessary to use mathematics and physics to build computer models of those dynamics. This together with the genomic data has produced a very powerful research tool, because you can not only look at molecules and proteins inside the cell, but also perturb them and measure the effects against your models' predictions.

Can you point to one event that gave birth to the 'new' field of systems biology?

Peer: In 2000 the molecular biologist Leroy Hood cofounded the Institute for Systems Biology in Seattle, USA. The term 'systems biology' had been out there before, but it was thanks to Leroy that it took off. The timing was right for it, because lots of quantitative data that you need for systems analysis were being produced. People coming from bioinformatics, or from protein and cell engineering, and others who were interested in self-organising principles – all were beginning to think in this more global way.

Eric: The notion of systems biology came from various directions and took shape a bit like a collective behaviour in self-organising systems...or fashion in general! However, one guy who has been important for systems biology at EMBL was Stan Leibler, a physicist who is now at the Rockefeller University in New York. Stan has always been interested in networks. He came here for a sabbatical around 1997, because of discussions he and I were having about self-organisation in the cytoskeleton and the structure and role of global network behaviours in living systems. During this stay he built the first artificial regulatory network inside a bacterial cell, with physics student Michael Elowitz. That triggered a whole series of network studies throughout the world. The former co-coordinator of EMBL's Structural and Computational Biology Unit, Luis Serrano, also became interested in such questions and influenced the lab quite significantly.

Will cell biology, structural biology and developmental biology ultimately merge into systems biology?

Peer: There are several buzzwords in systems biology. One is 'bridging scales', in other words bridging the

molecular and cellular worlds, which is very difficult, though we have big projects trying to do this. This speaks for a merge, but on the other hand I have already heard the terms 'molecular systems biology' and 'cellular systems biology' which again indicate specialisation and a niche-finding process. But essentially it is all biology!

Eric: To understand what life is about, you need people working at all the different scales – molecule, genome, cell and organism. At each scale, people work with slightly different concepts and techniques. That is why EMBL provides such a wonderful opportunity for the pursuit of systems biology, because all the different scales are represented here. Very few labs in the world are organised in this way.

Can you give some examples of EMBL's approach to systems biology?

Peer: In our Structural and Computational Biology Unit, for example, we have a project on mycoplasma, one of the smallest bacteria. The idea is to build up a picture of the entire bacterial cell, which only contains 700 different proteins, at atomic resolution. At the moment, you can see a cell with a microscope, but you can't really zoom inside it. You can also study single molecules, but not what they are doing in the wider context of the cell. We want to bridge the scales, and we are doing so using many different datasets and technologies. It's a very ambitious project, and we have eight or nine groups working on it, all from different backgrounds.



Systems biology allows scientists to tackle complex and dynamic systems, like the interaction of proteins during the yeast cell cycle.



Eric: In the Cell Biology and Biophysics Unit several groups are working on microtubules, the filaments that form the mitotic spindle. The spindle segregates a cell's chromosomes before it divides into two daughter cells. It is highly dynamic and the problem has been to understand its shape, which is critical to its function. How does the shape emerge from the collective behaviour of many molecules? We need to identify the molecules, to visualise the dynamic properties of the spindle, and then to model the system. Like Peer, we have brought interdisciplinarity to bear on the problem.

What is the future of systems biology at EMBL?

Eric: Twenty years from now, we will probably understand the systems properties of some aspects of cell organisation, such as the cytoskeleton and the regulation of gene expression. Cells are very robust, but at the moment nobody really understands how they manage to produce the right amounts of the different cellular proteins. We also know very little about the link between the shape and function of the cell, and gene expression, which is extremely important for understanding evolution and development in multicellular organisms.

Peer: We will tackle more complex systems. After cellular organisation, the focus will be organ development. At EMBL now there are groups working at the interface between cell biology and developmental biology, trying to understand, for example, how simple organs are generated in the fish. Beyond that, we will need to understand

organisms, organismal communities and bigger ecosystems like Earth. Just like the butterfly that flaps its wings in Madagascar and causes a hurricane in America, different scales interact – from the molecule to the entire ecosystem – and we will need to explore those interactions.

Will our view of biology be changed or enhanced by the systems approach?

Eric: There are whole fields of research, mainly theoretical, which look at the collective behaviour of systems, selforganising principles and emergent properties, for example in insect societies. Until recently, these have been somehow cut off from molecular, cell and developmental biology, but now their concepts are beginning to have an influence on scientists working in these areas of biology. Evolution, ecosystems and food chains are starting to be understood in this way. There are lots of concepts that physicists have, that biologists don't have, but there is also lots of knowledge that biologists have that physicists don't have. The two worlds need to come together to understand the essence of life.

Peer: We're not changing our view, we're adding to it. Adding different skills or cultures to biology in a complementary way is always positive, whether it's physics, mathematics, engineering or chemistry. The timing has got to be right, though. We still need more people who think globally, who are not focused on their own narrow problems. This kind of thinking will come, in time.



François Nédélec with his cutting-edge computer simulations and some more traditional models of microtubules.

Beauty in symmetry

N FRANÇOIS NÉDÉLEC'S COMPUTER SCREEN, a rod-shaped yeast cell is pulsing and vibrating. Inside it, bundles of fibres or microtubules constantly appear, elongate and vanish, appear, elongate and vanish – creating a dynamic scaffold that seems to press out on the cell's ends while simultaneously massaging its nucleus. As the timelapse movie proceeds, it becomes clear that the cell is growing. As it does so, thanks to the fibres' massage action, the nucleus remains at its centre, and the cell retains its overall symmetry. "That's very important," François explains, "because a cell that is symmetrical works more efficiently."

Grasping a bunch of pencils he now starts moving them around on his desk like Mikado sticks, to help explain the problem his group is grappling with. In the cell system we have just been watching, *Schizosaccharomyces pombe* or fission yeast, microtubule bundles not only organise the cell structurally, but also help to coordinate its growth by delivering growth signals to its extremities. To do so, they must be ordered in stable arrays. However, microtubules are born pointing in random directions, and if they grew that way the result would be a mess. So how do they align themselves, and how is order created out of apparent chaos?

It's an important problem because microtubule arrays perform a wide range of critical functions in cells. Microtubules are polarised, with a growing 'plus' end and a shrinking or static 'minus' end. They can be organised with their plus ends aligned, in a parallel array, or alternating, in an antiparallel array – depending on the func-

EMBL ANNUAL REPORT 06.07

tion they are required to perform. In neurons, for example, parallel arrays are responsible for guiding packets of neurotransmitters along the axon, the neuron's long, slender projection. In dividing cells, by contrast, a bundle of antiparallel microtubules called the mitotic spindle segregates the chromosomes, pulling them in opposite directions into the daughter cells. But the problem is also a challenging one to study, for two reasons. Many different proteins are involved in the formation of microtubule arrays, and the dynamic interactions of those proteins are still poorly understood.

Until a few decades ago, the only way to investigate microtubule behaviour was to kill cells and look at them under a microscope. Now it's possible to attach green fluorescent protein to proteins of interest and watch them move around in living cells. This technique tells you about the dynamics of microtubules, but if you want to actually dismantle the microtubule system and understand how it is put together, you need a different approach – one that François has been pursuing for the last two years in EMBL Heidelberg, in collaboration with cell biologist Marcel Janson in Phong Tran's lab at the University of Pennsylvania, Philadelphia (currently based at Vrije University in Amsterdam, the Netherlands).

For some years now, Marcel has been studying microtubules in cultured *S. pombe* cells – a useful model system because bundles only contain three or four microtubules, as opposed to the hundreds in a human mitotic spindle. By removing a specific protein from that system, or starting from scratch and mixing purified components, he has Computer simulations are an important, new tool to study cellular organisation. Fission yeast cells often organise microtubules (grey) in a complex manner with the help of crosslinking proteins (green) and motor proteins (red).



been able to tease apart many of the essential components of microtubule organisation in yeast. François and his group take that experimental information and feed it into computer simulations they have built to model protein interactions, with the help of funding from the Centre for Modelling and Simulation in the Biosciences in Heidelberg. Their computer program, Cytosim, not only simulates the collective behaviour of the fibres and associated proteins, but also creates scenarios in which the molecules are given hypothetical properties, and then explores whether those scenarios lead to the construction of stable structures. If they do, the scenario can be tested experimentally.

The collaboration is therefore a prime example of the systems biology approach, in which the study of interactions between components of a system leads to the building of models which recapitulate all the interactions in that system. The models allow predictions to be made about the behaviour of the system which can, in turn, be tested. "The computer simulation and experimental approaches are intimately linked," says François. "Experiment shows you the steps that are necessary to explain what you see. With the computer model you can then remove the steps one by one, to show that they are also sufficient. You end up with the whole story."

By 2005, this approach had illuminated key components of microtubule organisation in *S. pombe*. Marcel's group had made the important discovery that new microtubules are not born, or nucleated, at random throughout the cell, but on already existing microtubules. They had identified a bundling protein, ase1p, which crosslinked antiparallel microtubules within bundles. And they knew that motor proteins called kinesins were somehow involved in sliding new microtubules around in the cell. One problem still bothered them though: their observation that some microtubules were able to slide in the cell, while others appeared stably fixed.

A light bulb went on when Damian Brunner of EMBL Heidelberg discovered that the kinesins that slide microtubules along each other only bind to the plus ends of those microtubules. "When we discussed our work over a coffee at EMBL in 2005, the importance of this finding became very clear," says Marcel. "Microtubule sliding will stop abruptly when its plus end slides off an underlying microtubule." Armed with this insight, they went back to the drawing board, and have now come up with a nearly complete picture of microtubule organisation in *S. pombe*.

Over to François and his pencils. When a new microtubule is born, he explains with the help of his visual aids, it is attached at one end to an existing microtubule. Because it is still short it may flip-flop, aligning itself alternately parallel and antiparallel with that other microtubule. Only if it lies down in the antiparallel direction do the crosslinks form to bind the two together. At the same time the new microtubule is growing, and a motor protein, kinesin-14, is pulling it along the underlying one. "You always have a constant number of motors pulling on the end, but the number of crosslinking proteins, the brake, is proportional to the length of the microtubule," he says, "so as the microtubule grows, it gets harder and harder to pull." The sliding slows down as the microtubule grows, and one microtubule is prevented from sliding completely past another, because the kinesin-14 is only bound at one end and requires a substrate to crawl along.

At this point, François abandons his pencils and reaches for an analogy: "In a tractor-pulling competition, the tractor moves forward, dragging a load which is gradually increased, making the job progressively more difficult." The result is an ordered, antiparallel microtubule array that confers structural stability and symmetry on the cell.



Microtubules start growing from nucleation complexes that associate with their minus ends (blue). Motor protein klp2p (red) slides growing microtubules (grey) past each other and bundling protein ase1p crosslinks antiparallel microtubules. The longer the microtubules grow, the more as1p binds to them. The developing braking force slows down the movement until the two forces are balanced and the sliding stops.

Rose Loughlin, who came to François' lab as an undergraduate from Harvard University and spent ten months there before joining the Biophysics Graduate Group at the University of California, Berkeley, was responsible for the computer modelling that showed these steps did indeed generate the desired end product. "We used Cytosim to show that the intricate opposition of microtubule bundling proteins and microtubule motors is sufficient for the establishment of a dynamic microtubule bundle with a stable centralised region of antiparallel microtubule overlap," she says. François has now turned his attention to a more complicated system, and the Holy Grail of his research: the mitotic spindle. "Having more microtubules is not so much of a problem," he says. "But there may be many more proteins involved in the spindle, and at the moment we don't know exactly how many are implicated." He has already started looking at the spindle in different organisms – the worm *Caenorhabditis elegans* and the frog *Xenopus* – though the group hasn't yet developed computer simulations for these species. He believes that many of the components of these systems will turn out to be the same, though they may be put together differently. Ultimately, he would like to understand how the mitotic spindle forms, and segregates chromosomes, in human cells.

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The house guest that stayed

Lars Juhl Jensen, Fabiana Perocchi, Lars Steinmetz and Julien Gagneur

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Foreground: A schematic diagram of the 876 proteins found in the mitochondria and the 9780 connections building up the mitochondrial network. Background: an electron microscope image of a mitochondrion.

BOUT TWO BILLION YEARS AGO, ancient bacteria were engulfed by free-living, single cells and formed symbiotic relationships with them, living as bacterial colonies inside those cells. The cell came to depend on its mitochondria, as those colonies became known, mainly to produce its energy but also to perform other functions such as regulating programmed cell death – a natural part of the cell's life cycle. Because of that dependency, mitochondria now contribute to a large burden of human disease, which is why Lars Steinmetz would like to understand how they work.

He studies mitochondria in yeast, because yeast is a eukaryotic organism, its cells have a nucleus, placing it in

the same branch of life as humans, and its mitochondria are similar to those of other eukaryotes, including humans. His goal is to construct a map of all the interactions that take place between mitochondrial proteins in a cell, and to understand why disruptions in those networks lead to disease. He takes a systems biology approach to the problem, drawing on information generated by different molecular biological techniques, across a wide range of organisms.

Over the course of evolution, the mitochondria transferred many of their genes to the cell's nucleus, or simply lost them. Although the yeast Saccharomyces cerevisiae has around 800 mitochondrial proteins, only eight of these are encoded by mtDNA.

"The mitochondria are particularly attractive for that purpose, because their function has been highly conserved through evolution," says Lars. "Not only can we test technologies in yeast, but also we can transfer some of what we learn directly to humans. The prediction of human disease genes is one example of that."

But mapping mitochondrial interactions is not easy, for one simple reason: not all mitochondrial proteins are encoded by mitochondrial DNA (mtDNA). Over the course of evolution, the mitochondria transferred many of their genes to the cell's nucleus, or simply lost them. The result is that although the yeast *Saccharomyces cerevisiae* has around 800 mitochondrial proteins, only eight of these are encoded by mtDNA – the others are encoded by nuclear DNA. In other words, the mitochondria must recruit proteins from other parts of the cell to perform their functions. Just over half of the mitochondrial proteins in yeast are known, but that's not enough to build a picture of mitochondrial function.

Lars wants the global view. He made a first stab at getting it in 2002, while still at Stanford University in the USA, where he was involved in constructing a set of deletion strains of *S. cerevisiae*. In each strain, one gene was deleted and replaced by an identical 'dummy' cassette of DNA and a molecular barcode – a tag that meant the strain

> could be identified by DNA microarray, or gene chip, analysis. By growing all the strains together and varying environmental parameters – the food source, for example – Lars and his colleagues could see how they fared compared with one another, and measure the contribution of each deleted gene to the strain's fitness, or ability to reproduce.

> Using this method, they identified almost 500 genes whose deletion impaired mitochondrial function, around half of which were new. They then switched techniques, to proteomics, puri-

fying all the proteins in the organelle and using mass spectrometry to identify them by the mass of their components. Again, they came up with around 500 mitochondrial proteins. When they compared these with the 500 identified by the deletion screen, however, they were surprised. "Here were two global datasets in which we found significant enrichment of mitochondrial components, and yet they overlapped for less than 30 percent of the proteins," Lars says.

This set him thinking. "The deletion screen identifies components which, when knocked out, produce a mildly defective mutant, but it might not show up redundant fac-





Schematic summary of the integrated systems biology approach used to create a map of all mitochondrial interactions.

tors – factors whose loss can be compensated for by other enzymes, for example. The proteomics approach, on the other hand, identifies all the proteins which are found in the organelle, but it's biased towards those that are expressed there in larger quantities. It occurred to us that these different types of information might be complementary, and that there might be an advantage in combining them."

They generated one more dataset themselves, by identifying the fraction of yeast mitochondrial genes which are transcribed into messenger RNA – a predictor of those which eventually generate proteins – and then they went mining the literature for large-scale datasets that other groups had published. They came up with 24 in total, which had been derived using different high-throughput techniques, including computational models that predicted mitochondrial proteins from the signalling pathways associated with them, and phylogenetic studies that inferred yeast mitochondrial components from those known to exist in other species. "Some of these produced very powerful datasets, but none was able to capture all the mitochondrial proteins on their own," he says.

At that point the problem became a computational one, and he called in Lars Jensen, a member of Peer Bork's computational biology group in EMBL Heidelberg, to work with his PhD student Fabiana Perocchi in integrating the 24 datasets and extracting some meaningful information from them. Using computer programs which are capable of 'learning' from the information they process, they came up with a list in which every gene in the yeast genome was ranked according to the probability that its protein product was linked with the mitochondria.

To their delight, they found that almost all of the genes encoding the 500 mitochondrial proteins they already knew about were near the top of the list. Their computational method seemed to work. To test just how reliable it was, however, they took a handful of proteins that the computer had ranked highly, but which were new to them, and put them through some experiments. In collaboration with Uwe Ahting and Holger Prokisch of the Institute of Human Genetics at Munich's Technical University in Germany, they tested the ability of live yeast mitochondria to import these proteins when mixed with them in a dish. This screening method showed that 13 out of 16 of their candidate proteins were indeed imported, confirming their status as mitochondrial proteins – not bad considering the screen itself is not 100 % sensitive, says Lars Steinmetz.

Identifying mitochondrial proteins is one thing, but to know how they contribute to disease you need to know what they do in the healthy cell. Here the researchers had a helping hand in the form of a database that has been under development at EMBL since 2000. STRING, the Search Tool for the Retrieval of Interacting Genes/proteins, is a collection of known and predicted

"It is becoming increasingly clear that biology cannot be understood by studying individual molecules one at a time, nor by studying an entire biological system using a single experimental technique."

protein-protein interactions across a large number of organisms.

Using this database, the two Lars, Fabiana and their colleagues were able to place their putative mitochondrial proteins in the context of known protein-protein interactions, and hence to start to trace out, step by step, the pathways that mitochondria use to perform their vital functions. At last they could begin to build their map.

A survey of the map reveals certain patterns that are likely to hold for humans as well as for yeast. For example, the older the gene, the more important its role, with genes of ancient bacterial origin being more likely to control core mitochondrial functions such as respiration, and to be implicated in disease. To date, says Lars Steinmetz, one in ten of the genes which are known to be involved in heritable diseases has been found to code for mitochondrial proteins. "The mitochondria are implicated in a whole range of disorders from muscular to neurological diseases," he says. "Tissues which have high energy requirements tend to be affected, but the age of onset and the severity of the symptoms can be quite diverse."

To try to disentangle some of this complexity, researchers will need to pay attention to those mitochondrial proteins that modulate disease pathways, as well as to the actual components of those pathways. Here the map will prove invaluable because of the broader connections it reveals. Proteins that interact with known disease-causing proteins may themselves be implicated in disease, at a stroke increasing the number of candidate disease genes which warrant investigation. And the map can be considered a work in progress. As more genome-wide datasets are collected, it can be updated, simply by integrating the new data with the existing datasets and running the computer learning algorithms on the combined whole.

"A global approach to mitochondrial function was the only way to proceed because of the large number of components, the complexity of their interactions and their involvement in so many crucial functions in the cell," says Lars Steinmetz, adding that he thinks this systems approach will now be adopted for other organelles and organisms. Lars Jensen agrees: "It is becoming increasingly clear that biology cannot be understood by studying individual molecules one at a time, nor by studying an entire biological system using a single experimental technique," he says. "Integration of different types of highthroughput data will play a key role in biology in the future."

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Liliana Minichiello from EMBL Monterotondo

Joining the dots

N THE FILM *MEMENTO*, an insurance investigator named Leonard suffers a blow to the head, and as a result he is unable to remember anything that happens to him. This makes it difficult for him to complete his mission, which is to track down his wife's killer, because he is liable to forget each new lead he turns up. To compensate for his disability, he takes snaps with

a Polaroid camera, annotates them, and tattoos important facts on his body.

Leonard has a classic case of anterograde amnesia, which means that he has difficulty learning new information. The condition is a core symptom of Alzheimer's disease, the most common of dementias, yet it is hardly ever depicted in the movies. Hollywood prefers the much rarer condition, retrograde amnesia, which causes a person to forget information

he has already stored, because it offers richer plot possibilities. It was this condition, for example, that in Alfred Hitchcock's *Spellbound* made Gregory Peck forget what he was doing posing as a famous psychiatrist, about to take over the mental asylum.

Although the scriptwriters of *Memento* were a little hazy on the science, Leonard mistakenly says he has a shortterm memory problem, the film vividly depicts just how crippling an inability to learn can be, which is why scientists would like to understand the molecular basis of



learning, in order to be able to devise treatments for when it goes wrong. For a long time, they have suspected that the mechanism might be long-term potentiation (LTP) – the strengthening of connections between brain cells, or synapses, through repeated activation. But the evidence has been largely circumstantial: whenever learning was

> observed, LTP was never far away. Liliana Minichiello and her team have now caught LTP redhanded, strengthening synapses in mice as they learn.

> "Since LTP was first described over 30 years ago, people have been trying to work out exactly what it means," says Liliana, whose group is based at the Mouse Biology Unit in EMBL Monterotondo. "Many studies have looked at behaviour in living mice, and LTP in living slices of mouse brain. What has been missing has

been simultaneous studies of the two, in a living mouse model."

In collaboration with Agnès Gruart and colleagues at Pablo de Olavide University in Seville, Spain, they set out to provide that missing link. The Seville team is expert in associative learning, a kind of learning in which you learn to associate two stimuli, for example a certain radio jingle with the evening news. They wanted to test the ability of mice to learn associations as they simultaneously recorded LTP in a brain structure that is known to be important



for learning – the hippocampus. And they did this both in normal mice and in mice that carried defective versions of a molecule known to be important for LTP.

At least some forms of LTP are triggered when a receptor lying across the membrane of hippocampal neurons, TrkB, is activated by a molecule or molecules binding to it. It turns out that TrkB mediates different signalling pathways inside the cell, mainly via two different binding sites known as the PLCgamma (PLC γ)-site and the Shcsite. In previous work, Liliana's group had shown that only one of these, the PLC γ -site, is implicated in LTP. A specific mutation in the Shc-site affects other signalling pathways, but does not impair hippocampal LTP. Together with Agnès' group, she and researcher Carla Sciarretta had already created transgenic mice lacking either the Shc-site or the PLC γ -site of TrkB receptor. Now it was time to test their memory.

"The challenge for us was to record brain activity in a very small animal," explains Agnès. "We had done it in rabbits, cats, even rats, but in mice, everything is just that much closer together." They wanted to measure the response of neurons in one particular region of the hippocampus to stimulation of neurons in another, via electrodes implanted in both. Neurons in the two regions are connected, and the size of the response would reflect the strength of those connections and hence provide a measure of LTP.

To make things more difficult, they wanted to do this while the mice were being tested on a task of associative learning, in which a mildly uncomfortable stimulus to the eye was preceded by a light or an auditory tone. Healthy mice soon learn to close their eyes defensively in response to the warning signal. To obtain a precise measure of that learning, the researchers applied another set of electrodes to the skin over the eye muscles, to get a read-out of those muscles' contraction in response to the warning signal. All this had to be done in awake, active animals, without restricting their ability to move too much. That meant experimenting with the way the stimuli were presented, the number of electrodes and various other parameters. "Only after a year did we begin to see good results," said Agnès, "but it was worth it for what we saw, and also because we can now adapt the protocol to test other types of learning."

There were two striking findings. First, the TrkB PLC γ site defective mutants learned less well than the TrkB Shcsite defective mutants, and second, the TrkB PLC γ -site defective mutants showed less LTP than the TrkB Shc-site defective mutants. Liliana is cautious in interpreting these findings: "We cannot say for sure that learning and LTP are the same thing," she says, "but what we definitely can say is that the TrkB PLC γ mutation is implicated in both learning and LTP in an *in vivo* model."

Commenting on the research, the journal *Nature Reviews Neuroscience* suggested that other groups should apply the same combination of methods – behavioural, electrophysiological and genetic – to other mouse models, "and decipher other signalling pathways that are involved in learning and LTP." Liliana's group already has evidence that the same receptor, TrkB, and binding site, PLC γ , are involved in LTP and spatial learning – when a mouse learns its way around a new environment. And she has preliminary data suggesting that they may also be at the root of emotional learning – learning to be afraid of someone or something, for example – though in that case, a different brain structure is involved: the amygdala, which has long been implicated in emotional behaviour.

"In my opinion this is the right model to show that, truly, LTP and learning have a common molecular basis," she
says. "Moreover, using this model I can start to dissect at the molecular level how LTP works." She has now embarked on a series of experiments designed to tease out the signalling pathway in which PLC γ represents a key step, and to identify the molecule or molecules that bind to it, activating the pathway in the first place. The idea is to stop the mouse at different stages of a learning task and capture the protein profile of its hippocampal neurons at that point in time, using modern proteomics tools – again in the living mouse.

Though the TrkB receptor has not yet been implicated in Alzheimer's disease, one of the proteins that binds to it, brain-derived neurotrophic factor, has. And Liliana has no doubt that her research will throw light on this and other devastating neurological diseases. By way of an example, she points to a case in the medical literature of a boy who DNA tests revealed carried a mutation in the TrkB receptor. Among other defects, he showed learning impairments very similar to those of the TrkB $PLC\gamma$ mutant mice.

It seems increasingly likely that the fictional character Leonard might also have been suffering from a disruption of LTP in his brain – though in his case it was the result of trauma. Liliana's latest findings may not clinch the question that has been taxing neuroscientists for three decades, but the evidence that LTP is the basis of learning is now so strong that a more difficult question to answer might be: what is LTP for, if not learning?

Gruart, A., Sciarretta, C., Valenzuela-Harrington, M., Delgado-García, J.M. and Minichiello, L. (2006). Mutation at the TrkB PLC-gamma docking site affects hippocampal LTP and associative learning in conscious mice. *Learning and Memory* 14 (1): 54-62

Long-term potentiation (LTP), the strengthening of synapses between nerve cells in specific parts of the brain, is the molecular mechanism underlying learning. Some forms of LTP are triggered when a receptor called TrkB is activated by messenger molecules. Via a specific site the receptor activates a protein called PLC_{γ} which then sets off a cascade that leads to the strengthening of the electrical signal that is passed from one cell to the other and allows mice to learn.





Foteini Mourkioti, Paschalis Kratsios and Nadia Rosenthal, Head of EMBL Monterotondo

Pump it up

UMANS HAVE AN INNATE ABILITY to repair themselves, but tissue that grows back after injury rarely looks as good, or works as well, as the one it replaced. Why not? For years scientists blamed the immune system, especially inflammation, for causing scar tissue to form instead of healthy tissue. More recently, however, it has become clear that inflammation plays a much more complex role in regeneration. Without it, a wound cannot heal, but too much hinders new growth. Could it be, then, that by modulating inflammation, one could actually enhance repair?

Yes, says Nadia Rosenthal, Head of the Mouse Biology Unit in EMBL Monterotondo, whose group has been searching for ways of doing just that. When muscle regenerates, progenitor or 'satellite' cells lying dormant in muscle fibres are stimulated to form new muscle. A strong inflammatory response can prevent them from doing so. But, she says, "by dampening that inflammatory response in some way, we can allow the balance to shift back towards repair and away from a scar." The potential therapeutic applications of shifting that balance are obvious. Muscle can be damaged by injury, but it can also waste away, and wasting or atrophy is a major problem of old age, certain neurodegenerative diseases and cancer. "People forget that even before cancer spreads, muscle atrophy sets in and can be lethal, for example if it affects the respiratory muscles," says Foteini Mourkioti, a postdoc in Nadia's lab.

The group's latest research focuses on the role of a molecule called nuclear factor kappa B (NF- κ B) in repair,

and is the result of a collaboration which could only have come about because of the unique structure of EMBL. "It was a situation where, once again, the proximity of many groups working on the mouse had synergistic consequences," says Nadia.

Next door to her lab, Manolis Pasparakis – who is now at the Institute for Genetics at the University of Cologne, Germany – was studying the role of NF- κ B in inflammation. "Nadia and I had often discussed the possibility of starting a project that would try to shed some light on the function of NF- κ B in muscle, because we both believed that it must be important in muscle homeostasis or maintenance," explains Manolis.

An important regulator of inflammation, NF- κ B is also a transcription factor, which means that it works by switching on genes in a cell's nucleus. Manolis had genetically engineered mice that lacked an enzyme, IKK2, which as part of a larger enzyme complex, controls the access of NF- κ B to the nucleus. Without IKK2, NF- κ B cannot reach those genes and so cannot perform its normal function. NF- κ B is extremely important in many signalling pathways in many tissues (see box). To explore its specific role in muscle, Manolis created a mouse strain in which IKK2 was selectively knocked out in muscle – not in any other tissue. "For something which affects as many pathways as NF- κ B does, that's an invaluable model," Nadia says.

Manolis' team was disappointed, however, because the IKK2 knockouts didn't appear to differ from normal mice – they were born and developed normally. It took Foteini's

trained eye to notice that, once mature, they were beefier than regular mice. "These animals not only have bigger muscles, their muscles are stronger as well," says Nadia, whose collaborator at the University of Pavia in Italy, Roberto Bottinelli, has developed sensitive methods for measuring the strength of individual muscle fibres.

The critical question for Foteini, Nadia and the team was, could the deletion of IKK2 also enhance repair following atrophy or injury? To test this, they created a mouse

model of atrophy by severing the sciatic nerve and so removing all nerve input to one hind leg, leaving the other as a control. As in paralysed patients, the muscle in the now immobile leg began to atrophy. Within a month, its weight had shrunk to 30% of that in the 'good' leg, in normal mice. In the IKK2 knockouts, however, the shrinkage was much less pronounced – the mice retained about 60% of their muscle. This resistance to atrophy was enhanced



when the researchers repeated the procedure in genetically engineered mice that lacked IKK2, but also expressed larger than normal amounts of a protein called insulinlike growth factor 1 (IGF-1). Nadia's group had previously shown that IGF-1 can powerfully enhance regeneration in muscle by stimulating satellite cells to form new muscle, and IGF-1 is now in advanced clinical trials for the treatment of motor neuron disease. In mice that lacked NF- κ B but overexpressed IGF-1, the immobilised leg retained 80% of its original muscle weight. "That's a 50% increase in capacity to maintain muscle without any nerves, over normal mice," says Nadia. "By any standards, that's dramatic."

In another experiment, muscle damage induced by injection of a toxin was more rapidly repaired in the mice lacking IKK2, with even further improvement in the presence of the IGF-1 gene. Other research has suggested that IGF-1 may work in part by modulating the influence of

> IKK2 on NF-κB, but these findings indicate that it can't only be exerting an effect on repair via NF-κB – otherwise the researchers would not have seen an additive effect in the NF-κB mice that also overexpressed IGF-1. Nadia suspects that the extra regenerative potency provided by IGF-1 may result from a difference in the way cells respond to circulating IGF-1, than to IGF-1 that they synthesise themselves – though that hypothesis remains to be tested. Some of the original NF-

 κ B knockout mice are now about a year old, and they still appear healthy. "There must be a cost but we don't know what it is yet, the animals don't appear to be compromised in any way," she says. "The simple message from this paper is that interfering in a central pathway that is normally associated with inflammation, namely the NF- κ B pathway, had only a good outcome." However, she adds, "what's interesting about other work that's ongoing in the lab, is that it's not a simple situation."

In March 2007, former EMBL Group leader Manolis Pasparakis and colleagues published their discovery that NF- κ B, that master regulator of pro-inflammatory responses, is responsible for controlling the integrity of the intestinal lining in mice, as well as the interaction between the host's immune system and the helpful bacteria which colonise its gut. They suggested that one potential cause of inflammatory bowel disease could be disruption of NF- κ B signalling in the intestine.

The finding is just the latest addition to the list of known physiological functions of NF- κ B, a transcription factor which switches on genes in the cell nucleus. A month earlier, Manolis – who is now based at the University of Cologne – and his team found that NEMO, an enzyme which helps to release NF- κ B to the nucleus, suppresses the growth of cancer cells in the liver. And in September 2006, the group showed that NF- κ B-dependent gene expression in the central nervous system contributes to certain autoimmune diseases, such as multiple sclerosis.

Taken together, these findings demonstrate that the role of NF- κ B in contributing to or protecting from disease depends on which tissue it is acting in, and emphasise Nadia Rosenthal's point that a gene cannot be properly understood until its functions in all tissues are known.



Another strand of her research concerns the heart, and again in collaboration with Manolis' group, PhD student Paschalis Kratsios and others in the lab have been studying mice that lack NF-KB essential modulator (NEMO) another component of the enzyme complex that releases NF-κB to the nucleus, that also contains IKK2 – but only in heart muscle. Once again, these mice appear healthy at birth, and even continue to thrive into adulthood. But preliminary findings suggest that when they reach the mouse equivalent of old age, their hearts begin to sicken. "At eight months they look for all the world like a 60-yearold man who is coming down with heart failure," says Nadia. To a scientist that's exciting, she adds, because it means that an underlying deficiency of NF-KB signalling in the heart could explain why some people are more prone to heart disease than others, even if they indulge similarly in a fatty diet, lack of exercise and other known risk factors for the disease. That's only speculation for now, but an animal model of mature-onset heart failure which they appear to have stumbled upon in the NEMO knockouts - could allow them to test the idea.

The message from all this research seems to be that what the heart muscle needs for longevity and health – $NF-\kappa B$

Escher mice by Nadia Rosenthal

– is dispensable in skeletal muscle. Drugs already exist that inhibit IKK2, but the group's findings suggest that taking such drugs systemically could be dangerous, because they could increase some patients' risk of heart failure. That may mean that the only way to enhance repair while avoiding potential adverse effects will be to treat patients with gene therapy – by selectively blocking NF- κ B in the affected muscle. "Scientifically, it's a wonderful lesson in how we cannot understand genes in their entirety until we look at how they act in different tissues," Nadia says.

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At the tips of the tree

OST OF LIFE ON EARTH is invisible. Animals, plants and fungi reside only on a tiny fraction of the branches of the tree of life - the rest are teeming with microorganisms. But although microbes underpin the ecology of the planet, most evade the biologists' microscope because they are impossible to culture in the lab. Peer Bork - whose group develops computational techniques for analysing genomes - has found a novel way of interrogating 'metagenomic' data, giving him a glimpse of these elusive ecologies and the communities of organisms that live there.

Metagenomics is a new biological approach that lets researchers identify microbes without having to culture them. In fact, the microbes don't make it to the lab at all. Instead, genetic material is brought straight from an environment (say, a patch of soil, or the seabed), to the lab, inserted directly into plasmids - circular pieces of DNA that can be taken up and replicated by bacteria and sequenced, providing a picture of the whole community of organisms that live there. This 'shotgun sequencing' technique generates vast quantities of sequence data, which puts huge emphasis on data analysis. Peer says that his group are developing new ways of sifting through and interpreting this metagenomic data. "We want to find out which microbes are present and where yet-unknown species fit into taxonomy and evolution."

Peer studied the sequences of DNA fragments collected from four very different environments – agricultural soil, water from the ocean surface, whale bones from the seabed, and acidic water from a disused iron mine. The researchers wanted to identify fragments representing microbes that had not previously been described, compare these with known organisms, and place them on the high-resolution 'Tree of Life' that was recently published by Peer's group. So, from the thousands of pieces of DNA collected, the researchers picked out those containing sequences similar to 31 known protein-coding 'markers' – genes that have relatives in all of the organisms that have been sequenced so far (from bacteria, to yeast, to humans). By aligning the marker sequences of the comFour microbial communities are mapped onto the same reference tree of life: agricultural soil (ochre), ocean surface water (blue), acidic water from a disused iron mine (red) and whale bones from the seabed (green).

munity microbes with the same markers in known organ-

isms, the researchers placed the microbes onto the most likely positions on the tree, using software that they have made freely accessible.

Surprisingly, most of the newly discovered microbes had rather little in common with other sequenced organisms. In fact, some were so unusual that they seem to have branched off at the very root of the tree, where the three known 'domains of life' – the bacteria, the archaea and the eukaryotes – first split.

Their data also shed light on how whole communities change and evolve. Different environments had different evolutionary features – for example, organisms in the soil seem to be evolving more slowly than those in the ocean's surface. The researchers also found that on the whole, similar microbes are found in similar environments, and that dramatic changes in habitat happen surprisingly rarely. Peer says that this finding contradicts the long-standing belief that every microbe can potentially live everywhere: "It is not easy to enter a new environment and compete with the established communities in it."

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Doing the twist

N THE VERY BEGINNING a fruitfly - like any other animal – is nothing more than a single cell. But this one cell contains all the information it needs to develop into a multicellular organism with specialised tissues and organs. The manual that guides this development is the cell's DNA, identical copies of which it passes on to all the cells of the emerging body. But if they work with the same instructions, how come a brain cell differs from a heart cell, which is different from a blood cell? "Not every cell follows the same parts of the manual and the order in which they read different chapters also varies," explains Eileen Furlong. Her group at EMBL Heidelberg studies muscle development in fruitfly embryos to uncover the regulatory mechanisms that give cells their unique identities. "Different genes are transcribed at different times in the various cell types. The precise pattern of genes that are active over time determines the progression of development."

A special class of proteins, called transcription factors (TFs), makes sure the right genes are active at the right time. They bind to regulatory regions on the DNA, known as cis-regulatory modules (CRMs), and like molecular bookmarks label genes that should be transcribed. Twist is a TF that guides the formation of the mesoderm, the embryonic tissue that gives rise to muscle, gut and heart. Mutant flies that lack Twist don't develop any mesoderm and when expressed abnormally in other tissues Twist directs any cell to adopt a muscle-like fate. "Somehow this single protein is responsible for the entire process of mesoderm development. Twist acts as a master regulator," Eileen says. "Yet, only eleven of its target genes were known – far too few to explain this complex process."

To bridge this gap Eileen and her group set out to systematically identify all the genes that are controlled by Twist. Applying a systems biology approach integrating genetics, biochemistry and computational biology, PhD student Thomas Sandmann identified all the CRMs that Twist binds to at different stages of development. To analyse this genome-wide collection of DNA sequences he turned to Charles Girardot, software developer in Eileen's lab, and took advantage of the infrastructure set up within EMBL's Functional Genomics Centre. "The Centre's infrastructure and expertise for analysing genomics data was crucial for our study. If you consider the Genomics Core Facility with all its machinery as the hardware of genomics research, the Functional Genomics Centre adds the necessary software you need to make sense of your data," Eileen explains the role of the Centre that she coordinates together with Group leader colleague Lars Steinmetz. Apart from technical support the Centre offers regular meetings to encourage exchange of ideas and skills between researchers across all EMBL Units. The overall aim is to promote a stronger culture of functional genomics – the search for patterns, properties and functions in entire genomes.

Thomas' search for such meaningful patterns was very successful: he found more than 2000 CRMs bound by Twist. Through them, Twist directly controls around 500 genes at various stages of mesoderm development. Among these are genes required for cell proliferation and cell migration, but also genes encoding other TFs through which Twist affects vast cohorts of genes. By integrating all the information gathered about Twist's targets with other data generated in the Furlong lab, the group could draw a map of how the genetic regulation of mesoderm development changes over time.

"The map highlights Twist as a central control point, regulating an unexpectedly large number of genes in a dynamic manner," Thomas describes. "The genes activated by Twist vary over space and time. Some are activated specifically early in development, others only later." To make things even more complicated Twist does not act strictly hierarchically as master regulators are often thought to do. "Instead of kicking off a cascade of TFs, which then activate target genes, Twist acts in combination with these regulators. Often genes are only transcribed when Twist and another TF bind to their CMR together. This is called feed-forward regulation and allows Twist to affect almost every gene involved in early mesoderm development," Eileen concludes.

It is complex regulatory interactions like the Twist network that allow the development of different cell types despite shared genetic information. Since many of the mechanisms directing embryonic development in the fruitfly have been conserved by evolution, similar regulatory networks are likely to also govern the development of humans and other vertebrates.

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A window on the cell

'CELL' IS A CURIOUS WAY of describing the building block of multicellular life. The word was first coined by the 17th-century polymath Robert Hook, who first saw, with his basic light microscope, how a thin slice of cork was formed from tiny box-like structures. He named them cells, after the small bare rooms that monks lived in at the time.

Hook was studying a piece of dead cork, so it's perhaps not surprising that the word he chose described a rather bare, static and lifeless structure. Yet we now know that cells are in fact quite the opposite: busy, dynamic, animated systems, teeming with molecules that interact to produce complex living behaviour.

But for a long time, this molecular hubbub remained invisible to biologists. Although inventions such as electron microscopy allowed them to peer ever-closer at the structures within the cell, the method of preparing samples meant that they mostly got to look only at dead specimens. They had to perform biochemical experiments in the test tube to deduce how molecules within the cell interacted with one another. In many ways, they were working blind.

It wasn't until the mid-1990s that the method of 'live imaging' allowed biologists to tag molecules with harmless, visible molecular labels and watch how they moved in living cells. This was a revolution – and a revelation. Now, at last, biologists could sit and watch the nuts and bolts of living systems at work.

It also offered them the chance to move on from blind deduction and let them see how their molecules and systems of interest fitted into the big picture of cells and organisms as wholes. In the future, live imaging should even liberate biologists from the shackles of having to do biochemistry experiments in the test tube – instead, they will be able to watch biochemical reactions as they happen *'in vivo*', within a living cell.

It's these two methods – 'big picture' systems and *in vivo* biochemistry – that EMBL scientists are working on to realise the vision of how complexity builds in living organisms. Teams in Heidelberg are pioneering new technologies to push back the frontiers of live imaging, which their colleagues are using to explore the molecular structure of the cell and how it changes as the cell responds to its environment. Other researchers are seeing how cells fit into the bigger picture, by using live imaging to trace how cells behave in groups, such as how they migrate during embryonic development. The more they discover, the more it is clear that what they are seeing is less like a monk's cell and more like a living room.



Views on moving molecules

Live imaging has opened the cell up to scientists' eyes. Kota Miura, who runs the Centre for Molecular and Cellular Imaging (CMCI) in Heidelberg, and Rainer Pepperkok, Head of the Advanced Light Microscopy Core Facility (ALMF), describe the view from where they are.

What can be seen now with microscopes that couldn't be seen 150 years ago?

Rainer: A microscope is no more than an array of lenses, and there is a physical law, Abbe's Law, which says that you cannot see with a resolution greater than about half the wavelength of the light that is entering the microscope. Though the quality of optics may have improved considerably, scientists using microscopes in the 19^{th} century were therefore, in principle, able to see as much as we can today. What has made it easier for us has been the advent of digital technology, which facilitated the analysis of the images. This has especially revolutionised how we can track movement with microscopes.

Kota: Imaging single molecules using the light microscope only became possible with the evolution of highquality cameras and digital image processing techniques, which meant that we could study protein dynamics in living cells. In the 1940s people were already able to film moving cells, but they were using very inefficient techniques to analyse the images, for example measuring movement by overlaying stills that had been taken with a high-speed shutter. Twelve years ago, when I was analysing cell movement, I still had to put a transparency over an image of a cell, draw the outline by hand, then cut it out and try to measure the geometrical centre of the cell. It wasn't until more recently that computers took over these tasks. Overall, radical improvements in efficiency have increased both the quantity and quality of the data we get from microscopes, and the advent of digital analysis techniques means that seeing through the microscope has become measuring with the microscope.

The 1980s saw the birth of immunofluorescence microscopy. What difference did that make?

Rainer: Immunofluorescence microscopy has been a revolution. Though the fluorescence technology itself has nothing to do with microscopy, it allowed us to label molecules specifically. Originally, antibodies coupled with fluorophores, or fluorescent probes, would bind to your molecule of interest, and so highlight it under the fluorescence microscope.

Kota: Conjugating fluorescent probes with antibodies was hard work and time-consuming. But then, in 1994, along came green fluorescent protein (GFP), a naturally fluorescing protein that was originally found in jellyfish and corals in the 1960s. Once the reporter gene which encodes the GFP has been combined with the gene of interest, and the resulting DNA construct inserted into a cell or organism, that cell or organism expresses the gene and does most of the work for you. All you have to do is visualise the fluorescence under the microscope, which is also easier to quantify than with the antibody approach.

But in terms of resolution, the amount of detail you could see, you were still bound by Abbe's law?

Rainer: Yes. These limitations were only overcome when stimulated emission depletion (STED) microscopy was

developed. Put simply, fluorescence works by putting energy into a system, which is then spontaneously emitted. Imagine a bucket filled with water and then tipped up – that's fluorescence. But with STED, this is not a stochastic process; you decide when the bucket is tipped up. In this way you can achieve higher spatial resolution. The resolution that is now being claimed for STED is 20 to 30 nm, compared with 200 nm previously. This opens up a whole new world, because many cellular structures are smaller than 100 nm.

How are fluorescence microscopy techniques being applied at EMBL?

Rainer: They are being applied across biology, for example developmental biologists might use them to track cells and elucidate their role in developmental processes, or to study mutants generated by knockout techniques. One particularly remarkable application has been some work by Philippe Bastiaens, who left EMBL at the beginning of this year and is now at the Max Planck Institute for Molecular Physiology in Dortmund. He uses a very elegant method called fluorescence lifetime imaging (FLIM), which enables one to track proteins' behaviour over time, and even more important to monitor their interactions. That made it possible to study how signals are propagated in a cell. Before we could see cell structures, organelles, but we couldn't see the many interactions and states of soluble factors that swim around a cell. Now we can.

What are the technological constraints on live imaging now?

Kota: We can now see single molecules and watch how they move in space and time. Using a confocal microscope, we can capture them in 3D. We have not yet per-



Microscope image of a Drosophila embryo

fected the tracking of molecules in 3D over time – tracking in 2D is already tricky – but image analysis will evolve in years to come to support this. Moreover we now are getting better at extracting numerical data from image sequences, although how these data should be interpreted is still a matter for debate in biology. We probably need new physical theories to deal with biological dynamics, although these too may come along in the near future.

Rainer: The constraints still lie very much within the analysis. We are better at generating data than at analysing it, because each question we ask – and we ask thousands every day here at EMBL – requires a different assay and ultimately a different image analysis.

Is that why EMBL has the CMCI, a centre that is concerned only with imaging-related issues?

Kota: Exactly. Different projects have different aims. The basic technology – the microscope and analysis tools, which are provided by the ALMF at EMBL – is the same, but each research group takes the components it needs, modifies them and assembles its own technology to answer the question it is asking. Experiments designed in the knowledge that these components are available may achieve better results. The job of the CMCI is to provide support in the processing and analysis of imaging data, and to make scientists aware that these basic components exist. We organise seminars and courses to explain the technology, and these meetings also serve a strong networking purpose. Novel approaches arise through the exchange of information and ideas, and the technology in turn advances.

Rainer: To give an example, I am involved in a project on high-content screening microscopy – that is, microscopybased screening with different cellular assays. The idea is to build up a very detailed molecular picture of the cell. The assays we are using are fairly simple, nevertheless the quantity of data we are generating is so vast – tens of terabytes – that it becomes impossible for a few people to analyse them, and this is where the CMCI comes in.

What's next in live imaging?

Kota: The unification of biochemistry and microscopy. Traditionally, to work out an enzyme's function, you mix a solution of it in a beaker with a reagent to get a product whose biochemical properties you can then measure. With modern microscopy, we can study the biochemistry of the same enzyme directly inside the cell. That's important because an enzyme's characteristics vary depending on its local environment, for example whether it is closer to the nucleus or to the periphery of the cell. In biology in general, the relationship of structure and function is very important. Now we can begin to tease this apart. ■

A living screen success

GENETIC SCREEN is a method for finding genes and working out what they do. As screens have become more and more sophisticated, researchers have revealed genes with subtle effects on the inner machinery of cells. Now, Jan Ellenberg, who coordinates the Gene Expression Unit, and Rainer Pepperkok, Head of the Advanced Light Microscopy Facility, have taken this a step further and found a way of screening living cells. Together with their colleagues, they have refined a high-throughput method of disrupting genes in individual cells and have developed a system for capturing vast numbers of time-lapse images of these cells.

The researchers begin by fluorescently labelling carefully chosen proteins inside the cell that will allow them to follow the process that they're interested in - such as protein secretion or chromosome dynamics. They then systematically suppress thousands of genes individually in different cells using a powerful new tool called RNAi. When a small piece of RNA - called a 'small interfering RNA' or 'siRNA' - enters a cell, the cell recognises it as foreign and breaks it up, after all, a piece of RNA could be an invading virus. But the cell also gets rid of any of its own 'messenger' RNA that is identical to the foreign sequence. Messenger RNA is the template from which proteins are translated, so a specific siRNA will block the production of a specific protein. The researchers spot siRNAs in a grid or on glass plates, and on top of these they lay the fluorescently labelled cells, which absorb the siRNAs.

If a gene is important to a particular cellular process then its suppression will disrupt that process. So once a cell has taken up the siRNA, Jan and Rainer watch the effects of gene knockdown on the cell's internal machinery. For example, if they are interested in chromosome dynamics, they tag a chromosome-associated protein with a fluoresSmall interfering RNAs are powerful tools to disrupt gene expression. When they enter a cell, it recognises them as foreign and breaks them up and, with them, all messenger RNA with identical sequence. In this way the production of specific proteins is prevented.

cent label, then capture time-lapse images of the labelled proteins inside the cell. Crucially, the cells are still laid out on plates, and are not in test tubes, so the researchers can image the living cells directly as they grow. They have developed an automated microscopy system (now commercially available) that records a two-day-long movie of each cell, together with software that picks out any important features of this movie. They then test for correlations between any unusual dynamics within the cell and the genes that have been suppressed.

They've shown that this method works – but what do the researchers hope to find? Jan's lab is interested in finding genes that affect cell division and the organisation of the nucleus, and Rainer's lab is working out how cells produce and secrete proteins. Jan says, "Division is a rare and dynamic event in cultured cells, so the effects of mitotic genes can only be captured by time-lapse imaging of live cells." The researchers have lost no time in using their new methodology. In a big EU project called MitoCheck they are suppressing every single gene in the human genome and investigate their roles in mitosis, the division of a cell's nucleus. And as Rainer points out, "To get through all the approximately 22,000 human genes, a high-throughput approach like ours is vital."

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Sometimes you need to resort to unconventional methods to understand science: Jochen Wittbrodt explains eye development using a hammerhead shark.

A wandering eye

IN THE BEGINNING, there is a flat piece of tissue. The tissue folds and finally becomes a tube – the neural tube, to be precise, which is the starting point for the development of the brain. Before the brain develops any further, however, the eyes must extricate themselves by bulging out from either side of the tube – not unlike the outsized ears of Mickey Mouse, says Jochen Wittbrodt, who studies vertebrate eye development in Heidelberg.

Eyes are among the earliest recognisable structures in an

embryo, and although their development has been studied as a model for all organ development in vertebrates for more than a century, surprisingly little is known about the cellular processes that drive it. Among the most intriguing of these is the migration of cells out of the neural tube to form the early optical structures. Do they travel en masse, or singly? Do they move



Medaka, in Japanese 'useless fish with big eyes', is now a popular model organism for biologists.

actively, or are they more like passengers, propelled along by the surrounding tissue?

These are some of the questions that absorb Jochen and his team, and using state-of-the-art techniques, provided in part by the core facilities at EMBL Heidelberg – in particular the Advanced Light Microscopy Facility, run by Rainer Pepperkok – they now have a near-complete picture of the earliest steps in the development of the eye in a vertebrate, a small fish called a medaka. "We wanted to image the process rather than just describe it," says Jochen. "By seeing the process unfold, you suddenly understand much more than by looking at static pictures." Visualising migrating cells in a 3D structure over long periods of time is, however, not simple.

In 2001, Felix Loosli in Jochen's lab discovered that cells in the neural plate that go on to form the eye produce a protein called Rx3. Since Rx3 gave these retinal progenitor

> cells (RPCs) their stamp of identity, the team generated medaka embryos in which Rx3 was labelled with green fluorescent protein. They used another fluorescent label in the same embryos – this time a red one attached to a nuclear protein – to highlight all the neural plate cells. Now, in theory, they would be able to see how the green RPCs moved through the red

neural plate during the first stage of eye development – the evagination or bulging of the eyes' precursors, the optic vesicles.

Since no good 3D tracking software currently exists, the laborious task of tracking the labelled cells fell to Martina Rembold, then a PhD student in the lab. Using a confocal microscope she optically 'cut' the embryonic neural plate into 50 slices and looked at the positions of the labelled



cells in those slices at timepoint zero. She repeated the process two minutes later, by which time the cells had moved slightly, and continued this procedure over about eight hours, or 240 timepoints in all.

"If you calculate 50 sections per timepoint with two channels, red and green, over 240 timepoints, that gives 24,000 images," says Martina. "In collaboration with Richard Adams of the University of Cambridge in the UK, who developed a special program for cell tracking and analysis, I would go through all of these pictures, find one cell at the beginning and try to follow its path through time. I marked and recorded the position of that cell at every timepoint, and I repeated the procedure for many cells – 200 to 300 per movie – to reconstruct the process of optical vesicle formation."

Before the neural plate becomes a tube, the eye field starts out as a single field. Chemical signals emanating from the plate's midline split it in two – one field for each eye – and then all the cells in the neural plate start to migrate towards that midline, causing the plate to fold. The first surprise for Jochen's group when they watched Martina's movies, however, was that not all the cells move towards the midline at the same rate – as was conventionally thought. Some of the cells on the outside of the eye field slow down as they approach it, and never enter the fold at all. As a result, these cells, which already produce Rx3 and therefore 'know' they are RPCs, form 'pre-evaginated' optic vesicles which must then be enlarged. At this point, the movies showed that some of the cells that had been drawn into the fold now began to migrate out again, becoming interspersed with the cells that had stayed out, so expanding the vesicles. Jochen thinks that Rx3 not only defines eye cells, it also determines how attractive they find the signal in the midline. "The signal is attractive for cells on the outside until they get to a certain proximity, when it becomes repellent," he explains. "The result is that those closest to the midline come in and go out again."

The next question was whether the process they were watching was active or passive. Were the cells migrating actively, or were the optical vesicles being forced to evaginate, like balloons blowing out as a result of pressure building up in the developing neural tube? To test this, the researchers made use of a medaka mutant called Eyeless, in which the eyes never develop. Using the gene that is mutated in Eyeless, they created genetic mosaics – medaka embryos that were either normal with transplanted mutant RPCs, or mutant with transplanted normal RPCs.

In Eyeless, when the neural plate folds, all cells migrate towards it at the same pace, and there is no slowing of the cells in the eye field. The tube also seems to be more tightly bound at its outer edges, and to lack the flexibility which allows the optic vesicles to evaginate in the normal



Schematic of the gradual eye formation process in a medaka embryo. First, cells move towards the midline of the embryo, with the cells that will form the eye lagging behind. Then the optic vesicles bulge out gradually when individual cells migrate outwards possibly repelled by cues located at the midline. Below: Optic vesicle formation over time as seen through a confocal microscope. The cells that will form eyes are labelled green, other cells red.

While he was working as a postdoc in Jochen Wittbrodt's lab, the discovery of the importance of the transcription factor Rx3 in vertebrate eye development drove Detlev Arendt to look at its evolutionary implications (see page 20).

Insect and vertebrate eyes use rhabdomeric and ciliary photoreceptor cells for vision, respectively. Not only are these cells structurally very different, they also process light differently. Could both have been present in the common ancestor of insects and vertebrates, an extinct creature named Urbilateria?

In 2002, Jochen, Detlev and their colleagues found a powerful clue that they were: a living descendant of Urbilateria, a tiny marine worm called *Platynereis dumerilii*, has both cell types-rhabdomeric photoreceptors in the eye and ciliary photoreceptors in the brain. Having identified the *Platynereis* equivalent of Rx3, Rx, the group found that Rx was present in ciliary photoreceptors – those cells that *Platynereis* shares with vertebrates – but not in rhabdomeric photoreceptors.

Combining those findings with the new developmental results, Jochen now speculates about how the vertebrate eye might have evolved. "The problem with the ciliary photoreceptor in the vertebrate lineage is that initially it was buried in the brain," he says. "To make sure it got out, perhaps ciliary photoreceptor specification became linked with the morphogenesis that was necessary to build a proper retina. That link was probably Rx."

In the eponymous medaka mutant Eyeless, the neural tube of the embryo seems to be rigidly bound and shows none of the bulging that, in the normal embryo, presages eye development. Jochen thinks of this mutant as the evolutionary 'ground state' – the normal developmental pattern before natural selection introduced the innovation of the vertebrate eye. "It's a cool theory," he shrugs, "but there's no way to prove it."



embryo. The researchers labelled the transplanted and normal cells with different fluorescent labels and, once again, created movies of eye development. "This was the really fun part," Jochen smiles.

The movies showed clearly that normal RPCs transplanted into a mutant embryo still moved in towards the midline, and then out again – as if attracted and then repelled by a chemical signal there. Jochen was surprised that the relatively static, mutant tissue in which those normal cells were embedded didn't present more of an obstacle to them: "They go through it like a hot knife through butter," he says, adding that this observation could have implications for scientists' understanding of metastasis, or the spread of tumour cells through healthy tissues.

There remained the possibility that the normal RPCs were being pushed through that tissue by a gradient of 'stickiness', being passively drawn towards the 'stickiest' cells in the eye field. Martina's observations ruled this out, however, because if cell adhesion was the mechanism driving migration, clusters of cells would be affected similarly by local adhesion factors. In fact, though the cells' migration was coordinated, it was clear they were moving as individuals – indicating, once again, that matters had been oversimplified in the textbooks. Though organogenesis has generally been considered a mass migration phenomenon, it can also be seen as resulting from the migration of individual cells. When they published their findings in the summer of 2006, Jochen and his colleagues were overwhelmed by the responses of their scientific peers. "People really liked the idea that individual cells are migrating in organ formation," he says, "and I think it might apply to many cases of organogenesis. Of course there are exceptions like the formation of the lateral line organ in zebrafish. But individual cell migration has been seen in heart, kidney and pancreas and I guess, if you just look closely enough, you will see it in other organs too."

The group is now looking at the next stage of eye development – when the evaginated optic vesicle subsequently invaginates, to form the cup that will eventually wrap around the lens. This, too, turns out to be a migratory phenomenon, says Jochen. More generally, he believes that his group's research represents a signpost for the direction in which biology is evolving. "This is a story that starts with a mutant and moves down to cell biology, which is happening more and more," he says. "Eventually the fields will merge and cell biology will be done in the organismal context."

Rembold, M., Loosli, F., Adams, R.J. and Wittbrodt, J. (2006). Individual cell migration serves as the driving force for optic vesicle evagination. *Science* 313(5790): 1130-1134

Arendt, D., Tessmar-Raible, K., Snyman, H., Dorresteijn, A.W. and Wittbrodt, J. (2004). Ciliary photoreceptors with a vertebrate-type opsin in an invertebrate brain. *Science* 306(5697): 869-871

Pay attention at the back

ne of the remarkable aspects of mammalian development is the large distance that newborn neurons must travel to reach their final destination in the brain. They push past, through and over other cells, covering as much as several centimetres, in the human brain. The cell's dynamic scaffold or cytoskeleton makes this migration possible, but not until recently did scientists suspect that the cytoskeleton might continue to play a part in the functioning of the mature brain. Walter Witke now has evidence that it does.

Walter, whose group is based at the Mouse Biology Unit at EMBL Monterotondo, is interested in a molecule called profilin, whose function is to elongate the filaments made of another protein, actin – the main constituent of the cytoskeleton. These filaments are formed when single actin molecules or monomers come together, and profil-in's job is to tow the monomers to the growing end of the filament.

With postdocs Pietro Pilo Boyl, Alessia Di Nardo and others, Walter has genetically engineered mice to produce a mutant that lacks one member of the profilin family. Mice have three profilins, two of which are found in the brain. Because the cytoskeleton is known to be important in brain development, the researchers half-expected that mice lacking profilin 2 would not be viable-but to their immense surprise, they were. "Development and neuronal migration are totally unaffected," says Walter, "the brain is perfectly made."

The differences only became apparent later, in their behaviour. The profilin 2 knockouts were hyperactive. When novel objects were placed in their cages, they proved to be much more inquisitive about these than their normal counterparts, but also much quicker to lose interest in them. When the researchers took a closer look at their brains, they discovered a possible explanation.

Two neurons communicate via a junction or synapse, which consists of pre- and post-synaptic boutons or neuron terminals separated by a tiny gap. The pre-synaptic bouton contains vesicles loaded with brain chemicals, or neurotransmitters. When an electrical signal reaches it, the vesicles fuse with the membrane, releasing their contents into the synapse where they bind to receptors on the post-synaptic side and trigger another electrical signal. "The two boutons are full of actin," says Walter. "Why is



not completely understood, but actin may be important in the release and recycling of neurostransmitters."

One reason for believing so is that in front of the vesicles, electron microscopy images reveal a dense zone of actin filaments. "It looks like this barrier exists to limit access of the vesicles to the membrane," says Walter, "but in the profilin 2 mutants, the barrier is disordered and you have a constant flow of vesicles to the membrane."

One neurotransmitter, glutamate, increases the excitability of neurons and is known to mediate novelty-seeking behaviour. Sure enough, the researchers found that more glutamate was released into the synapses of the profilin 2 knockouts, whose neurons were also hyperactivated.

The synapse and pre- and post-synaptic boutons can be isolated as a unit called the synaptosome. The team extracted synaptosomes from a brain region called the striatum, which is important for movement control, kept them alive in buffer solutions and measured the remodelling of the actin cytoskeleton in the mutants compared to normal mice. Without profilin 2, they found that the actin network could not form in the synaptosomes.

Walter believes that during evolution, profilin 2 might have acquired a very specific function: controlling the release of neurotransmitters at synapses-something that requires high precision. He suggests that his findings may throw light on what is happening in the brains of children with attention deficit hyperactivity disorder, who share many of the symptoms of his profilin 2 knockouts. "What is interesting is that the cytoskeleton is also influencing higher brain function and complex behaviour," he says.

Pilo Boyl, P., Di Nardo, P., Mulle, C., Sassoè-Pognetto, M. Panzanelli, P., Mele, A., Kneussel, M., Costantini, V., Perlas, E., Massimi, M., Giustetto, M., Vara Rivera, H. and Witke, W. Profilin2 contributes to synaptic vesicle exocytosis, neuronal excitability, and novelty-seeking behavior. *EMBO Journal*, in press.

Ingenious cell dynamics

E VERY CELL NEEDS INTERNAL SCAFFOLDING to help it keep its shape, move and divide. The 'cytoskeleton' is made of a sophisticated array of filaments, and one type of filament – the microtubules – has particularly impressive dynamic properties. Before a cell divides, the long, stable microtubules that make up the cytoskeleton become short and dynamic, and transform into the 'mitotic spindles' that attach to chromosomes and pull them to opposite sides of the cell. Eric Karsenti wants to know how this microtubule switch – from a stable state to a dynamic state – is orchestrated.

Some details are already known. Several proteins – known as microtubule associated proteins (or MAPs) – attach to microtubules and influence their dynamics; some MAPs stabilise the fibres and others destabilise them. But Eric suspected that the key to understanding how the switch occurs would be the way that these proteins interact with one another. So he, together with researcher Philippe Bastiaens, devised a technique that allows them to investigate the interactions between several proteins simultaneously.

They decided to study these interactions using extracts of frog eggs – a favourite system for biologists wanting to observe the dynamics of cell division inside a test tube. Researchers normally analyse interactions between proteins by 'fishing' for a target protein, and finding out which other proteins the target is stuck to. To the extract, they add beads coated with an antibody that sticks to a 'target' protein. When the bead is removed from the extract and washed, researchers can analyse the target protein and any other proteins that the target interacts with. But Eric felt that this technique – called 'immunoprecipitation' – was not sensitive enough for the protein interactions that interested him. Interactions between MAPs are weak, and could be disrupted by the washing process.

Philippe came up with a solution. Together with Philipp Niethammer – now based at Harvard Medical School – they developed a way of examining the interactions between MAPs directly in frog-egg extracts, a technique that they call 'visual immunoprecipitation'. As before, they coat beads with antibodies that bind to a specific target MAP, but this time they tag each of the potential binding partners of the target MAP with a fluorescent label. When one or several of the tagged proteins bind to the beads, different combinations of proteins produce different fluorescent colour readouts. The fluorescent labels let the



Microtubule aster in a cell that is resting between two divisions.

researchers identify the interactions between MAPs directly under the microscope, without taking the beads out of the test tube.

And what did they find? One particular MAP is a 'destabilising factor' called XKCM1. Together with another member of Eric's lab, Iva Kronja, the researchers figured out that when the cell is resting between cell divisions, 'stabilising' MAPs attach to and trap XKCM1, suppressing its destabilising activity. Then, just before cell division, these stabilising MAPs let XKCM1 go free, causing fibres to become short and dynamic and to form mitotic spindles. "This shows that microtubule assembly during cell division is largely regulated by dynamic interactions between MAPs," says Eric. He describes visual immunoprecipitation as a new way of looking at regulatory processes in cells. "It allows us to investigate the effects of a dynamic network of proteins, and not just the activity of one molecule."

Niethammer, P., Kronja, I., Kandels-Lewis, S., Rybina, S., Bastiaens, P. and Karsenti, E. (2007). Discrete states of a protein interaction network govern interphase and mitotic microtubule dynamics. *PLoS Biology* 5(2): e29

Roadworks on the motorways of the cell

A CELL IS A BUSY PLACE. In a permanent rush hour, molecules are transported along a motorway system made up of filaments called microtubules. But unlike our roads, microtubules are not stable. Instead they constantly grow and shrink and are flexibly assembled wherever a cargo needs to go. The filaments are kept stable only while the transport of molecules is in progress.

To investigate how this temporary stability is brought about, Group leader Damian Brunner and EMBL alumnus Andreas Hoenger studied how microtubules form in fission yeast. First the basic building blocks, small proteins called tubulins, assemble in a single line to form socalled protofilaments. Several protofilaments then combine to build a large tubulin sheet, which folds into the tube-like structure of microtubules. Combining molecular techniques with a unique electron microscope setup based at the ETH in Zürich, they discovered that a protein called Mal3p is crucial for holding the filaments together. Mal3p binds in a single line at the so-called lattice seam, which forms as the two sides of the tubulin sheet fold into a tube, seals the tube and stabilises it at its weakest point. "It is the first time that we've found a protein that specifically binds to the microtubule seam," says Andreas, who is now at the University of Colorado. "Until now the function of the seam has been unknown and it has been largely ignored as an odd and irrelevant part of the microtubule. Our experiments now reveal it as a central spot where microtubule stability can be regulated."

Linda Sandblad, who started out her PhD in Andreas' lab and continued her work with Damian, investigated what happens to microtubules in the presence of varying amounts of Mal3p. "Without Mal3p, microtubules are unstable and likely to disassemble, while in its presence they grow into long filaments," she reports. "This behaviour suggests Mal3p is a key regulator of microtubule stability. Controlling its presence allows fast switches between growth and shrinkage, which are essential for rapid and flexible cellular transport."

Mal3p's location along the microtubule seam is crucial, because here it can confer stability without obstructing the traffic of motor proteins along the filament. Apart from its stabilising role Mal3P could also play a more active role in transportation. "Motor proteins move along microtubules through direct interaction with tubulin. They transport cargo similarly to trucks driving on motorways," explains Damian. "The line of Mal3p along the seam potentially creates an alternative track on the filament, along which a specialised type of motor protein could move, just like creating a railway track along a motorway. This dual system could make transport more diverse and efficient." In line with this idea, motor proteins have been found for which motor activity is enhanced by Mal3p.

The new insights gained into cellular transport and the stabilisation of microtubules in yeast might help shed light on how similar processes work in humans. Mal3p is highly conserved across species and its human counterpart plays a role in various clinical conditions, such as colon cancer and neurodegenerative diseases.

Sandblad, L., Busch, K.E., Tittmann, P., Gross, H., Brunner, D. and Hoenger, A. (2006). The Schizosaccharomyces pombe EB1 homolog Mal3p binds and stabilizes the microtubule lattice seam. *Cell* 127(7): 1415-1424

Andreas Hoenger, Linda Sandblad and Damian Brunner found the Swiss solution to fixing a microtubule.

The great migration



Darren Gilmour, Guillaume Valentin and Petra Haas



VERY YEAR IN MAY, vast herds of wildebeest move from the dry East African plains towards the woods in search of food. They return in November, when the short rains come to water the plain, and so their annual migration is completed. Each wildebeest behaves according to its instincts for survival, which tend to push it towards the company of other wildebeest. Once a herd has formed, it behaves as a new unit, according to its own rules. Cells have a similar herd instinct. They are genetically programmed to work with other cells, but there is no strict set of genetic instructions that determines the precise behaviour of every cell in the group. "We realise now that many of the events that occur after fertilisation are somehow free and plastic, and based on self-organising principles," says Darren Gilmour.

As a developmental biologist, Darren would like to understand those principles, because the mass migration of cells gives rise to the sculpting of intricate and complex organs such as the respiratory system and blood vessels. Since the cells within these migrating groups appear to be very similar in terms of the genes they express, knocking out or down single genes tends to block migration in the whole group. He has to take a more subtle approach, finding ways to disrupt the cells' social networks, and looking at how that affects their ability to migrate and build organs. "It's sociology, basically," he says. "We want to know who tells who what to do, and how." In Heidelberg, Darren's group explores these principles in the lateral line primordium of the zebrafish, a transient structure that can only be seen in early development, and whose function is to equip the embryonic fish with hair cell organs – sensory organs which enable it to detect the presence of other fish based on minute changes in water pressure. "The primordium is a cluster of more than 100 cells which creeps along under the skin of the animal, like a slug," Darren explains. "It starts behind the ear, moving towards the tail, and as it goes it leaves a trail about two cells thick, punctuated by little clusters of cells that will eventually become hair cell organs."

As model systems go, the zebrafish is an answer to biologists' prayers, being transparent and therefore amenable to live imaging of cells which have been labelled with fluorescent proteins. The lateral line system is relatively easy to engineer genetically, and it is also a gift to those interested in cell migration, because it makes use of a signalling pathway that is very important in human health, and therefore well-studied. Primordium cells express the receptor Cxcr4b, which responds to the chemical signal SDF1a, the zebrafish equivalent of a human signal, stromal-derived factor 1 (SDF1). Cxcr4b is a protein which sits across the cell membrane and, in humans, is necessary for HIV infection. As well as playing a role in normal development, it is also implicated in metastasis, or the spread of tumour cells.



In a zebrafish mutant that has reduced Cxcr4b signalling, Darren's group found that the primordium loses all directionality – there is no forward movement and no slug trail left behind. Moreover, the static structure seems to stop producing cell clusters, as if it has to move and expel those clusters in order to generate more. One of the most inter-

esting features of the mutant, however, is that even though the primordium's migration is stalled, a closer look reveals that its cells are still moving, tumbling over one another in an apparently random fashion. "It looks as if the cells are confused," says Darren, "like monkeys in a sack." And that, he adds, is the key to how the system works.

To find out if they could rescue the mutant and restore forward motion to the primordium, the researchers

created a genetic mosaic by transplanting some normal, 'seeing' cells, which expressed the Cxcr4b receptor and could therefore respond to SDF1a, into the otherwise 'blind' mutant tissue. The normal and mutant cells were tagged with red and green fluorescent proteins, respectively, so that they could track their movements. Very quickly, through the tumbling motion of the cells in the primordium, the seeing cells found their way to the front, and the primordium once again moved forward. What was strange about these experiments, says Darren, was that not all of the sensing, Cxcr4b-expressing cells found their way to the front. As soon as some had moved there, the random tumbling motion stopped, the primordium moved forward and those sensing cells that had not made it to the front remained where they were – as if

"It's not the genes that say, you're the leader, you're the follower," Darren says. "Rather the genes equip all the cells with the receptor, and after that it's a case of, fight amongst yourselves." frozen in ice. "We think that the system finds strength in instability," he says. "It rolls around and makes random movements, and in doing so it ensures that the cells that can lead get into the position where they do lead. Whenever they pull, the others freeze."

But if not all sensing cells become leaders, what defines a leader? Whatever it is, it doesn't appear to be differences in the activity of

genes. "It's not the genes that say, you're the leader, you're the follower," Darren says. "Rather the genes equip all the cells with the receptor, and after that it's a case of, fight amongst yourselves." He and his co-worker Petra Haas think leadership is more likely to be defined by the unstable nature of the migrating group. The random tumbling of the cells gives some a temporary bias over others, in terms of their proximity to the source of the signal. They then latch on to it, and if they are able to maintain the activation of their receptor, they move to the front. However, that is not the end of the story, by any means.

One theory of cell migration is that the leading cells somehow carry the followers, which passively allow themselves to be swept along. Darren is sceptical of what he calls the hitchhiker model, however. He prefers to think that the leaders point the others in the right direction, and the primordium is internally organised such that those others then actively follow. And he believes that a similar principle will apply to the development of all organs. "If you look at the branching of blood vessels, I predict that there will be a small number of cells at the tip which sense the signals from the environment, and through cell-cell interactions they somehow influence the behaviour of the others," he says. "The others don't have to see the world." He gives the analogy of a falcon ambushing a flock of starlings. Not all the starlings have to see the falcon in order to respond appropriately by fleeing; they only have to detect the panicked response of their neighbours.

So far so good, but the story gets more complicated still, because it turns out that not all the followers behave in the same way. In their live-imaging experiments, Darren and his team have noticed that as the front of the primordium noses forward, the cells in the rear slow down, causing the tissue to stretch. PhD student Guillaume Valentin has now identified another receptor, expressed by those rear cells, which also responds to SDF1a, but which triggers a



In a genetic mosaic, wild-type cells (red) find their way to the tip of the primordium and project in the direction of migration, pulling mutant cells (green) with them.

A genetic mosaic, is made by taking cells expressing the Cxcr4b receptor (red) out of a wild-type embryo and transplanting them into a mutant lacking functional Cxcr4b (green).



different kind of behaviour to that elicited by the SDF1a/Cxcr4b pathway – giving the cells at the back a certain degree of independence from the Cxcr4b-expressing leader cells.

When the primordium is moving forward, depositing cell clusters at the back, the general motion of cells through the structure is backwards. As cells fall back inside it, Darren suspects they may switch on this second receptor. Perhaps, he speculates, the combination of the two cell populations expressing different receptors gives rise to a kind of inchworm motion, in which the front and back of the primordium move forward alternately. Some evidence for this comes from experiments with zebrafish embryos in which the activity of the second receptor is reduced, and in which the second half of that coordinated movement is lacking. "The leading edge pulls and pulls, but the back doesn't come with it, so eventually it gives up and tumbles back," he says.

If he is correct, the new findings raise questions about how dynamic information is transmitted through large groups of cells. It becomes necessary to understand not only signalling, but also the propagation of forces through cell populations, in terms of cell-cell interactions. This is where Darren now hopes to take his research. For now, though, he is excited by the discovery of a second SDF1a receptor in the zebrafish primordium. "The idea that the cells inside can also sense is entirely novel," he says. Whether the same principle applies to wildebeest is another matter.

Haas, P. and Gilmour, D. (2006). Chemokine signaling mediates self-organizing tissue migration in the zebrafish lateral line. *Developmental Cell* 10(5): 673-680

Valentin, G., Haas, P. and Gilmour, D. (2007). The chemokine SDF1a coordinates tissue migration through the spatially restricted activation of Cxcr7 and Cxcr4b. *Current Biology*, in press.

An architectural plan of the cell

IKE OUR BODIES every cell has a skeleton that provides it with a shape, confers rigidity and protects its fragile inner workings. This scaffold, called the cytoskeleton, is built from different types of protein filaments, including long, tube-like structures called microtubules. Microtubules are very dynamic and consist of constantly growing and shrinking rows of elementary proteins called tubulins. To increase their rigidity, microtubules associate

in bundles and interact with stabilising proteins in complex networks. These networks are essential for many cellular processes and a precise plan of their architecture would be a big step towards understanding the diverse roles they play in a cell. But such a plan is difficult to achieve.

"To really understand the architecture of the cytoskeleton you have to see the entire cell in three dimensions," says Claude Antony, who heads EMBL's Electron Microscopy Core Facility and also leads a team studying the cytoskeleton. "At the same time you need a very good resolution to investigate its fine structures. It is impossible to obtain such detailed images of a eukaryotic cell with normal microscopes."



The electron tomogram of a complete yeast cell reveals the cellular architecture. It shows plasma membrane, microtubules and light vacuoles (green), nucleus, dark vacuoles and dark vesicles (gold), mitochondria and large dark vesicles (blue) and light vesicles (pink).

only suggested. When a cell is not dividing, a microtubule bundle consists of 4-5 individual filaments that are physically connected with each other via minute bridges likely formed by proteins. The nature of these bridges and their role in the formation of microtubule bundles is what Claude's PhD student Helio Roque is trying to find out. They contribute to crosslinking microtubules into complex networks, in which the orientation of the filaments is

> crucial. Microtubules are polar structures: their two ends grow and shrink at different rates. The study created a precise map indicating the location of all growing and shrinking microtubule ends in the cell.

> "Our 3D image of fission yeast can serve as a reference map of the cell for all biologists interested in its architecture," says Johanna. "You can extract information about all sorts of cellular structures from it or use it to place findings into the spatial context of the cell."

Yeast is one of the most commonly used model organisms in biology. It has many similarities with higher eukaryotes, including multicellular organisms. Many of the insights gained into its cellular organisation are likely to apply also to mammals.

To bridge the gap between global overview and structural detail of a cell, Claude's team collaborated with yeast and electron microscopy expert Richard McIntosh at the University of Colorado. Using a technique called electron tomography, Johanna Höög, a PhD student in Claude's lab, created the first 3D image of a complete eukaryotic cell at a resolution high enough to resolve the cytoskeleton's precise architectural plan. She took pictures of sequential sections of a fission yeast cell from many different angles through an electron microscope and combined these snapshots into a 3D reconstruction on the computer. A similar principle is used to generate brain scans.

Thanks to the image, the researchers could for the first time see directly what previous studies in fission yeast had In mammalian nerve cells, for example, microtubule bundles similar to those observed in yeast are essential for the transmission of the signal from cell to cell. The study also has implications for Claude's Electron Microscopy Core Facility. "Research like this expands the limits of what we can do with electron microscopes. It drives the development of instrumentation and technology which we can then make available in the Core Facility so that other scientists can benefit," concludes Claude.

Höög, J.L., Schwartz, C., Noon, A.T., O'Toole, E.T., Mastronarde, D.N., McIntosh, J.R. and Antony, C. (2007). Organization of interphase microtubules in fission yeast analyzed by electron tomography. *Developmental Cell* 12(3): 349-361



Close encounters

OWADAYS, DOCTORS CAN OPERATE on a fetus in the womb, to correct congenital defects they judge would be too advanced to correct after birth. One of the striking aspects of fetal surgery is that, at least until a certain point in pregnancy, it leaves no superficial trace. Since the fetal immune system is not yet active, the inflammatory cells which are involved in the formation of angry red scar tissue after birth don't infiltrate the wound as it heals. The result is an invisible seam, as if the surgeon's knife had never entered.

Something similar happens in the final stages of development of the fruitfly embryo. After it has acquired its segmented form, and while the head is still taking shape, a yawning, eye-shaped opening remains in the epithelium or skin covering its back. At last, the epithelium stretches and the hole closes from both ends, like a zipper, until no evidence of it remains. Dorsal closure, as this process is called, has long been considered a model for wound healing, though in the absence of any scar it most closely resembles fetal wound healing.

When Damian Brunner was studying fruitfly development for his PhD, the signals that initiate dorsal closure were a hot topic of research. "But the signal is only the trigger," he says. "After that, you see dramatic changes taking place in the cells. To me, those changes were far more interesting." He started looking at the mechanisms that shape cells in another organism, fission yeast, in which microtubules play a key role as general orienters of the cell – giving it a front and a back, for example (see page 41). Meanwhile, in other fields, evidence was growing that microtubules weren't just the cell's housekeepers; they could also be recruited for specialised activities to do with shaping the space beyond the cell – activities that might include dorsal closure. That, in a nutshell, is how Damian came back to study the fruitfly *Drosophila* with his group in Heidelberg, and in particular the role of microtubules in dorsal closure. "Everyone thought that microtubules in non-dividing cells were just permissive, that they merely transported things in the cell," he says. "That was also all they were believed to do in dorsal closure."

It was already known that dorsal closure seems to start with the contraction of the amnioserosa cells that occupy the eye-shaped opening in the embryo's back. That contraction coincides with the elongation of the epithelial cells on either side of the opening, along an axis perpendicular to it – a stretching, thinning effect which is aided by the contraction of a band around the epithelial cells, made from another protein called actin. In 2002, another piece was added to the picture when researchers showed that microtubules in dead epithelial cells that had been fixed or 'frozen' in their elongated state were aligned in parallel arrays along their longitudinal axis.

With postdoc Ferenc Jankovics, Damian set out to image dorsal closure in living fly embryos in which the microtubule constituent protein tubulin had been labelled with a fluorescent marker. Though live imaging of dorsal closure had been done before, their interest in microtubules represented a particular technical challenge: "The main difficulty was that we wanted to study very small objects,



Zipping up a fly embryo.

at the subcellular level, in the huge *Drosophila* embryo," Ferenc explains. "Another difficulty was that we were studying a very dynamic process, microtubule organisation, so we needed good time resolution." After four months of trial and error, and with the invaluable support of the Advanced Light Microscopy Facility in Heidelberg, they found the optimal conditions for creating confocal microscopy movies of dorsal closure, meaning that they could now watch it unfold in real time.

As the epithelial cells stretched and became thin, Damian and Ferencs saw the microtubules inside them become increasingly aligned until they formed stable, fairly rigid, parallel arrays. At the zippering stage, the cells' actin machinery – which they had labelled with a differently coloured fluorescent protein – began to form protrusions from the leading edge of the epithelial cells nearest to the opening, and these protrusions were then infiltrated by microtubules. "As in many cell types, actin is the force creating and shaping the surface, but the microtubules can crucially modify this system," Damian says. The protrusions would seek out protrusions from cells on the other side of the opening and, when close enough to fuse, intertwine with them like the teeth of a zip. The hole closed, and very soon all evidence of the cells' fusion had vanished, leaving a seamless join. Within 30 minutes of the completion of zippering, the microtubule arrays had dismantled themselves and the epithelial cells had shortened again, returning to their pre-dorsal closure shape. The whole process lasted less than three hours, with the final zippering stage taking about an hour.

The researchers now suspected that microtubules were involved in dorsal closure, but were they essential to the process? To investigate this, they treated embryos with a drug which selectively degrades microtubules. The amnioserosa cells contracted as before, the epithelial cells stretched and the two sides of the opening approached each other. After this promising start, however, the epithelial cells' elongation collapsed, they became roundish again and zippering failed. Those experiments showed that microtubules were only essential for the final phase of dorsal closure, the zippering, but since the drug had destroyed microtubules in all the embryonic cells, they needed to test that the microtubules in epithelial cells alone were driving the process. So they created transgenic embryos which expressed a protein known to chop microtubules into little pieces, and they created them in such a way that the protein was only expressed in alternating bands of epithelium.

The results could not have been clearer: in the bands where the microtubules remained intact, the epithelial cells fused and the zip closed. But in the bands in which they had been destroyed, the zip could not close and small holes remained in the epithelium. "The epithelial cells have these protrusions coming out, they search for each other and they fuse very exactly with a partner on the other side," says Damian, adding that similar protrusions are seen in wound healing. "The protrusions contain microtubules, and they need those microtubules to be effective."

He would now like to explore the mechanisms that direct that fusion process, and to know precisely what initiates microtubule reorganisation. Is it the contraction of the amnioserosa cells, the stretching of the epithelial cells or specific signals from neighbouring cells? "Maybe a combination of factors is required," he speculates. "First the pulling stretches the cells, but then you need the microtubules to keep them stretched – that's my favourite hypothesis."

The answer could have implications for scientists' understanding of zippering events in other organisms, including humans – from the closure of the neural tube in the embryo, to the wrapping of the optic cup around the lens during eye development, and of course to wound healing, both before and after birth.

Jankovics, F. and Brunner, D. (2006). Transiently reorganized microtubules are essential for zippering during dorsal closure in Drosophila melanogaster. *Developmental Cell* 11(3): 375-385

The microscope image of the dorsal opening of a fly embryo shows alternating stripes of epithelial cells with aligned microtubule bundles (green) and epithelial cells treated with microtubule-destroying drug spastin (blue). Labelled in red is the protein actin that lines the border of cells, particularly the amnioserosa cells occupying the eye-shaped opening.



An early mover

AMILIAR

textbook drawings of animal and plant cells show DNA packaged neatly inside a

two-layered membrane that defines the nucleus. But drawings cannot convey one of the most striking properties of this 'nuclear envelope' – its remarkable dynamics. On top of its sophisticated structural and regulatory functions, the nuclear envelope is continually remodelling itself. Before cell division, the envelope needs to break down to release the chromosomes, and at the end of cell division it has to be reformed. Iain Mattaj has spent years teasing apart the complex functions of the nuclear envelope, and so its dynamics present a particularly intriguing problem – how are the components of the envelope dispersed and reassembled every time a cell divides?

Iain's lab at EMBL Heidelberg works on a protein called Ran, which is needed to help move the gene replication and regulation machinery in and out of the nucleus through protein channels called nuclear pore complexes. But Iain recently found that Ran also has an important role in nuclear dynamics, which piqued his interest in other players responsible for coordinating nuclear remodelling. Now, he and his lab have found another protein – called MEL-28 – that helps the nuclear envelope to remodel itself when the cell divides.

Postdocs Vincent Galy and Peter Askjaer began by investigating what happens to MEL-28 at the beginning of cell division. After the chromosomes have replicated, the nuclear envelope breaks apart and the chromosomes are pulled to opposite sides of the cell by fibrous structures called mitotic spindles. Some of the nucleoporins – the proteins that normally form the nuclear pore complexes – accumulate at the kinetochores, the regions on the chromosome at which the mitotic spindles attach. The researchers labelled MEL-28 with a green fluorescent protein and watched its progress inside the cell. They were intrigued to see MEL-28 accumulating at the kinetochores with the nucleoporins. What MEL-28 does at the kinetochores is still a puzzle, but Iain points out that when this protein is missing, the chromosomes do not segregate properly to daughter cells. "We wonder whether MEL-28 is needed for the mitotic spindles to attach properly to the chromosomes."

Nuclear pores on Mars.

But MEL-28 also seems to have functions at the end of cell division. After the cell divides, the genetic material, the chromatin, decondenses, and around it accumulate the components of the future nuclear envelope – the nucleoporins and small pieces of membrane. Cerstin Franz and Rudolf Walczak found that MEL-28 arrives at the chromatin very early in this process, where it interacts with some of the nucleoporins as they accumulate there. In fact, MEL-28 is essential for recruiting the nucleoporins to the chromatin – without it, the nucleoporins never get to the chromatin and the nuclear envelope that forms has no nuclear pore complexes.

Iain describes MEL-28 as an anchoring platform for nucleoporins. "It looks as though MEL-28 functions as a 'seeding' point for the assembly of the nuclear pore complexes." He says that MEL-28's interaction with chromatin is controlled by Ran. "But we're keen to find out why this interaction only happens at the correct time in cell division, i.e. how it is regulated by the cell cycle."

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A switch between life and death

HAT DO BANTAMS AND HIPPOS have in common? One is a microRNA and one is a protein, but both shed some light on one of the great unsolved questions of biology: how do developing organisms and their tissues know how big to grow? MicroRNAs are tiny RNAs with a 'hairpin loop' structure that are crucial for gene regulation. Developmental biologist Steve Cohen recently stumbled across a microRNA with a powerful effect on the size of fruitflies. Now, he's worked out how this microRNA fits into an important pathway that controls growth.

The story started a few years ago when Steve and colleagues became intrigued by a mutation – called bantam – that caused flies to be very small. For a while, it wasn't clear what the gene encoded; it was only when Julius Brennecke, a PhD student in Steve's lab at the time, recognised that its RNA sequence must produce a microRNA that things began to get interesting. MicroRNAs are tiny gene regulators that stick to the tails of specific messenger RNAs – the mobile genetic 'templates' from which protein sequences are translated – and block protein production. The bantam microRNA clearly affected growth, but the researchers did not know what it did or how it was controlled.

Then postdoc Barry Thompson noticed that overexpressing bantam (forcing a tissue to produce more bantam than usual) had the same effect as disrupting a potent growth regulator called the Hippo pathway. The Hippo pathway has attracted a great deal of interest because all of its components are conserved in humans, and several are involved in cancer. This pathway regulates the activity of a protein called Yorkie, which promotes tissue growth. Steve and Barry wondered if the functions of Yorkie and bantam might be connected.

So the researchers genetically engineered cells either to overexpress yorkie, or to underexpress bantam, or to simultaneously overexpress yorkie and underexpress bantam. From this, they showed that when yorkie is overexpressed, the resulting overgrowth of tissues is caused by its The picture of a *Drosophila* brain shows how bantam expression relates to cell proliferation. Proliferating cells are marked in red and the cells expressing bantam are all that is not blue.

activation of bantam. In other words, bantam is responsible for the powerful action of the Hippo pathway.

But what about bantam's targets? "The hunt for bantam's targets led the lab in an exciting new direction," Steve says. Alex Stark (in Rob Russell's lab in the Structural and Computational Biology Unit), collaborated with Julius to develop bioinformatics approaches for recognising which messenger RNAs are targets for microRNAs. They found that a gene called Hid, which is involved in both cell division and cell death, is an important target of bantam (although the researchers know there must be others). They were also astonished to find that, overall, as many as a third of all messenger RNAs are regulated by microRNAs.

These findings are all the more remarkable because microRNAs lay completely undetected until about ten years ago. "MicroRNAs are small targets for conventional mutagenesis, so they have tended to be missed by genetic screens," says Steve. "Now we realise that they have important roles in development and disease." The discovery of bantam and the wealth of microRNA targets across the genome opens the door to understanding these roles.

Thompson, B.J. and Cohen, S.M. (2006). The Hippo pathway regulates the bantam microRNA to control cell proliferation and apoptosis in Drosophila. *Cell* 126(4): 767-774



A delicate balancing act

THE AIRWAYS OF THE HUMAN LUNG are covered with a thin film of liquid which, like so many systems in biology, is in a constant state of flux. To maintain that liquid layer, the cells lining the airways secrete fluid containing chloride ions, and absorb fluid containing sodium ions – the two components of salt. This dynamic equilibrium is critical to the airways' health, because it enables them to adapt to changing circumstances. In the fetus, for example, active secretion helps drive the expansion of the developing lungs. If infection later causes oedema or water retention in the lungs, the balance may shift towards absorption.

One disease which fatally disrupts this equilibrium is cystic fibrosis (CF). A genetic mutation means that although a key chloride channel is present in the cells of the lung's lining or epithelium, it doesn't occupy the correct position in their membranes. "The absence of that chloride channel means too little chloride is secreted and the lung's epithelial surface dries out," Carsten Schultz explains. "The mucus solidifies and can't be coughed off. Opportunistic infections set in and though they can be treated, these days patients still only have an average life expectancy of 25 to 30 years."

Carsten's background is in medicinal chemistry, but the current interests of his group in the Gene Expression Unit in Heidelberg fall squarely into the domain of cell biology. He aims to elucidate molecular pathways that regulate epithelial chloride channels. The chloride channel that is affected by CF is called the CF transmembrane conductance regulator. Thinking laterally, however, the Schultz group has focused its attention on a less well-studied chloride secretion pathway – one involving calcium-activated chloride channels (CaCCs).

"The goal of our research is to visualise the whole of this signalling cascade, from the occupation of the receptor which activates it to the opening of the chloride channel," says Carsten. "Any component of this pathway is a target for us, and we will prepare fluorescence sensors to visualise it in living cells, to see how it interacts with other components. We believe that by doing so we can eventually help people develop drugs against CF that positively influence chloride secretion via the CaCCs."

The CaCCs activate chloride secretion in response to elevated levels of calcium ions in epithelial cells. One of the later steps in the CaCC pathway is the activation of enzymes which phosphorylate, or attach a phosphate group to, the chloride channel, causing it to open. In 1994, scientists found that if epithelial cells were treated with a cellular protein called annexin A4 at high calcium concentrations, the annexin A4 clotted together to form a flat grid beneath the cell membrane, preventing one key enzyme from getting to the chloride channel and phosphorylating it.

Those findings seemed to suggest that annexin A4 could be acting as an off switch for the CaCC pathway, making it potentially interesting to Carsten and his team. By genetically engineering cultured human cells, they tagged annexin A4 molecules with fluorescent protein labels, or fluorophores, and tracked the fluorescence under a confocal microscope. This meant they were able to see how the



The membrane of lung epithelial cells contains channels (blue) that secrete chloride ions. These channels move sideways within the membrane. Under normal conditions the protein annexin A4 (red) is evenly distributed inside the cell.

annexin A4 responded to artificially raised calcium levels in the cell. "The annexin A4 is evenly distributed inside the cells, and upon elevation of calcium you see it translocate, first from the cytoplasm or internal fluid of the cell to the inner leaflet of the membrane and then, with a certain delay, from inside the nucleus to the inner leaflet of the nucleus," says Carsten.

The grids underneath the cell membrane looked pretty solid, so they used a technique called fluorescence recovery after photobleaching (FRAP – see box) to detect an exchange of annexin A4 molecules within the grids, and between the grids and the rest of the cell, in order to test just how immovable and rigid they really were. They saw hardly any movement. "More than 95 percent of the annexin A4 molecules were at the membranes and the cytoplasm was pretty empty," says Carsten. "The grid is really solid, which makes us believe that annexins are involved in stabilising the membrane when something really dramatic happens to the cell, like an inflammatory incident or mechanical injury."

To their surprise, they found that they could drive the annexin A4 aggregation into reverse by treating the cell with tricyclic drugs – drugs that in humans are used to treat the psychiatric disease schizophrenia. Using another technique called fluorescence resonance energy transfer (FRET – see box), they highlighted the aggregation of annexin A4 under high calcium conditions, and watched what happened when they added the tricyclics. "We could see very clearly the dissolution of the grids and the restoration of even annexin A4 distribution in the cytoplasm, through the breakdown of FRET," says Carsten.

"We think that annexins are part of an innate repair system for cells, and that when in distress – as in the CF lung – epithelial cells might try to recruit that system to physically strengthen the epithelium," says Carsten. "This may have implications for chloride secretion, because with the grids in place the chloride channels can't move as much any more – including those channels which are not directly affected by the disease."

The findings also suggest that the annexins might play a more positive role – perhaps their intended physiological role – in wound healing after mechanical injury. In a more recent series of experiments, the researchers looked at the behaviour of annexins in cells that had been damaged by poking with a sharp glass needle. "We find that annexin A4 concentrates very locally where the tip of the needle punches a hole into the cell," Carsten says. Different members of the annexin family appear to be active at different stages of wound healing, potentially forming separate grids around the site of injury and so, over time, adding strength.

Because annexins are important in a range of bodily processes besides chloride secretion, notably wound healing, Carsten thinks they are unlikely to make good therapeutic targets for CF. Nevertheless, he believes this work could lead to a better understanding of the pathways involved in chloride secretion, and hence to the design of more specific drugs with fewer side-effects – and not just for CF.

The findings could also throw light on how tricyclics exert their therapeutic effects in the brain, where annexins are present in abundance. "One could speculate that communication between neurons is inhibited in the


When a cell is in distress it elevates its calcium levels (Ca²⁺), which causes the annexin A4 to translocate to the membrane and form a flat grid. The grid prevents the chloride channel from opening and stops its motion within the membrane. While this mechanism might strengthen the membrane after injury it has negative consequences in cystic fibrosis.

schizophrenic brain by abnormal annexin aggregation," Carsten says. "Tricyclics may remove that inhibition by dismantling the annexin grid." He has no evidence for that theory, but if he is right, it could help explain one of the long-standing mysteries of tricyclic therapy – why the drugs can take several weeks to produce their positive effects in patients.

Piljic, A. and Schultz, C. (2006). Annexin A4 self-association modulates general membrane protein mobility in living cells. *Molecular Biology of the Cell* 17(7): 3318-3328

The last decade has seen an explosion in the number of biological applications of one particular kind of genetic engineering: the addition of tags, based on naturally fluorescing proteins originally found in jellyfish and corals, to cellular proteins. This is achieved by combining the reporter gene, which encodes the protein fluorophore, with the gene of interest in the same DNA construct, and inserting that construct into a cell or organism. The fluorescence of the resulting fusion protein can then be visualised in live cells under a fluorescence microscope.

Fluorescence resonance energy transfer (FRET) – in which fluorophores that come into close proximity transfer energy between themselves, resulting in an amplified fluorescence effect – is just one widely used application of this basic technology. Another, which Jan Ellenberg and colleagues at the Gene Expression Unit in Heidelberg were among the first to apply to living cells, is fluorescence recovery after photobleaching (FRAP) – when the movement of intact fluorophores is observed into an area of a cell where others have been bleached by a targeted laser beam.

Jan's team is currently applying a novel variation on FRAP that is based on photo-activation rather than photobleaching. "We're using this technique to explore dynamic movements of proteins at very high time resolutions, in the millisecond range," he explains. "For example, we're interested in diffusion and molecular crowding in the nucleus. Macromolecules like DNA are not evenly distributed in the nucleus, and their density may affect processes such as the ability of transcription factors to turn on genes. By attaching fluorophores to specific molecules in the nucleus, we can ask them to report on crowding for us."

'Intelligent', computerised microscopes are also under development, which will enable techniques such as FRET and FRAP to be applied in a high-throughput manner to the scanning of living cells. Such a microscope would be equipped with automatic recognition software that would enable it to illuminate the correct portion of the cell in FRAP, for example. Though the technology is in its infancy, Jan says it won't be long before it's in routine use.



Structuring the bigger picture if you reall

ern structural biology, go and look at a Monet. At first glance, the link between the work of a French Impressionist painter and the science of studying the shapes of molecules might not be so obvious. But look a little closer and you will see how: just as Monet's brush-strokes work together to create an overall impression, so the structures and functions of proteins and other molecules synergise to create a bigger picture.

Impressionist painters didn't rely on mixing their paints on a palette to achieve just the right colour. Instead, they applied paints straight to the canvas, juxtaposing different colours so that they would merge in the eye of the observer and create the right shade. If you peer too closely at the canvas, all you will see is different-coloured brush-strokes lying next to each other. Pull away, and the whole picture comes alive.

Structural biology used to focus very much upon the cellular equivalent of brush-strokes: the structures of individual molecules. Such studies were, and still are, extremely valuable. Molecules are like tiny machines, each with intricate working parts. Understand the structure, and you understand more about how the machine works.

Increasingly, however, just like art critics scrutinising Monet's handiwork, biologists are trying to understand how these machines interact with each other and how they all come together to generate the big picture of how a cell works. Seeing how structures work as part of systems will not only shed new light on how those systems operate, but also open up avenues for scientists to manipulate them – developing new drug treatments or understanding what causes disease. Researchers at EMBL Grenoble, for example, have used structures to uncover vulnerable spots in the biology of viruses such as rabies and HIV, and given new insights into how certain drugs exert their effects on the proteins they target. Other teams are determining the structures and functions of proteins that can fail, causing cancer.

The global approach will also help scientists predict a protein's probable function from its structure. Work like this, now being done by teams at the EBI, will save a lot of time and money, allowing scientists to go straight to the right experiment to confirm the function of a newly discovered protein. So from studying the details, to putting the final picture together, EMBL scientists, like Monet, are creating an impression of real life.



Stephen Cusack, Head of EMBL Grenoble

All for one

Stephen Cusack, Head of the Grenoble Outstation, explains how structural biology is set to merge with the bigger picture and how EMBL will tackle the challenges this presents.

Structural biology has received an increasing amount of attention in the past ten years. Why is it so important?

The structure of a molecule itself can be very beautiful, but just seeing what it looks like is not the main goal. The ultimate aim is to understand biological processes. Proteins and other molecules are very much like tiny machines, with intricate working parts and mechanisms. Being able to see how these parts work in atomic detail is really going to help scientists understand a molecule's biology, or help with a medical aspect, for example, understanding how a genetic disease mutation can affect a protein's function or how a drug can inhibit a key viral or bacterial enzyme.

In the past, structural biologists studied single proteins in isolation, such as myoglobin, which transports oxygen in the body's muscles. Nowadays, researchers, and especially people at EMBL, are interested in seeing how these proteins work as parts of systems, as part of processes within the cell as a whole.

Our group works on a number of proteins related to RNA metabolism: how different RNA molecules in the cell are matured and transported and 'translated' into proteins. These processes involve the interactions of many different groups, or 'complexes', of proteins. Work like this is bringing structural biology closer to molecular cell biology and the new discipline of 'systems' biology, which aims to explain how the millions of proteins and molecules within a cell interact in a complex network to allow the cell to function.

Can you give an example of how the work at EMBL Grenoble is starting to merge with cell and systems biology?

One key example is our project on the influenza virus polymerase. This is an enzyme that makes copies of viral RNA in an infected cell, meaning it is essential for the production of new viruses. It is also essential for the production of viral proteins.

We are interested in it for three reasons. First, it is interesting from a purely structural and mechanical point of view: it is made of three main parts, or subunits, and functions like a machine to synthesise RNA; how does it work? Second, it interacts with proteins in the host cell. Working out how and why it does this is key to understanding more about the disease and why, for instance, bird flu polymerase has to adapt by mutation to perform optimally in human cells. Finally, it is a good potential drug target, and new drugs to treat flu are badly needed. There is a very real risk that one of the bird flu strains currently in circulation will adapt to humans, triggering a pandemic.

We succeeded in crystallising and studying a fragment of one of these subunits (see page 120). After solving its structure, we turned to cell and systems biology. Together with experts in live-cell imaging at EMBL Heidelberg, we studied the behaviour of the fragment in living cells and worked out how it interacted with a key host protein. This work has given the first clues as to what the role of these mutations might be, and they almost certainly have rather subtle effects. They rebalance how the polymerase works in avian versus mammalian cells – a real systems biology question. It shows the power of structural studies in gaining an insight into how structure affects function which in turn affects biology.

We have now managed to make and study other fragments of the polymerase. One of these is directly involved in the production of viral RNAs which get translated into proteins and is an obvious target for a new drug. Seeing the structure of this fragment is crucial to finding the key working parts that can be blocked by drugs.

Where do you see structural biology going in the future?



For me, the real future is not doing structural biology in a test tube, but doing structural biology in a cell. We will move towards understanding structures and how they are relevant within the cell environment – by studying molecules and complexes inside living cells. To do so, structural biology will need to become truly interdisciplinary.

Again, the influenza project shows how this is already happening. Together with Jan Ellenberg, who heads the Gene Expression Unit at EMBL Heidelberg, we plan to track the production of these three subunits of the viral polymerase in the cell, to see how they are transported and assembled. We can use a technique called fluorescence correlation microscopy to watch how these proteins join together, and relate this to their structures. We can then see how mutations affect this and also which host cell proteins might be necessary.

In the future, structural biologists will still be looking in the test tube, but they will want to look in more detail at how molecules interact in living systems.

How is EMBL Grenoble preparing to meet the challenges of this new trend?

We're not trying to be a centre for structural genomics – an approach that aims to determine a structure for the product of each protein-coding gene in the genome, whether its function is known or not. Instead, we are focusing on studying a smaller number of proteins in greater detail, especially those of medical importance. Although structural genomics has its value in broadly surveying the protein world and maybe turning up something novel and exciting, I personally think our more selective approach is more satisfactory and more integrated into systems biology.

We are developing a range of technologies that will allow us to answer these more selective, systems-orientated questions, as well as building on the excellent resources and expertise here and in the neighbouring partner institutes of the PSB (Partnership for Structural Biology). Some of our innovations focus on automated highthroughput technologies that allow scientists to perform hundreds of experiments in parallel. Darren Hart's ESPRIT system is one of these: it allows scientists to quickly identify and make working parts, or domains, of proteins that are suitable for structural study. This is such an improvement on the older methods that we have a long queue of researchers who want to use it.

Other technologies that speed the process of research include the high-throughput crystallisation platform developed by Josan Márquez. This system forms part of the ongoing collaboration we have with the Hamburg



Inside the synchrotron bikes are the quickest way to move between the beamlines of the 844 m long storage ring.

Outstation to coordinate our technologies and expertise. Scientists from both outstations meet up every year for a 'bilateral meeting' to exchange ideas and know-how to push the development of such technologies forward. We are working, for example, on projects to develop more sensitive detectors to collect the X-rays scattered by crystals, and robots that change samples and align crystals correctly in the X-ray beams.

Anyone who wants to do structural biology in future needs access to all these kinds of platforms. In the future, I think there will be a number of major centres that will try to have a number of platforms for doing interdisciplinary structural biology. Such centres can be good for training – training and user aspects are part of EMBL's mission. We're collaborating with other institutes nearby to build on our strengths as a centre for integrated structural biology and also plan that this will become part of a network of such labs within Europe, as envisaged by the new European Road Map for Infrastructures (ESFRI).

Can you tell us more about these collaborations?

EMBL already has extensive collaborations with neighbouring facilities in the form of the PSB. This includes: the EMBL Outstation; the European Synchrotron Radiation Facility (ESRF), which produces some of the world's most intense X-ray beams; the Institut Laue Langevin (ILL), which produces neutron beams; and the Institut de Biologie Structurale, a French national laboratory supported by the research council CNRS and the French Atomic Energy Commission.

In 2001, Rob Ruigrok, a former Group leader at EMBL, founded the Institut de Virologie Moléculaire et Structurale (IVMS) as part of the University of Grenoble. But the IVMS was only intended to be a temporary arrangement on the road to setting up a mixed international research unit affiliated to a French university. We spent last year discussing a contract with the French authorities and have now set up the Unit of Virus Host Cell Interactions (UVHCI) between EMBL, the University of Grenoble and CNRS, which started at the beginning of 2007. The Unit is strongly founded on productive scientific collaborations between EMBL and the former IVMS on different aspects of virus host-cell interactions. Good examples are Winfried Weissenhorn's work on HIV budding, joint work on Epstein-Barr virus proteins, which also involves the Grenoble hospital, and of course the influenza virus project. We have just secured EU funding for this project to build a critical mass of expertise in the form of a three-year STREP (Specific Targeted Research Programme) - the Host Cell Variants of Influenza Polymerase project - called FLUPOL. This is all part of our strategy to combine state-of-the-art technical platforms for integrated structural biology with important biological and medical problems.



Winfried Weissenhorn in his former lab at EMBL Grenoble.

Locking the cradle

T IS PERHAPS ONE OF THE MOST FEARED diseases in the world. Once they appear, the classic symptoms stumbling, drooling, fear of water, paralysis - almost always portend death. Only five people have ever survived rabies, and all but one suffered permanent neurological damage. Although vaccination and post-exposure preventive treatments have dramatically reduced the impact of the disease in Europe and North America, there is still no cure. Rabies remains a serious threat in many developing nations, killing up to 70,000 people every year. Worryingly, rabies and similar viruses may now be reemerging in developed countries, spread to people by contact with bats. So the discovery by Winfried Weissenhorn, Rob Ruigrok and their colleagues of an avenue to attack the virus is very timely. The teams, based at EMBL Grenoble and the neighbouring Unit for Virus Host Cell Interactions (UVHCI), have uncovered the structure of the protein that cocoons the virus genome and hides it from the body's immune system until it has had a chance to copy itself. It may be possible to use drugs to lock the genome inside this protective cradle, thus preventing viral replication.

But the work has implications beyond the realm of the single disease rabies. It turns out that many other viruses, including the Ebola, Borna disease and measles viruses, have similar cocoons for their genomes, meaning that this finding could also shed light on where such viruses came from and how they evolved. The rabies virus, like several other viruses, forms its genome out of a single strand of RNA, a chemical relative of DNA. But the strand does not code directly for the proteins needed to make the virus.

Instead, it is a complementary sequence, a kind of chemical photographic negative, of the sequence needed. So before the virus can make proteins, it first has to convert this 'negative strand' of RNA into 'positive' ones that can be translated into proteins. It also needs to make more copies of its genome to turn into more viruses.

For the virus, these processes are fraught with danger. Mammalian cells, including human cells, contain defence systems that attack and destroy foreign RNA. So the virus hides its vulnerable genome by packing it tightly inside a nucleocapsid, a shell made of a protein called nucleoprotein, to protect it until it can get inside the cell and do its job. As well as shielding the genome, nucleoprotein helps to control the balance between protein production and viral replication, and so plays a key role in the virus life history.

Until now, however, the only clues scientists had about how nucleoproteins worked came from fuzzy electron microscope images that showed how the nucleoprotein molecules polymerise on the genome to form nucleocapsids, but revealed little about the structure of the protein itself. To find out more, Rob Ruigrok of the UVHCI and Winfried Weissenhorn, who recently moved from his Group leader position at EMBL Grenoble to the UVHCI, collaborated to make crystals of nucleoprotein and determine its structure using the high-intensity X-ray beams available at the ESRF.

The project began in Rob's lab, where his team had been working on the nucleoproteins of a number of negative-



strand RNA viruses since the mid-1990s. The rabies virus just happened to be the one that turned out to be the easiest to work with. Aurélie Albertini, a PhD student in the lab, had succeeded in getting insect cells in tissue culture to produce rabies nucleoprotein. The protein wrapped itself around the host cells' RNA molecules, forming rings containing between nine and 13 protein molecules. Rob's group had realised earlier that these behaved as miniature nucleocapsids, and electron microscopy studies revealed they could be used to study the structure of nucleoprotein arranged around RNA.

Aurélie started to work on producing nucleoprotein-RNA crystals, a project that was later joined by Amy Wernimont, a postdoc in Winfried's lab. But the molecules were not playing ball, and both Aurélie and Amy struggled for a long time to get crystals that would allow Amy to determine the structure at a resolution of 4 Ångströms, which is sharp enough to distinguish how the protein folds, but not quite enough to show the sequence of amino acids in the protein. One problem was that the cells she was working with only produced tiny amounts of the nucleoprotein.

Fortunately, Josan Márquez's high-throughput crystallisation facility was at hand to help solve the problem, using only small protein samples. "The crystallisation robotics were absolutely wonderful," recalls Winfried. "If we had needed large samples, we would not have been able to screen for the right crystallisation conditions." The team eventually found the right conditions, and managed to tweak them to gain 3.5 Ångström resolution – enough to construct a detailed model of the protein's structure. Help also came from Raimond Ravelli, a team leader in the Instrumentation Group at EMBL Grenoble, who assisted with data collection, fine-tuning the X-ray exposure time to get the best results and with continuous advice at various stages of the structure solution process.

The results revealed that nucleoprotein clamps itself completely around the RNA, locking it away like family jewels in a bank vault. "It's not accessible for any other enzyme to attack it," says Winfried. The protein is formed of two main working parts, or domains. One, called the CTD, cradles one side of the RNA and also sticks to the CTDs of other nucleoprotein molecules, helping to form a helical cocoon. The other domain, the NTD, sits on the other side of the RNA and does not make extensive contact with the other nucleoproteins. The overall structure is like a clamp squeezing around the RNA and keeping everything else out. "The nucleoprotein will prevent the RNA from being recognised by the innate immune system," says Winfried. "But how does it become accessible for replication and translation?"

The answer lies in two stray-thread-like structures that protrude from either domain. These could act as hinges, swinging the NTD region up and bending the nucleoprotein clamp open, allowing viral enzymes access to small sections of the genome at a time. One particular protein, called phosphoprotein P, may be involved. It links RNA polymerase, the enzyme needed to copy the genome, to nucleoprotein, and may bind to one of the hinges to swing the NTD out of the way. This hinge mechanism suggests a way to tackle viruses like the rabies virus with drugs that interfere with it. "If our concept that it opens up is correct, we could jam it shut," says Winfried. "This would block viral replication." Locked inside its protein cradle, the viral genome would be rendered powerless and eventually be disposed of by the cell.

The findings could also give some insight into how negative-strand RNA viruses evolved, says Winfried. Related virus species can have very different genome sequences, making it hard to draw any conclusions about their evolutionary history from sequence comparison alone. Structures, on the other hand, are a different story. The same physical structure can be built from a variety of gene and amino-acid sequences. So even if genes evolve and change dramatically, the structures they encode can reveal deep evolutionary links between viruses. Electron microscopy pictures of N-RNA polymers of other negative-strand RNA viruses, such as the measles virus, the Marburg virus and a crystal structure of a Borna virus, suggest that their nucleoproteins have a similar hinged clamp structure. This suggests these viruses use a similar tactic to the rabies virus for shielding their RNA, and so could also perhaps be targeted by drugs that jam their cocoons shut. It also suggests they share a common ancestor, says Winfried. "From the sequence analysis, you wouldn't think they were related," he adds. "I think there was probably some ancestral nucleocapsid, but then they diverged as the viruses evolved to infect different kinds of cells."

Albertini A.A., Wernimont A.K., Muziol T., Ravelli R.B., Clapier C.R., Schoehn G., Weissenhorn W., Ruigrok R.W. (2006). Crystal structure of the rabies virus nucleoprotein-RNA complex. *Science* 313(5785): 360-363



The space-filling model of the nucleoprotein reveals that the RNA is completely clamped at the interface of the NTD (top) and the CTD (bottom) and thus is not accessible to degrading host enzymes or the polymerase.

Six nucleoproteins (in different colours) bind side-by-side on one RNA molecule (black). The protein is formed from two main working parts: the CTD, which cradles one side of the RNA and also sticks to the CTDs of other nucleoprotein molecules, and the NTD, which sits on the other side of the RNA and does not make extensive contact with the other nucleoproteins.



Matthias Wilmanns, Head of EMBL Hamburg, and Paul Tucker

Fighting an old enemy

C HE GELIDA MANINA" – How cold your little hand is! Rodolpho, an impoverished artist in Puccini's opera, *La Bohème*, sings a love song to Mimi, a girl he has just met. The two characters meet and fall in love in a garret in 1830s Paris, enchanting the opera's audience with their touching love story. But the relationship is doomed:

Mimi has tuberculosis (TB) and gradually perishes, coughing and cold. Come the final scene, when a griefstricken Rodolpho casts himself over Mimi's deathbed, crying out her name, there is scarcely a dry eye in the house.

Even so, *La Bohème*, written in the 1890s, has lost some of its impact for a modern European audience. Much as we might sympathise with poor Rodolpho, the idea of someone actually dying from TB seems a little unreal. In our modern age of vaccines and antibiotics, it feels like a disease from a bygone age. Indeed, in the 1950s and 1960s, such was the success of antibiotic therapy that many experts pre-

dicted that TB was destined to be eradicated worldwide.

But two things have weakened our therapeutic defences against TB. The first was that patients often failed to complete their courses of antibiotics. The second was the arrival of the global HIV epidemic, which decimated the immune systems of millions worldwide. These things gave TB the opportunity to develop resistance to our drugs, and it did so with alarming rapidity.

Soon, strains of the bacterium appeared that resisted one or more antibiotics. Now, a highly lethal form, dubbed XDR-TB for 'extremely drug-resistant TB', has emerged,

> which resists nearly all antibiotics – one strain has been found that resists them all. If it became widespread, such a strain could render doctors as helpless to treat patients as they were back in the 1800s. "It's alarming," says Matthias Wilmanns, Head of EMBL Hamburg. "The emergence of the XDR-TB strain is a major problem."

> Matthias heads a project that forms part of biomedical science's counteroffensive against TB. The project, called the X-MTB project, is run by a consortium of academic and industrial institutions in Germany, and is coordinated by the EMBL Hamburg outstation. It aims to discover the

structures of TB proteins that are promising candidates as drug targets. And new drugs are desperately needed: no new therapies to tackle TB have been brought to the clinic for more than 40 years.

Matthias's team is part of a global effort to apply structural genomics – the science of determining the structure of





EMBL scientists, here Thomas Gehrmann, build and operate beamlines to provide crucial services for users at the Hamburg Outstation.

every protein encoded in an organism's genome – to *Mycobacterium tuberculosis*, the bacterium that causes TB. While the largest consortium, the US TB Structural Genomics Consortium, has been taking a broad approach and scanning the entire genome, Matthias and his collaborators have focused their efforts on finding structures for proteins that have already been singled out as promising drug targets. Knowing a protein's structure is invaluable to drug developers, because they can design drugs to interact with the key working parts of the protein.

The X-MTB consortium began in 2003, funded by the German Ministry for Science and Education, the BMBF. With the help of consortium member Stefan Kaufmann of the Max Planck Institute of Infection Biology, the team selected 242 proteins to work on. Of particular interest were proteins that are involved in TB's ability to survive in the body for decades.

About one third of the world's population is infected with TB, the majority harbouring dormant infections that are kept in check by the body's immune system. Up to three million people die each year from acute infection or dormant disease that has become reactivated. This dormancy or 'latency' is a particular problem because most antibiotics only kill bacterial cells that are actively dividing. So finding drugs that inhibit TB's long-term survival in the body is a key goal.

To date, the X-MTB project has been remarkably successful: thanks to its experimental and technological setup, the X-MTB team has disproved a long-standing dogma in X-ray crystallography that it was only possible to get a structure determination efficiency of 10%. "We have shown that it is possible to get about a third of all targets selected purified and crystallised," says Matthias.

Key to this success was the open-access, high-throughput crystallisation facility at the Hamburg outstation, coupled with the innovative system the team used to produce the proteins. "The conventional wisdom was that it was very difficult to get structures," says Matthias. "With this project, we have been able to show that if you produce a good pipeline, you can get almost too many structures."

Thanks to these efforts, the X-MTB consortium has so far produced structures for 35 key TB proteins. The function of many of these was unknown, but the consortium has already made good progress in working out what some of them do. In a number of cases, the structure has actually helped to reveal the function of the protein.

One such case is LipB, an enzyme involved in producing cofactors TB needs to grow and survive. Stefan singled it out as being a key target when his team found the enzyme was extremely active in infected human cells, especially those infected with multi-drug resistant TB. So Matthias's group set to work making and studying crystals and deter-





mined its structure. This revealed, unexpectedly, that there was another, small molecule called decanoic acid attached to the enzyme. Further studies showed that this formed part of LipB's active site and allowed Matthias and his team to deduce what kind of chemical reactions took place there, and from this, to work out what the enzyme actually does. "It's a kind of classical functional discovery, based on structure," says Matthias. Further work will be needed to see whether it is possible to block this process with drugs, he adds.

Other important avenues of attack include the sensing systems TB uses to probe and respond to its environment. Such systems are poorly understood. One, called the PrrA-PrrB system, helps the bacterium replicate and survive inside macrophages, the immune cells it infects, and is an obvious target for drug therapy. Last year, EMBL Hamburg scientists, led by Group leader Paul Tucker, published the structures of the two proteins, PrrA and PrrB, that constitute this system. These revealed how the structures of the proteins change as they interact – information that will prove invaluable for scientists designing chemicals to inhibit that interaction.

But such discoveries will not make a difference unless ways can be found to translate them into therapies. Historically, the pharmaceutical industry has not been terribly interested in developing TB drugs as it is perceived as being a disease of developing countries; countries that are too poor to buy enough drugs to allow companies to recoup the considerable costs of developing them, let alone make a profit. Now, says Matthias, there are encouraging signs that things might be changing. He is involved in efforts to find new ways of funding TB drug development, such as securing EU funding, possibly collaborating with labs in India and other developing nations. Even pharmaceutical companies are starting to show more interest, he says, now that China and India are set to become major economic powers.

But TB does not respect national borders, and it would be a mistake for developed countries to ignore the threat. It is a global disease that requires global action, lest it return, incurable, and make the sad tale of Mimi and Rodolpho strike a chord with Western audiences once more.

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It takes two

Jochen Müller-Dieckmann (left) and Josan Márquez (right) at the highthroughput crystallisation facilities in Hamburg and Grenoble.



C OMETIMES, THE BEST COLLABORATIONS happen over a beer in the bar." Josan Márquez, team leader at EMBL Grenoble, talks enthusiastically about the benefits of meeting up with his colleagues from EMBL Hamburg. Although both outstations are constantly in touch with each other to ensure the technologies they develop are complementary and compatible, there is nothing like a formally organised, face-to-face meeting to make sure



everyone is "singing from the same hymn sheet," he says. This is the rationale behind the 'bilateral meetings' that have taken place annually between Hamburg and Grenoble since 2003. They involve many people from different research groups and are coordinated by Josan and his Hamburg counterparts, team leader Jochen Müller-Dieckman and staff scientist Andrea Schmidt. A major theme of the meet-

ings is instrumentation, particularly the 'high-throughput' technologies, which allow hundreds of experiments to run at once, that both outstations are developing.

One such technology is automated, high-throughput methods for producing crystals for X-ray crystallography. "This is a major part of the contact between Hamburg and Grenoble," says Josan. "We are trying to get the best by putting experience in common." The two outstations are developing parallel systems that have different scopes, but which have common standards so that they can be used interchangeably if need be.

The system at Grenoble is geared towards supporting the 500 or so scientists from the Partnership for Structural Biology, a multi-institute collaborative enterprise involving EMBL, the European Synchrotron Radiation Facility (ESRF), the Institut Laue Langevin (ILL), the Institut de Biologie Structurale (IBS) and the Unit of Virus and Host Cell Interactions (UVHCI).

The Hamburg facilities, on the other hand, are openaccess, designed to be used mainly by scientists from all the European states, although scientists from elsewhere in the world can also apply to use them. "This fits in well with EMBL's mission to provide these services to the scientific community," says Jochen. EMBLEM acts as the intermediary between Hamburg and its users. Services are provided for free, but users pay for consumables and maintenance.

Either system has its advantages and disadvantages, says Jochen. "It depends on what you want to do." At Hamburg, for example, capacity is the main consideration – the facility can run 10,000 experiments per day – ideal for multi-construct projects that trawl for crystallisable versions of difficult proteins or for customized screening. Grenoble, on the other hand, focuses on building more detailed and accurate pictures of a smaller number of proteins, ideal for more targeted projects.

Both have a common aim, however: to tackle one of the biggest stumbling blocks that holds up progress in structural biology, namely getting proteins to form high-quality crystals. Until these automated systems were developed, scientists used to have to perform experiments by hand to determine under what conditions their proteins formed suitable crystals. "It's cumbersome and labourintensive," says Jochen. It also needs large quantities of protein, which can be hard to obtain. Now, researchers can use ten times less protein and leave the screening down to the machines. "It allows you to do more with less," says Josan. As well as this, both outstations have developed technology for helping users use the time they have on the beamlines much more efficiently, such as robotic sample-changers.

Future bilateral meetings will tackle the challenges of pushing the technology forward. A group has already been established, for example, to work on the problem of developing larger, more efficient detectors for reading the X-ray diffraction patterns that come from a crystal. Exchange visits could further cement the collaborative and entrepreneurial spirit that exists between the outstations, says Josan.

Putting a virus under stress

T HE DYNAMICS OF COMPLEX proteins poses some intriguing challenges to structural biologists like Bettina Boettcher. Bettina's lab specialises in working out the 3D structures of proteins – but has also developed some elegant methods for probing the dynamics of these structures. And recently, she turned her attention to the dynamics of the Hepatitis B virus.

Hepatitis B causes more than a million human deaths each year, so there is a great deal of interest in finding ways of controlling it. When the virus infects somebody it immediately replicates itself by hijacking that person's own gene-replication machinery. "There is huge interest in finding ways of obstructing this replication process," Bettina says. "And it is not only important to understand the key structures of the virus, it is also crucial to find out how they move."

So what makes the virus capsid so interesting? Like all viruses, Hepatitis B is pared down to the barest essentials. The mature Hepatitis B particle consists of a capsid shell that houses the double-stranded DNA, and an outer envelope made of lipids and proteins. But before a new virus particle can mature and become enveloped by the lipid membrane, its genome has to change state – from RNA into DNA – and somehow the state of the genome has to be signalled from the inside to the outside of the capsid. Bettina and

her colleagues wondered whether changes in the capsid structure might provide this signal.

The capsid is made of a single type of 'core' protein – these proteins join together in pairs to form dimers (two identical interlocked proteins), and the dimers fit together to form a spiky icosahedron (a shape with 20 faces). Bettina, with postdoctoral researcher Martin Ploss and Michael Nassal at the University Hospital Freiburg, wanted to find out how the capsid responded to stress applied at its sur-



Image reconstructions of the Hepatitis B virus capsid.

face. They reasoned that if stress applied from the outside could be conveyed to the inside, then perhaps changes inside the capsid could also be signalled to the surface.

The researchers applied stress in a novel way, by making genetic 'fusion constructs'. Michael took the genetic sequence that normally encodes the capsid core protein and added into it foreign pieces of sequence. One by one, he inserted an array of different proteins inside the capsid dimers, and Bettina and Martin examined how this distorted the capsid.

They found it to be surprisingly flexible. "The spikes were able to swivel a great deal to cope with the insertion of new sequence," said Bettina, "so the capsid architecture was incredibly tolerant towards massive structural alterations." This stress affected the internal organisation of the capsid, showing that information could be conveyed from the outside to the inside, and vice versa.

The flexibility of the capsid is important for understanding how Hepatitis B replicates, and so opens up new possibilities for controlling infection. But the practical implications of this work go far beyond Hepatitis B. "The capsid could also be used as a tool to deliver vaccines against other

diseases," says Bettina. Vaccination is all about presenting a person's immune system with 'antigens' – small pieces of an infectious agent – to prime the immune system against invasion. She explains: "The enormous flexibility of the Hepatitis B capsid could make it an ideal antigen display platform."

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In the land of the blind

The NUT

Klaus Scheffzek, Stefan Welti and Jeanette Seiler admiring a scientific artwork.

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N JOHN GODFREY SAXE'S POEM, *The Blindmen and the Elephant*, six blind men try to identify an elephant by feeling different parts of it, and come up with six wildly different descriptions. The poem has often been used as a parable for religious discord, but it might just as well apply to a very large protein named neurofibromin. Rather like a rogue elephant, abnormal forms of neurofibromin can have devastating consequences for people, which is why Klaus Scheffzek would like to know what it looks like, and what all its functions are. Ideally he would like to see its full-length structure, but since the tools don't currently exist that allow him to do that, he is forced to 'feel' his way slowly along it. Bit by bit, he is building up a more complete picture of it.



Matthias Wilm and Sven Fraterman at the mass spectrometer.

Mutations in the gene that encodes neurofibromin cause one of the most common genetic diseases in humans, neurofibromatosis type 1. Affecting one in 3500 people, this syndrome includes tumours of the nervous system, learning difficulties and skeletal deformations. The gene in question, NF1, is a tumour suppressor gene, and some mutant forms of it are unable to control another protein called Ras. Ras sits in the cell membrane and is involved in the regulation of cell division, and although it is important during development it must eventually be switched off, or it will lead to abnormal cell proliferation and cancer.

Until a few years ago, the only part of the neurofibromin protein that Klaus' team had been able to describe in any detail was a GTPase-activating protein, or GAP, which specifically binds Ras and may be involved in shutting it off. This suggested that a mutation in neurofibromin might prevent this GAP from communicating efficiently with Ras, leading to unchecked cell proliferation. "But the GAP domain only accounts for roughly 10 percent of the protein," says Klaus. "About the remaining 90 percent, we had ideas but no solid data. All we had to go on was the medical literature, which seemed to suggest that the rest of the protein somehow modulates that interaction with Ras."

Their attempts to crystallise other components of neurofibromin, and then to use a technique called X-ray crystallography to deduce their 3D structures from the pattern of X-ray diffraction through the crystal lattice, met with difficulties. After a number of failures to obtain good quality protein samples, they finally managed to obtain crystals containing a domain neighbouring the GAP module, called Sec14. When they looked at the X-ray diffraction data, however, they found that the crystals contained more than they had bargained for. Along with Sec14 came a neighbouring section of the protein, a pleckstrin homology (PH)-like domain. This, says Klaus, was exciting. "The presence of the Sec14 domain was predicted previously, and it is found in other signal regulators that control families of Ras proteins," he says. "But the PH domain was entirely unexpected, and though we suspected it might communicate with Sec14, its function is essentially a mystery to us."

Could the Sec14-PH ensemble be involved in neurofibromin's regulation of Ras? In structural terms, Sec14 consists of a cage which traps small fat molecules, or lipids. Klaus' team had been able to show that when mixed with a detergent – which has a hydrophilic or water-soluble head and a hydrophobic or fatty tail – the detergent molecule bound to the interior of the Sec14 cage. The cell membrane also contains lipids, and the researchers wondered if membrane lipids could bind to the cage, thereby contributing to the correct positioning of neurofibromin at the membrane, which is required for productive interaction with Ras.

To explore the chemical nature of lipids bound to Sec14-PH, Klaus sought the help of Matthias Wilm's group in Heidelberg, who brought state-of-the-art mass spectrometry techniques to bear on the problem. Mass spectrometry is used to identify molecules by the mass of their components, and Klaus wanted to identify potential ligands of



The schematic representation suggests a possible mechanism for how Sec14 (blue) and PH (orange) domains of the protein neurofibromin could interact to control the access of ligands to two different binding sites. The binding of ligand A to the Sec14-PH interface controls the access of ligand B to the Sec14 cage by blocking the lid that covers the cage. In the absence of ligand A a hinge movement grants the lipid ligand access to Sec14.

the Sec14-PH domain. This meant mixing the protein fragment with different lipids embedded in spherical membrane bodies or vesicles, or with small molecules that could inhibit the binding of those lipids – and then analysing the resulting protein-lipid complexes in the mass spectrometer, to see which lipids Sec14-PH preferred to bind to.

For Matthias and Sven Fraterman, a PhD student in his lab, this posed a number of technical problems. For example, the ability to measure the huge mass of the protein fragment comes at the expense of precision, but precision is needed to detect the tiny increase in mass caused by the binding of the miniscule lipid molecule. To identify the exact type of lipid that is involved, it then has to be separated from the protein by a special chemical procedure and examined alone in a high-precision experiment. "We were lucky in seeing the protein-lipid complex in the mass spectrometer at all, to prove that it is still intact and that all the lipid we see comes from the complex," says Matthias.

The resulting mass spectra showed that the ligands of the Sec14-PH domain belong to a class of lipids called glycerophospholipids, which are major constituents of cell membranes. However, different glycerophospholipids seem to bind to the interior of the Sec14 cage, and to the exterior – the interface between Sec14 and PH. Based on these insights, Klaus, PhD student Stefan Welti and the other members of their team sketched out a scenario for how the two domains might interact. "The structural situation suggests a regulatory communication between the



The diagram obtained from a highresolution image of neurofibromin shows the PH region in orange and the Sec domain in purple.

Sec14 and PH portions," Stefan explains. "In a somewhat simplistic model, binding of a ligand to the Sec14-PH interface may control access of the other ligand to the interior cage of the Sec14 portion."

According to this scenario, which still has to be clarified, a hinge movement of a lid covering the region through which the ligand enters the Sec14 cage could potentially play an important role. This, the researchers think, could be the mechanism by which Sec14 draws the entire neurofibromin molecule into the compartment of the cell membrane where the GAP domain can communicate with Ras. Recent studies suggest that Ras regulates different signalling pathways depending on where it is in the membrane. If so, then neurofibromin must be in the right membrane compartment before it starts to modulate Ras activity. The function of the Sec14-PH module could therefore be to localise the GAP activity of neurofibromin – a hypothesis that Klaus says will need to be confirmed by further experiments.

The researchers now have a pretty good idea of what around a quarter of the neurofibromin protein looks like. To try to gain some insight into the remaining three quarters, they have teamed up with Darren Hart, Head of the High-Throughput Team at EMBL Grenoble. Over the last three years, Darren has been developing a technique for randomly fragmenting a target's DNA sequence and then testing those sequences in a high-throughput manner, to identify those which generate soluble protein fragments. By virtue of being soluble, rather than precipitating out of solution, such fragments are potentially amenable to Xray crystallography and a range of other analytic techniques. The strategy, called expression of soluble proteins by random incremental truncation (ESPRIT), has the advantage that it can identify domains that evade rational prediction. By making and testing 30,000 random stretches of DNA per experiment, it aims to eliminate the tedious cycles of prediction and testing of individual DNA constructs whose protein products turn out to be insoluble. The challenge then becomes to identify the rare soluble protein 'hits' among all the junk, which Darren describes as a bit like finding a needle in a haystack. To do this, his team has combined sensitive labelling methods with robotics to allow them to screen in a rapid, automated way.

ESPRIT should be able to find not only the soluble domains that Klaus' team has already found, but also new ones – and not by actively looking for them, but by generating random fragments along the entire protein sequence from start to finish. In other words, says Darren, ESPRIT removes the hypothesis from the process, and he gives the analogy of the lottery: "The surest way to win is to buy all the tickets." In the land of the blind, the one-eyed man is king.

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Setting a trap

A NEW MECHANISM to attack hard-to-treat fungal infections has been revealed by structural biologists at EMBL Grenoble. The team, headed by Stephen Cusack, collaborated with a California-based biotech company called Anacor to work out exactly how one of their new compounds kills fungal cells.

Fungi are especially problematic because, unlike bacteria, their cells are very similar to ours. So it is hard to find drugs that target fungal proteins but also leave human proteins unscathed. What's more, fungi often infect body parts that are hard to reach with drugs, such as underneath the nail.

The collaboration began when Dickon Alley, a scientist from Anacor, got talking to Stephen at a conference in India in 2005. Anacor had discovered a small, boron-containing compound that turned out to be highly effective in treating a common chronic nail infection caused by fungi. They established that the compound kills fungi by blocking their ability to make proteins and more precisely that it works by jamming an essential fungal enzyme called leucyl-tRNA synthetase. But exactly how the compound, dubbed AN2690, did this presented company scientists with a mystery.

As luck would have it, Stephen and his team had studied the structure of leucyl-tRNA synthetase for many years. With the help of EMBL's technology transfer company, EMBLEM, his team and Anacor made an agreement to collaborate so that Stephen could work out how AN2690 interacted with the enzyme.

Leucyl-tRNA synthetase is involved in translation, one of the last steps in the process of turning a gene's DNA code into a protein. The process begins when the cell makes an RNA version of the gene's code, called messenger RNA. The cell reads, or 'translates' the messenger RNA and stitches the amino acids specified in the message together to make a protein, with the help of molecules called transfer RNAs, or tRNAs.

tRNAs act as matchmakers, linking the code in the messenger RNA with the correct amino acid. Leucyl-tRNA synthetase is one of a group of enzymes called aminoacyltRNA synthetases that attach the correct amino acid to each tRNA. Some of these enzymes have two main functional parts, or active sites: a site that links the amino acid to the tRNA, and a separate editing site that proofreads this process to ensure that the right amino acid has been added.

Stephen's team determined the crystal structure of leucyltRNA synthetase with bound AN2690 and found that the compound sticks in the editing site of the enzyme. When the end of the tRNA enters the editing site it makes a very strong chemical linkage with the boron atom in AN2690, thus trapping the tRNA on the enzyme literally for hours. This stops the usual high rate of turnover of the enzyme and thus stops protein synthesis, killing the cell. "It was a real 'Eureka' moment causing great excitement in Grenoble and Palo Alto! From the crystal structure, everything fell into place," says Stephen. As well as explaining how the drug worked, the structural work immediately suggested to Anacor chemists how they could modify the compound hopefully to increase its potency.

Anacor have now successfully completed phase II clinical trials with AN2690. Anacor scientists and Stephen believe that the approach could be adapted to target other aminoacyl-tRNA synthetases with editing sites and other pathogenic microbes. "It's my hope that we will end up with new antibacterial compounds to counter the problem of antibiotic resistance," he says.



Shaping up for the future

EVER JUDGE A BOOK BY ITS COVER", or so the old adage goes. Yet more often than not, we make assumptions about a person from their appearance – such as what they do for a living – rather than getting to know them and finding out what they are really made of. But while this approach may be shallow from a personal point of view, for biologists it is becoming a profound and invaluable strategy when it comes to making sense of the reams of information they now have about proteins. Looking at the 3D shapes of proteins, rather than just the sequence of amino-acid building blocks that make them, is helping researchers make educated guesses about what jobs these proteins do in the cell. This will let biologists focus their experiments in the right areas to verify these guesses, saving years of painstaking work.

Only ten years ago, the problem facing biology was that it couldn't get enough information about the content of an organism's genome. Today, the problem is that there is almost too much to deal with. Biologists now have the gene and amino-acid sequences for thousands of proteins, but have no idea what many of these proteins do. Sometimes, they can get a good idea of what a new protein does by comparing its amino-acid sequence with that of another protein whose function is known. If parts are similar, chances are, the new protein does a similar job. But many proteins do not compare with any of known functions. And trying to work out the function of each one in the lab would take years. So efforts are underway worldwide to develop bioinformatics tools that can better predict what a new protein might do. EBI Director Janet Thornton and her team are developing such tools and are

also involved in international collaborations and consortia working on the problem. Janet and her colleagues are developing ways of identifying parts of proteins that interact with other molecules, or ligands, and finding ways to predict what ligands a protein interacts with. The group is also involved in developing a bioinformatics server that runs several structural and sequence analysis approaches simultaneously, thus maximising the chances of matching a protein to a possible function.

This server, called ProFunc, is being developed as part of a programme run by the Midwest Center for Structural Genomics, or MCSG. The MCSG is funded by the US National Institutes of Health and is a consortium of eight academic institutions from the USA and Europe, including the EBI. "Our role in the consortium is to develop tools to use the structural data that have been determined to try to say something about the function of the proteins," explains Janet.

A look at the structural biology output of the consortium reveals why such tools are needed. As of September 2005, the MCSG had deposited 319 protein structures in the Protein Databank (PDB), an international protein database, and more than a third had poorly defined or unknown functions. In the PDB as a whole, there were some 1100 proteins out of a total of more than 32,000 labelled as having unknown function.

The ProFunc server aims to tackle this. "Anyone can upload a new protein and run algorithms to get some information on function," says Janet. The server is fully



automated, and runs a battery of analyses on structures uploaded by a user. It employs a number of analytical tests, some of which are being developed by Janet's group. The tests fall into two main camps: those that are based on gene or protein sequence, and those that are based on structure. The sequence-comparison systems search for similar sequences, or homologues, in the PDB and UniProt databases that have functional information attached to them. They can also scan for stretches of protein sequences called motifs that are characteristic of particular protein types, such as motifs that are involved in binding to DNA.

The structure-based analyses look for key structural elements, such as the way a particular protein segment is folded or the shape of clefts where enzymatic reactions often take place. "Part of it is trying to recognise a relative, another is finding unrelated but similar binding sites," says Janet. She and her team have been instrumental in designing ways to analyse the shape of clefts, including active sites, the regions of enzymes that bind to their targets and catalyse biochemical reactions. By building 3D models of known enzyme active sites, the team has been able to map the distribution of key amino-acids and electrostatic charges in the structure. This can then be used to search for clefts that create a similar biochemical environment, even if their overall amino-acid sequences look quite different.

The consortium published the findings from the first phase of the ProFunc project in early 2007. "The results show that it is very difficult to predict function from structure," says Janet. Nonetheless, it is possible to get useful leads: "It's often easier to get a general function, but specifics are harder," she says. During the first phase of the ProFunc project, the consortium found that structurebased methods, such as those that looked at overall fold structure, were the most useful when it came to assigning function to unknown proteins whose sequences could not be matched to known proteins. For example, fold analysis helped to confirm the probable function of a new protein found in Staphylococcus aureus, a bacterium that can cause life-threatening infections. Sequence comparison analyses had thrown up a list of possible functions, and the structural work helped to narrow it down to a single class of enzymes called monooxygenases.

Another consortium with which Janet's group collaborates is the Structural Genomics Consortium, or SGC. This aims to determine the structure of proteins with medical and therapeutic relevance and operates from the University of Oxford, the Karolinska Institute in Stockholm and the University of Toronto. Janet's team has been looking at a large family of human enzymes called the cytosolic sulfotransferases, whose structures have been determined by the SGC. The function of many of these enzymes remains a mystery, although they are known to bind 'small' molecules – chemicals such as drugs and metabolites that are not large biomolecules like DNA or proteins. Janet's study aimed to find out whether it is possible to predict what molecules an enzyme will bind, and so reveal more about its function. "We know what some of them do, so can we predict what the others do?" she says.

The team found that enzymes that bound similar sorts of molecules did indeed have similar structures, but that the converse was not true: the fact that two proteins have structurally similar binding sites does not mean that they will bind to the same compound. While this means that bioinformaticians have more work to do before they can predict what molecule an enzyme targets, it is reassuring from a medical point of view: it means that a drug that targets a particular protein is unlikely to cross-react with other, related proteins, so reducing the risk of side-effects.

In a related project, Janet's team has been developing a way of predicting what ligand an enzyme binds naturally in the cell, rather than what molecules it will bind when tested in the test tube. The focus of much of their efforts has been a large and diverse group of enzymes called short-chain dehydrogenases/reductases or SDRs, which perform many different metabolic roles in the cell. There are more than 60 human SDRs, and the function of only about half of them is known. Any clues about their ligands would help scientists focus their experimental efforts more efficiently. "This gives us good clues as to what the good experiment might be," says Janet.

As well as helping scientists work out what jobs a protein does, its appearance can also be used to probe its evolutionary relationships with other proteins. It's rather like using a person's family resemblance to trace their family tree – although in the case of proteins, these trees can go back millions of years. Gene and amino-acid sequences can change dramatically over long evolutionary distances, making it extremely hard to find homologues. Yet those sequences can still end up building similar 3D structures, says Janet, making it possible to make links. "You can see farther back in evolution with structure."

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Teatime at the landing





On the fringe

ONG BEFORE A FRUITFLY CAN FLY, it carries the *I* blueprints of its wings. Along with other appendages that only show up in the adult, such as legs and antennae, they are imprinted on tissue structures in the larva called imaginal discs. When the larva enters a pupa and undergoes metamorphosis, many of its tissues are broken down and reabsorbed. The imaginal discs, however, undergo rapid development, each turning itself inside out and elongating to become the chosen appendage.

What interests developmental biologist Steve Cohen is that, although the cells of the imaginal discs appear undifferentiated in the larva, their developmental fate has already been determined. It is written down in tissue boundaries, with cells on either side of a boundary destined to fulfil different roles in the adult. In the wing, one of these boundaries is created by a signalling molecule called Notch, and mild malfunctions of Notch result in a notched wing (hence its name). Understanding how Notch works has implications beyond the fruitfly, since it is important for cell differentiation in all animals and has been implicated in several diseases, including cancer.

In 1995, Steve's group in Heidelberg set out to discover how asymmetric Notch signalling could set up a boundary, and over a few years worked out the biological rules. An explanation at the molecular level followed. "In 2000 we showed that Fringe is an enzyme that puts a sugar onto the Notch protein and changes its activity in a profound way," Steve says. "Notch is made sensitive to one possible activator and insensitive to another. That change in activity is key to the asymmetric signalling, and makes the boundary between the two cell populations." The discovery of a role for Fringe attracted the attention of structural biologist Elena Conti, who is also based in Heidelberg. As Martin Jinek, a former student in the Conti group, explains, the Fringe proteins had long been a puzzle to them: "They are in a family of their own, which meant we couldn't infer their structure from similarities with other proteins," he says.

Now that the Cohen group had found a potentially important biological role for the Fringe family, the Conti group had the impetus it needed to investigate that structure, and the two collaborated in a project funded by the Human Frontier Science Program. "Solving the structure of Fringe provides a basis for starting to understand in molecular detail how Fringe recognises Notch to put the sugar onto it," says Steve. "Molecular specificity at this level is the key to understanding regulatory mechanisms in biology."

However, solving or deducing the structure of Fringe turned out to be far from straightforward. The first challenge was to obtain enough material. By engineering the protein so that it responded artificially to a certain cell signal, they forced insect cells to secrete it. They then had to purify the secreted enzyme. "We're talking about one milligram of protein in a bucket of insect cell medium," Martin smiles. "Purifying that is not trivial."

The hardest part was still to come, however. X-ray crystallography is a technique that uses the diffraction pattern of X-rays through a crystal lattice to probe the nature of that lattice, but it is a hit-and-miss procedure because there is



Focus on Drosophila breed

no way of predicting whether a protein will form a crystal or not. In this case the researchers were unlucky, and Fringe from the fruitfly *Drosophila* turned out not to be sufficiently stable in solution to form crystals. "Martin had the idea to turn to a homologue, and that was key to the success of the project," says Elena.

By a homologue, she means the equivalent protein in another species. Fortunately, the Fringe family of proteins is highly similar across species. Whereas *Drosophila* only has one member of the family, the mouse has three, with the colourful names of Manic Fringe, Lunatic Fringe and Radical Fringe. Thirty months after their first attempt to crystallise Fringe in the fly, Manic Fringe obliged them by forming crystals. "It took great perseverance to get to that point, which was all down to Martin," Elena says. Armed with the protein's structure, they could now peer into its sheets and helices, its loops and pins, to find out exactly how it modifies Notch.

The Notch protein sits across the cell membrane, with part of it inside the cell and part of it outside. In *Drosophila*, the natural binding partners of Notch are called Delta and Serrate, and when these bind to the external part of Notch they cause the release of the internal portion, which then travels to the nucleus to alter gene expression. Notch's extracellular domain consists of a large number of modules called epidermal growth factor (EGF) repeats. For it to function properly, a series of sugars has to be added to these repeats in sequence, through the action of enzymes called glycosyltransferases. One of these enzymes, Fringe, is responsible for attaching the second sugar – the one that is most important for defining which ligands Notch recognises.

It was the project of Ya-Wen Chen, a PhD student in Steve's lab, to investigate the precise nature of these Fringe modifications. She knew that Fringe catalyses the elongation of an 'acceptor' unit, a molecule of the sugar fucose attached to one of Notch's EGF repeats, by the addition of another sugar, N-acetylglucosamine, from a 'donor' complex. "Fringe has to recognise both the acceptor and the donor, put them into its catalytic pocket and do its magic," says Ya-Wen.

From the crystal structure of Manic Fringe, Ya-Wen and Martin could see where both the donor and the acceptor bind in the enzyme's catalytic pocket. Lining that pocket are a number of amino-acid residues – the building blocks of proteins – which looked to them to be structurally similar to the catalytic units in other, known glycosyltransferases. Could these residues be responsible for the 'magic' wrought by Fringe, which causes Notch to signal differently in different cells?

To find out if they were, Ya-Wen engineered *Drosophila* cells in which one or more of the residues were altered. She then tested the ability of Notch to bind Delta in those cells, by fusing Delta with an enzyme called alkaline phosphatase (AP). If Notch bound Delta, AP would be released into the cell medium where she could detect its presence. Hence, indirectly, she could measure the ability of Notch to bind its ligand, Delta. "If those residues are important to Fringe function, then without them Fringe activity will be low," Ya-Wen explains. "If Fringe activity is low, you don't get the proper modification of Notch. It won't bind Delta and the result is a low read-out of AP."

These experiments revealed that many of the residues in and around the catalytic pocket of Fringe are necessary for it to modify Notch correctly. There are still questions to be answered, and Ya-Wen would like to see her findings replicated in the living fruitfly. Nevertheless, a little over a decade after they first asked the question, how does Notch signal asymmetrically and so direct cell differentiation during development, the researchers now have their answer – and at the level of detail of single amino acids.

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Caught in the act

T WAS an audacious escape. The inmate sneaked past the guards and into the garbage truck that transported the refuse out of the prison building. Once outside the walls, he climbed out and ran off, free to embark upon a new spree of breaking and entering.

This is not a real-life jailbreak. It's the story of how viruses like HIV escape from cells once they have replicated. Now Winfried Weissenhorn and his colleagues at EMBL Grenoble and the University of Massachusetts have uncovered part of the mechanism that helps HIV break free, perhaps offering scientists a chance to thwart it. The findings also offer new insights into the basic problem of how cells bend membranes to achieve budding. Each HIV particle is encased in a membrane stolen from its host cell. Newly replicated viruses gather at the cell's outer membrane and bend it outwards. The membrane eventually pinches together, creating a tiny bubble with the virus inside it, which is then cut loose.

HIV does this by hijacking machinery the cell uses to dispose of unwanted plasma-membrane receptor proteins. The machinery normally works in a membrane-bound structure called the multi-vesicular body, or MVB. This houses many tiny bubble-like vesicles containing proteins marked out for destruction. They form by bending the membrane of the MVB away from the inside of the cell, and towards the MVB's interior. This looks uncannily like the outward bending that takes place at the outer membrane when viruses like HIV bud out from the cell. Proteins called CHMP proteins seem to be involved in driving the final pinching-off of the budding vesicle and are recruited by HIV to bud from a cell. But no-one knew exactly how the CHMP proteins did their job, or how HIV might hijack them. To find out more, Winfried, who has now moved to the Unit for Virus Host Cell Interactions (UVHCI) next to the Grenoble Outstation, and his colleagues decided to determine the structure of CHMP3 using X-ray crystallography. But it wasn't easy: "We screened hundreds of crystals to find one that diffracted well enough," recalls Winfried. With the help of the highthroughput crystallisation facility at EMBL Grenoble, and

The schematic drawing shows the life cycle of HIV. The virus fuses with the plasma membrane and injects its RNA genome into the host cell. In the cytoplasm the RNA genome is turned into DNA, transported into the nucleus and then copied and transcribed. Among the viral proteins produced is Gag, which assembles at the plasma membrane and recruits the viral genome and host machinery that helps the virus bud from the membrane.

plasma membrane

Cytoplasm

nucleus

nuclear membrane

Raimond Ravelli, a team leader in the outstation's Instrumentation Group, the researchers eventually managed to find a single crystal that worked. The structure revealed that CHMP3 folds itself up quite flat and that it can pair up with other CHMP3 proteins to form a lattice structure. CHMP3's flat surface is dotted with a lot of positive electrical charges, suggesting that it interacts extensively with the lipids in the membrane, which are negatively charged. The other members of the CHMP family of proteins have similar flat surfaces, but different patterns of electrical charges. This suggests that a lattice made of different CHMPs could move particular kinds of lipids around in the areas of the membrane with which they are interacting. Changing the composition of the membrane like this can cause it to spontaneously form vesicles.

Winfried and his team are now working to test this idea. "Now that we have a snapshot of how CHMP3 works, how does it interact with other proteins?" he asks. Once he knows that, perhaps biologists will one day be able to find a way of changing the locks on the cell's garbage system to stop viruses like HIV making a bid for freedom.

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The EMBL building and the new Carllvar Brändén Building on the Polygone Scientifique campus in Grenoble

Corsairs of the cell

T LOOKS LIKE SUCH A LITTLE THING. A small spelling mistake in the string of genetic letters that make up one of the genes in a virus. But in 1918, little things like this triggered a global disaster. A handful of such spelling mistakes, or mutations, allowed a bird-flu virus to jump into humans and spawn a deadly influenza pandemic. Today, scientists are nervously watching the current birdflu strain, H5N1, in case history repeats itself. It is probably only a matter of time before it does, although today we are hopefully more prepared to limit the consequences.

Despite a great deal of effort, researchers have only been able to work out how some of these mutations favour human-to-human transmission of bird viruses, thus potentially triggering a pandemic. Technical problems limited their ability to study the structure of some of the virus key proteins to work out how these mutations allowed it to jump species. But now, EMBL Grenoble researchers Darren Hart, Stephen Cusack and colleagues have solved one of the pieces of this puzzle. Thanks to a new technology invented by Darren, they have discovered that one of influenza's proteins uses an old pirate trick to help the virus plunder its host's cells, and that certain mutations may make it easier for the bird virus to pull this stunt in humans.

The teams focused their efforts on the virus 'polymerase', an enzyme that performs two crucial functions. First, it is needed for transcription, the first step in the process of turning the viral genetic information into protein. Second, it makes new copies of the viral genome which can then be incorporated into infectious progeny viruses.

EMBL ANNUAL REPORT 06.07

Ten of the mutations that helped the 1918 virus jump from birds to humans lie in its polymerase; seven of these have been seen individually in circulating H5N1 viruses.

Until now, however, no-one has been able to study the 3D structure of the polymerase in sufficient detail to work out what these mutations might be doing. To determine a protein's structure, biologists engineer lab-grown bacterial or insect cells to make the protein in large amounts in soluble form. They can then apply one of two key techniques: one called nuclear magnetic resonance spectroscopy, or NMR, which works on proteins in solution. This gives a less detailed structure, but reveals movement of regions within a protein. The other technique is X-ray crystallography, where researchers first crystallise the protein and then focus a very intense beam of X-rays on the crystal. This ultimately generates a 3D image in which each atom within the molecule can be placed with high accuracy. One can also co-crystallise two associating proteins to see which parts of them interact to stick them together. Previously, when scientists tried to crystallise the polymerase, it either formed insoluble clumps, or the cells were unable to make sufficient amounts to be useful.

When faced with this problem, structural biologists often dismantle their target protein into its key working parts, or domains, and study them individually, rather like mechanics stripping down a car engine. Domains are smaller than whole proteins and so easier for bacterial cells to produce in the right form.



But this strategy only works if you can work out which bits of your protein form well-defined domains. Traditionally, researchers compare the amino-acid sequence of their target protein with those of proteins with similar sequences whose domain structures are known. They use this information as a guide to where the domains lie within the target protein, cut the gene coding for the protein into pieces, or 'constructs' corresponding to these domains, put the constructs into cells and hope that the cells can make soluble protein from them.

This approach has worked well for many proteins, but can still be something of a lottery. Researchers have to painstakingly make and test each construct. All too often, constructs fail to yield soluble protein. Viruses pose an additional set of problems. Their ancestries are a bit of a mystery and they also evolve extraordinarily quickly, meaning that it can be difficult to compare viral proteins with anything else in a meaningful way.

So Darren went for a revolutionary approach. "The surest way to win the lottery is to buy all the tickets," he explains. "Rather than trying to rationalise the answer to a problem in the traditional way, we make all possible constructs in a single, giant experiment and test them all at the same time to see which ones give soluble protein."

It works like this: Darren and his team systematically cut the target gene into thousands of random fragments and put each piece into a unique bacterial cell. On the end of each piece, they attach the molecular equivalent of a furled flag. The cell then attempts to make the protein; if it is soluble, the cell unfurls the flag and signals the presence of the soluble protein, but if it is insoluble, the flag remains invisible and the cell degrades the protein.

Finding all these flag-waving cells by hand would take forever. So the team prints each bacterial cell onto a paperlike membrane and coaxes it to grow into a colony, separate from its neighbour. Using scanners and software adapted from genomics projects, they scan arrays of tens of thousands of spots for those cells bearing flags, and then use them to make soluble proteins for structural study. The system, dubbed ESPRIT (Expression of Soluble Proteins by Random Incremental Truncation), is a dramatic improvement over old methods.

Stephen Cusack, Head of the Grenoble Outstation, had been interested in the influenza virus polymerase for a long time but was not making progress in its structure determination for the reasons given above. He encouraged Darren and his colleagues to use influenza's recalcitrant polymerase as a test case for driving the development of ESPRIT. Funding from the EU FP5 Structural Proteomics project SPINE was crucial to get the project going.

The polymerase is a large, complicated structure, formed of three proteins, or subunits, called PA, PB1 and PB2. The team focused on PB2, and succeeded in producing a small soluble domain in large amounts. Since the protein did not crystallise on its own, they enlisted the help of colleagues at the neighbouring Institut de Biologie Structurale to study the domain with NMR, which can determine a protein structure without the need to grow crystals. This revealed, at the end of the domain, an ele-



polymerase domain (red) interacting with the human protein importin- $\alpha 5$ (blue) involved in nuclear transport.

ment known as a nuclear localisation sequence, or NLS. Such sequences exist in cellular proteins destined for delivery inside the nucleus. The NLS of subunit PB2 had been investigated before, but the correct sequence had been simply overlooked.

To find out more, the team turned to a human protein called importin- α 5, which normally collects cellular proteins labelled with an NLS and ferries them into the nucleus. A PhD student at the institute next door (UVHCI), Sebastian Boulo, had been trying to crystallise importin with little luck, but succeeded in co-crystallising it with the influenza PB2 domain. With Stephen, they were able to study the structure with X-ray crystallography to see exactly how the two proteins interact. This revealed that the domain sticks to importin by unfolding its NLS element and slinging it over the protein like a pirate casting a grappling hook over the stern of a ship. With the help of Jan Ellenberg, a Group leader in cell biology at EMBL Heidelberg who specialises in tracking the localisation of proteins in living cells, the team showed that PB2 then sails into the nucleus, where it clubs together with the remaining two polymerase subunits to commandeer the cell's resources to make new viruses.

When not in use, the grappling hook is tethered to the rest of the subunit by a chemical attraction between amino acids number 701 and 753. Intriguingly, one of the mutations that allows the H5N1 strain to jump from birds to mammals alters amino acid 701. "Our work has given the first clue as to what the role of position 701 might be," says Stephen. It's too early to say how mutations in this spot would help the virus adapt to humans, but it may be that the switch loosens the tether, making it easier for PB2 to grab importin and get into the nucleus.

Changes to two other amino acids in the domain have also been associated with virus adaptations to humans (including one from the 1918 strain). No-one yet knows how these might have their effect, but the team is now studying how these mutations alter the domain's structure and ability to be transported into the nucleus.

As well as offering insights into how bird flu adapts to humans, the work could aid the development of new drugs to combat H5N1, if it ever makes the leap. Polymerases are essential for the life cycles of viruses, which is why a number of antiviral drugs, such as those used to treat HIV (the causative agent of AIDS), target them. "It's been a very obvious target, but no-one has been able to get a handle on it," says Stephen. "Interfering with polymerase function could provide new ways to treat or prevent flu," he adds, "but this will require a detailed picture of the rest of the polymerase. This is what we are aiming for in our new EU-funded FLUPOL project. In a joint effort with other European laboratories in Madrid, London, Marburg and Lyon, we will explore both the structure and function of this key drug target and try to characterise other mutations implicated in bird-to-human transmission."

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Ringing the changes

In 2010, EMBL Hamburg will open the doors of a new structural biology facility, EMBL@PETRA-III. Matthias Wilmanns, Head of the Hamburg Outstation, and Thomas Schneider, Head of the new facility, outline their visions for the future of the project.

What is the vision for this new project and how did it come about?

Matthias: The vision is to provide state-of-the-art X-ray sources, or 'beamlines', and support facilities for use by the international community of structural biologists. The existing facilities at Hamburg are excellent, for example, they played a key role in a TB Structural Genomics Consortium Project, which discovered the structures of proteins from the tuberculosis bacterium. Even so, they are not yet up to the challenges being posed by the next generation of structural biology projects.

These concern the area of 'systems biology', which aims to understand how proteins function as part of intricate networks and how they come together to form massive molecular machines, or complexes. Studying the structure and function of many of these huge protein complexes is beyond the capability of currently available technology.

In February 2007, the German Federal Ministry for Education and Research awarded the Outstation nearly nine million Euros to build an Integrated Research Facility for Structural Biology on the planned new PETRA-III storage ring of the German Synchrotron Research Centre (DESY). This will be used as a source of X-rays, which researchers use to probe the structures of biological molecules. PETRA-III will be one of the most powerful radiation sources in the world, and the X-rays it produces will be sufficiently intense to tackle difficult protein complexes. Together with the sample preparation, beamline and computational technology developed by EMBL Hamburg, the facility is set to be a world leader in 21st-century structural biology.

What will the new set-up be?

Thomas: The new facility will be built in the shell of the current PETRA-II synchrotron ring. Its beam will be extremely small and extremely intense. Four of the 30 beamlines to be built will be used for biology, and of these, three will be built and run by EMBL. The fourth will be built by the Max-Planck Society and the Helmholtz Association, in collaboration with DESY.

Matthias: What's special about this project here in Hamburg is that life scientists have been asked to build and run the three beamlines. This is unique: at other synchrotrons, beamlines are built by physicists, who then allow biologists access for applications in life sciences. Thanks to the set-up here in Hamburg, the beamlines and technical support will be specifically orientated to the needs of biologists. We have the right mindset for sensing the needs of the most challenging biology.

That all sounds great, but why do biologists need more beamlines? There are already many in existence worldwide.

Matthias: We're not adding to these existing beamlines: we're creating something new and different, as a substitute for our aging beamlines at the storage ring DORIS. I like to use the analogy of buying a car: we're not replacing the old car, we are buying a completely new model. The beamlines at PETRA-III will be world-leading, and we



anticipate that they will attract users from many different countries, particularly those in Europe. What's more, unlike many beamlines elsewhere, ours will be open for anyone from anywhere in the world to use, based on scientific merit. Most of our users will be academic scientists, but we may also allow industrial clients to buy beamtime.

Thomas: Another very important thing we're adding is the on-site integrated facility for sample preparation. Preparing samples for experiments with synchrotron radiation is not something many biology labs do everyday; you need the expertise and the critical mass to do it well. Towards this end, we have the high-throughput crystallisation facility on-site. This is dramatically cutting the amount of time it takes to produce good protein crystals and is also enabling scientists to work with proteins and protein complexes previously thought to be un-crystallisable.

As well as providing this expertise, we will provide facilities where users can get the best out of the data that result from their experiments. We have a critical mass of experts and resources in computational analysis, for example. We can become a significant force internationally there and we will have all the steps of a structural biology experiment under the same roof, from sample preparation, to data processing and analysis.

What will the three new beamlines offer?

Thomas: Each will have a particular function or feature. The first will be dedicated to a technique known as small angle X-ray scattering, or SAXS. This is a technique that has been around for a while, but was little-used until recently, because of the experimental and computational challenges it presents. It is going through a renaissance at the moment, largely thanks to the efforts of Dmitri Svergun, one of the scientists here at the Hamburg Outstation. His work on software to interpret SAXS data has revolutionised the way these data can be used.

The advantage of SAXS is that, unlike in X-ray crystallography, you don't have to crystallise your protein to study its structure; you can do so in solution. This means you can study proteins that don't crystallise, including large protein complexes. What's more, proteins in solution are in a more natural state compared with those in crystals, so there is a higher chance that what you see with SAXS accurately mimics what happens in real life. The key disadvantage is that the resolution is much lower: you can only really discern the shape of the protein or complex, rather than its atomic-level structure.

Even so, the information you get is invaluable. You can gain an understanding of how large protein complexes form, and work out how the components are talking to each other. This is important if you want to talk about how structural biology fits into systems biology.

The higher intensity of the new PETRA-III beams will allow scientists to use more dilute solutions, which are more like the conditions inside a living cell. They will also be able to alter the conditions within the solution – such as its acidity or alkalinity. It should eventually be possible to take sequential snapshots of a sample, allowing scientists to watch an enzyme react or a protein complex as it forms in real time.



Artist's impression of the new PETRA-III hall.

Matthias: I think SAXS is the technique of choice for big systems projects. There isn't really any alternative for scientists studying massive molecular complexes in the cell, such as the proteasome, a machine that degrades waste proteins in the cell.

Thomas: Agreed. And although SAXS only gives a relatively low-resolution picture, we can use other techniques, such as X-ray crystallography, to fill in the gaps.

What about the other two beamlines?

Thomas: The second beamline is a 'microfocus' beamline, like the ones currently in use at ESRF in Grenoble. Microfocusing means that the beam is tightly focused and enormous numbers of photons are delivered in a very small cross-section. A smaller-sized beam means we can study smaller crystals. With the PETRA-III beamline, we can realistically talk about using crystals of between 5 and 10 micrometres in size; that's about a quarter of the width of a human hair. Many protein complexes only form small crystals – it often seems as though the more interesting the system, the smaller the crystals it makes!

Matthias: It's a big problem, and one that has hampered the study of these systems up until now. Many crystals are tiny, tiny, tiny – hard to see even with the strongest light microscope. This means the demand for state-of-the-art microfocus beams is huge. Some scientists from the USA, for example, have been travelling to Grenoble to use the microfocus beam there. **Thomas:** The key feature of the third beamline is that, in addition to providing a small and intense beam, we will be able to change, or tune, its wavelength. Probing a crystal with different wavelengths reveals much more detail about its structure and composition than experiments at constant wavelength.

What is the timescale for the construction and running of EMBL@PETRA-III?

Thomas: The experiments on the old PETRA-II ring stopped in June 2007. The official start date for building the new ring will be 2 July 2007. We will be able to start test runs of the new beamlines in 2009, and will be open for business in 2010. In the meantime, we will continue to use beamlines on DORIS, another ring on-site, so will still be able to offer beamline access to our users while construction is underway.

How will you coordinate this new project with your colleagues at EMBL Grenoble?

Matthias: We have already started to have 'bilateral meetings' (see page 104), annual meetings to coordinate things like synchrotron instrumentation, data analysis and highthroughput technology platforms. But there are big plans for even more coordination in the pipeline, such as collaborations and exchange projects. What's clear is that the two units belong to the same organisation, and we need to make sure that Hamburg and Grenoble not only complement, but synergise with each other.

A Year in the Life of EMBL

April

ELLS workshop in France



The European Learning Laboratory for the Life Sciences (ELLS) took their LearningLABs for the first time to Grenoble on 3-4 April. Scientists at the French Outstation hosted local science teachers for a series of talks and presentations, concentrating on the area of structural biology. LearningLABs aim to combine cutting-edge science with simpler activities that are transferable to the classroom.

Mouse biologists meeting in Monterotondo

Scientists from four Japanese institutes were welcomed to EMBL Monterotondo on 19-20 April for a Mouse Biology Meeting. The visit was part of the collaboration agreement that EMBL signed in August 2005 with Japan's National Institute for Basic Biology (NIBB). During the meeting, local group leaders and the visitors presented their research on mouse limb and head development as well as transgenics.



June

Lab Day 2006

Scientists from all units gathered in Heidelberg on 9 June for another EMBL Lab Day, a chance for all scientists to convene and learn about their colleagues' activities. The event included postdoc seminars, a ceremony for this year's graduating predocs and a presentation of ELLS' new project, SET-Routes. Only one day before, on 8 June, the Postdoc Association and EMBLEM organised a Career Options Day. The event gave an overview of non-research career possibilities, such as those in scientific writing, industry, patenting and policy making.



A visit from Scandinavia



On 14 June, the Board of Directors of the Swedish Research Council visited EMBL to find out about EMBL's special culture and discuss future plans with the Director General. The delegation of 28 enjoyed two days in Heidelberg with a fully packed schedule including discussions and presentations.

2006

Croatia becomes EMBL's 19th Member State



EMBL's Member State count is now up to 19 after Croatia signed the accession contract in June 2006. The joining of Croatia is an impor-

tant first step towards expanding EMBL's membership to Eastern Europe. The delegate representing Croatia in the EMBL Council is Professor Krešimir Pavelić from the renowned Ruder Bošković Institute in Zagreb.

July

Spanish journalist visits EMBL

For a week in July, EMBL hosted the Spanish journalist Pilar Gil Villar, who took the opportunity to zoom into various scientific projects at EMBL. The visit was part of the European Initiative for Communicators of Science, funded by the Max Planck Institute for Biophysical Chemistry. Pilar, who lives in Madrid and writes for the science magazine Quo, got acquainted with new scientific methods, instruments and, last but not least, the day-to-day life in the lab.

EMBL attends ESOF



On 15-19 July, Munich hosted the second EuroScience Open Forum (ESOF), an international conference to engage the public, journalists and young scientists in cutting-edge research. EMBL was also there to present its work and opportunities for young scientists as an EIROforum member organisation.

Group leaders in new Partnership talks



In mid-July, several EMBL group leaders and Core Facility heads met researchers from the Station Biologique de Roscoff (SBR) at its location in Brittany, France, for a three-day mini-conference. Both parties explored new areas of collaboration and a possible EMBL Partnership. While the SBR would benefit from access to EMBL Core Facilities and the possibility to increase their activity in developmental biology, EMBL would gain access to their diversity of marine species.

August

A new start-up company to develop anti-cancer drugs



EMBL scientists, EMBLEM and EMBL's venture vehicle, EMBL Ventures GmbH, founded Elara Pharmaceuticals GmbH, a start-up company that will translate basic research findings into new anticancer drugs. Elara is a spin-off company dedicated to drug development and will follow up on promising small molecules that have shown powerful anti-cancer actions in screening experiments.

ECM in Leuven

Crystallographers from all over Europe descended on the university city of Leuven, Belgium, for a week in August for the 23rd European Crystallographic Meeting. EMBL was represented with a stand in the exhibition area, and scientists were in attendance to let visitors know about the institute's facilities. EMBL's attendees also contributed to the conference with talks and presentations.

2006

September

New EMBL partnership in Barcelona



A new EMBL partnership with the Centre for Genomic Regulation (CRG) in Barcelona is set to advance the understanding of complex biological systems. Funded by the Spanish Ministry for Education and Science for the next nine years, the EMBL/CRG partnership is dedicated to systems biology, an emerging field that focuses on understanding and engineering complex biological systems.



Faculty Retreat in Hamburg

Faculty members from all five EMBL sites met on 13 September in Hamburg for their annual retreat. The Faculty Retreat gave group leaders a chance to



present their work and get feedback from others. And it showed once again that nothing can replace face-to-face encounters and a joint discussion about the current research projects, away from the bench.

Second EMBL postdoctoral retreat



More than 60 postdocs from all EMBL units came together for the 2nd EMBL postdoctoral retreat from 30 September to 2 October in Aalen, Germany. The

programme featured talks by invited speakers and EMBL postdocs, poster presentations and joint discussion of the postdoctoral experience at EMBL. The motivation behind the event was to strengthen the links between postdocs in different units.

October

Work begins on the ATC



A groundbreaking ceremony on 6 October for EMBL Heidelberg's Advanced Training Centre welcomed special guests including Annette Schavan, Germany's Minister for Education and Research, sponsor Klaus Tschira and Baden-Württemberg's Minister for Science, Peter Frankenberg. The building, in the structure of a double helix, will feature an auditorium for 450 people, a display area, teaching labs and seminar rooms.

A fruitful get-together



This year, the predocs' organisational committee chose Prague as a perfect backdrop to a weekend of discussion for PhD students from all EMBL sites. The talks during the predoc retreat covered a wide spectrum of scientific topics, ranging from metagenomics and proteomics to gene silencing in *Drosophila*. During the Prague meeting, the PhD students also got a chance to learn the rules of successful paper submission. After only two events, this forum for communication has already become an established part on the PhD programme.

Science Days in Rust



EMBL took part in Germany's biggest science festival on 12-14 October when the European Learning Laboratory for the Life Sciences (ELLS) team exhibited at 'Science Days 2006' in Rust, near Freiburg. ELLS demonstrated its microarray game to the young audience and a series of posters about the movers and shakers in the history of biology. The festival welcomed more than 24,000 visitors over three days, including children of all ages, teachers and families.



Bridges between neighbours

Thirteen young group leaders from the German Cancer Research Center (DKFZ) met EMBL Heidelberg and EMBL-EBI faculty for the 2nd DKFZ-EMBL retreat on 12-13 October in Herxheim, Germany. The scientists discussed different aspects of research at all three sites in four separate sessions and developed ideas of how to tighten the scientific bonds between the institutes.

November

A beautiful mind?



At the 7th joint EMBL/EMBO Science and Society Conference on 3-4 November, 'Genes, brain/mind and behaviour', more than 300 scientists, students and interested laypeople came from all over the world to discuss this fast-growing area of discovery. Talks covered the genetic basis of depression and the social and ethical implications of these research areas within the life sciences. Twenty-five invited speakers explored the current state of genetics, neurotechnologies and neuroethics in four separate sessions followed by lively panel discussions.

Approval for future

The approval of the budget and the scientific programme for the coming five years was the big news from the EMBL Council meeting, which took place in Grenoble on 21-22 November. After more than a year at the drawing board, the EMBL Programme and the Indicative Scheme 2007-2011 received the go-ahead from delegates. The new Indicative Scheme will represent a significant increase in member state funding, particularly for data resources at EMBL-EBI.

Grant approval for SET-Routes

SET-Routes, a project to promote women in science, received an EU FP6 Science and Society grant allowing it to spread its message in schools and universities throughout Europe. Coordinated by EMBL, EMBO and CERN, the programme aims to mobilise women in science, engineering and technology (SET) and to encourage pupils, students and graduates to pursue an education or career in science.



From predocs to predocs

The 8th International EMBL PhD Student Symposium, 'Biology of Disease: A Molecular Battlefield', was held in Heidelberg at the end of November, close to the end of the EMBL PhD course. The symposium, fully planned and carried out by EMBL predocs, started with a public lecture about avian influenza infecting humans and ended with an award ceremony for the joint EMBO/EMBL Science Writing Prize. Eighteen prominent speakers and around 130 guests came to share their insights on topics ranging from food-related diseases to mental disorders.

December

'EB-eye' launch



EMBL-EBI's new, faster and easier-to-use website was launched on 11 December. As well as an attractive design, the new resource boasts an exhaustive search engine which allows instant searches of all EMBL-EBI's databases from a single query. Genomes, genes, proteins, structures and biological functions can all be explored through a single simple interface. 'EB-eye' is the result of a survey to find out users' preferences.

Board of US professors at EMBL

Twenty professors and associated professors of biochemistry and chemistry from various US universities were given an introduction to EMBL, its training, outreach and technology transfer activities on 8 December. The event was organised by the German Academic Exchange Service (DAAD).

Howard Hughes Holiday Lecture



Head of EMBL Monterotondo, Nadia Rosenthal, was invited to deliver the 2006 Howard Hughes Medical Institute Holiday Lecture. She gave two presentations on 'Adult Stem Cells and Regeneration' and 'Stem Cells and the End of Aging', covering basic research with an emphasis on clinical implications. By delivering the lecture, Nadia joined a prestigious list of former speakers including Nobel laureates. Originally designed as a lecture series for local high-school students, the event now attracts many thousands of audience members worldwide.

January

Burns' Night Supper

As it does every year, EMBL celebrated Scotland's most famous poet with a full-blown Burns' Supper in January. The numerous guests at EMBL Heidelberg were first allowed to sample the traditional Scottish haggis rounded off by 'bashed neeps' and 'champit tatties' and then went on to practice the vivid Scottish Ceilidh. Following tradition, the gastronomic and dancing affairs were accompanied by literary extras including a recitation of famous Burns' poems.



February

Kick-off for new facility at PETRA-III



On 1 February, it was officially announced that the German Federal Ministry for Education and Research (BMBF) will provide funding of Euro 8.8 million for EMBL@PETRA-III. The new facility is an Integrated Centre for Structural Biology on the new high-energy storage ring at the Deutsches Elektronen-Synchrotron (DESY), one of the world's most powerful synchrotron rings. This new addition to EMBL Hamburg's existing facilities will start operating in 2010.

Predocs' choice

Within the series of Distinguished Visitor Lectures, the EMBL PhD students invite a speaker of their choice each year. In February 2007, their guest was Alain Fischer, from the Necker University Hospital in Paris. Alain Fischer talked about 'Genetic defects of the immune system as a good model for bench to bed-side research'. Fischer was awarded the Luis-Jeantet Prize for medicine in 2001 for his use of stem-cell techniques to treat immune deficiencies.



EMBL's busy events calendar



To show the USA what European molecular biology has to offer and to raise its profile internationally, EMBL took part for the

first time in the annual meeting of the American Association for the Advancement of Science (AAAS), held in San Francisco on 16-19 February. The AAAS is the world's largest general science organisation. Once a year it brings together scientists, policy-makers, teachers, journalists and members of the public in a big science fair. At the end of February, EMBL also attended the third annual meeting of the EU IT BIOXHIT conference in structural biology, held in Didcot, UK.



Far East connection

Visitors from Japan's National Institute of Basic Biology (NIBB) on 25-28 February had a comprehensive tour of various EMBL facilities and learned about the Core Facilities and the outreach and training activities at EMBL. After signing an agreement on academic exchange in 2005, both institutes now strive to strengthen the administrative ties between them.

March

Scientific Writing Course for predocs



In a survey last year, EMBL PhD students expressed a strong desire for communication training. EMBL responded to the predocs' wish and organised a three-day course on scientific writing and publishing on 21-23 March, entitled 'Effective Writing for Life Sciences Research'. Tutor Jane Fraser, an experienced British freelance scientific writing trainer, explained the rules of a good scientific story. The practical sessions covering style, writing and editing exercises were very much appreciated by all attendees. The course was funded by the Marie Curie E-STAR grant.

New Scandinavian liaison

A new partnership in Scandinavia complements EMBL's worldwide scientific network. The Nordic EMBL Partnership for Molecular Medicine will have three nodes: one in Oslo (Norway), one in Helsinki (Finland), and a third in Swedish Umeå, which will be called the Laboratory of Molecular Infection Medicine. The Swedish Research Council agreed to help the Umeå department expand with six new research groups, but the exchange will also proceed in close collaboration with the other nodes. The joint agreement was accompanied by mutual visits of the Scandinavian partners in Heidelberg and vice versa.

EBI at Cambridge Science Festival

Thirty volunteers from EMBL-EBI and their next-door neighbour, the Wellcome Trust Sanger Institute, took 2000 people through an array of activities at the 2007 Cambridge Science Festival in March. This time, the children were invited to investigate all things big and small, from giant ladybirds and monsters in the plant world to tiny pollen grains. The Cambridge Science Festival is one of the most popular science events in the UK with plays, exhibitions, demonstrations and workshops for the whole family to make science and technology more accessible – for big and small.



2007

April

Science on Stage



Around 500 science teachers from 27 European countries took part in the second EIROforum's 'Science on Stage' festival, held in Grenoble from 2-6 April 2007, which was also attended by the EMBL-based ELLS team. The large-scale festival was full of spectacular experiments and examples of innovative teaching methods. The organisers put together a four-day programme of parallel sessions, with workshops and presentations ranging from 'Motivation in Science', to 'Physics Experiments at Less Than 1 Euro' and 'How to Surprise Leonardo and Einstein'.



Girls' Day at EMBL

Eleven-year-old Eva Heinzmann joined Mustafa Uyguner, EMBL Heidelberg's main housekeeper, and his colleagues for 'Girls' Day' at the end of April. Her visit was part of a national initiative to give schoolgirls a taste of what are thought to be traditionally 'male' jobs. Eva, who attends the Realschule in nearby Wiesloch, spent a busy day helping to refurbish labs and painting columns with Andreas Schlecht – a challenge that she clearly enjoyed.



Piano recital at EMBL



Ragna Schirmer, one of Germany's most renowned young piano artists, gave a concert featuring Bach's Goldberg Variations in EMBL Heidelberg's large Operon on 19 April. The recital was part of the international music festival 'Heidelberger Frühling', which for the first time ever entered into a collaboration with EMBL. More than 300 guests found their way up the hill for the concert. Instead of paying an entrance fee, visitors were asked for donations for the aid organisation 'Helfer ohne Grenzen' which supports orphans, refugees and impoverished families in crisis regions.

Notes

Notes

Notes

Index

Antony, Claude
Arendt, Detlev 22, 69
Bastiaens, Philippe64
Benes, Vladimir18
Besir, Hüseyin18
Birney, Ewan
Bork, Peer 37, 48, 58
Böttcher, Bettina 105
Brunner, Damian 42, 73, 81
Cameron, Graham 11, 15
Cohen, Stephen 18, 46, 85, 117
Conti, Elena 19, 117
Cusack, Stephen
Ellenberg, Jan 65, 89, 94, 123
Franz, Thomas 19
Furlong, Eileen 59
Gannon, Frank 19
Gilmour, Darren76
Gross, Cornelius9
Hart, Darren
Karsenti, Eric
Le Novère, Nicolas6
Lewis, Joe
Lopez-Serrano, Rodrigo 11, 15
Luscombe, Nicholas6
Márquez, José Antonio
Mattaj, Iain 29, 84

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