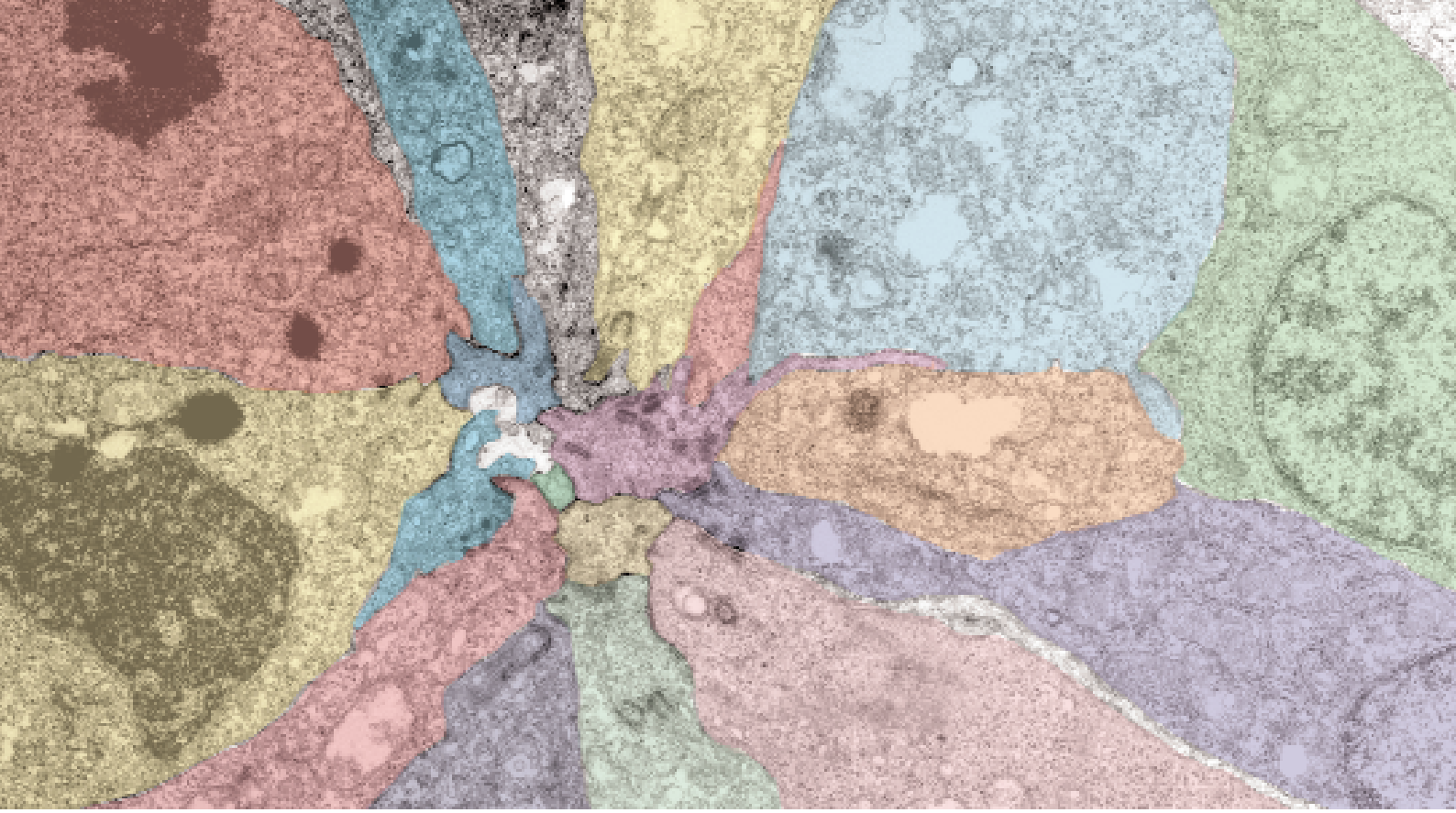

Annual Report

2008-2009

EMBL

Annual Report 2008-2009

European Molecular Biology Laboratory

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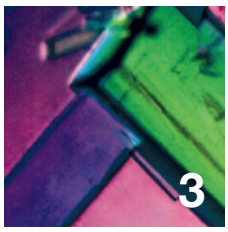



BL 2009

At EMBL, because of the combination of our staff turnover system and the remarkable pace of discovery in Molecular Biology, it often seems that the only constant is change. It therefore comes as something of a surprise that this year was marked by two anniversaries. The EMBL International PhD Programme, one of the mainstays of our research efforts, celebrated its first 25 years in 2008. It remains one of the most competitive PhD training schemes for molecular biology worldwide and we are proud to say that it has now been copied in multiple high-quality institutes and universities throughout Europe, usually with our help. In June 2009, the Monterotondo Mouse Biology outstation celebrated its 10th anniversary. The Unit has grown continuously in size, research breadth and service to become a critical aspect of EMBL's activity. I would like to thank current and former outstation Heads Nadia Rosenthal and Klaus Rajewsky for their hard work and commitment.

More normally for EMBL's rate of change, two research Units in Heidelberg have new leadership this year. I would like to welcome Jan Ellenberg as new Head of the Cell Biology and Biophysics Unit. He is working alongside Eric Karsenti this year before Eric steps down in 2010. Jan led the Gene Expression Unit from 2006 to 2008 and his leadership was highly commended in the Unit's last scientific review. Eileen Furlong and Lars Steinmetz are the new Joint Heads of Gene Expression. Aside from their outstanding research performance, Eileen and Lars have already played important roles in directing EMBL's Centre for High Throughput Functional Genomics and elsewhere, Lars as Academic Mentor for the PhD Programme and Eileen as one of the organisers of the annual labdays and faculty retreats. I am convinced that both Units are once more in very capable hands and will continue to thrive as they develop new scientific profiles.

I would like to congratulate three senior colleagues on major honours they received this year. Anne Ephrussi was elected member of the prestigious French Académie des Sciences, Janet Thornton was honoured with the Protein Society's Dorothy Crowfoot Hodgkin Award and Peer Bork received one of the Nature Awards for Mentoring in Science, an new prize that highlights an important aspect of scientific leadership that is often undervalued.

On a broader scale, European science currently finds itself in a critical phase. One of the keys to our future research competitiveness is the ability to provide scientists throughout Europe with access to cutting-edge research infrastructure. EMBL provides such infrastructure in the life sciences, for example the EMBL-EBI data resources and the structural biology beamlines at the Grenoble/ESRF and Hamburg/DESY outstations. The European Strategy Forum for Research Infrastructures (ESFRI) process is driving a broad discussion of research infrastructure strategy across disciplines and EMBL has taken a very active role by coordinating or participating in several projects within ESFRI's European Roadmap for Research Infrastructures. We have done so because in my view any future improvement of Europe's capacity in research infrastructure involves adopting a pan-European integrated approach that transcends national interests, much in the way that EMBL has operated in the life sciences.

I invite you to enjoy our report. It is intended to provide a set of windows through which you will see the lively and exciting place that is EMBL.

Iain W. Mattaj
Director General

State of the Laboratory

Research

This section is intended to provide a brief overview of the most important research developments at EMBL over the past year. In the following chapter, the Scientific Report showcases research highlights of 2008/2009 in more detail.

Reorganisation of Research Units in Heidelberg

Over the past year, two research units in Heidelberg have seen changes in their leadership. Jan Ellenberg, Head of the Gene Expression Unit from 2006-2008, has been appointed as Joint Head of the Cell Biology and Biophysics Unit. Since the beginning of 2009, Jan has been coordinating the Unit alongside Eric Karsenti. Eric will step down from the Head of Unit position at the end of the year to enable him to devote more time to his leadership of the Tara Oceans project, a major effort to gain insight into ocean biodiversity and its response to climate change. Together with the Ellenberg group, the labs of Carsten Schultz and Christian Häring, whose research lies at the thematic interface of both Units, have moved from Gene Expression to Cell Biology and Biophysics. The coordination of Gene Expression is now in the hands of Eileen Furlong and Lars Steinmetz, who took up posts as Joint Heads of the Unit in January 2009.

Reflecting the true EMBL spirit that encourages scientists to identify and pursue new research trends early on, and guided by the comments and recommendations of a very favourable scientific review of the Unit in May 2008, the newly appointed Unit Heads have already started to revamp the strategies and directions of the Gene Expression Unit. The overarching theme of the unit will remain 'bridging the gap from genome to phenotype' and will be pursued by combining wet-lab and computational methods. While the Unit will continue to draw on its traditional strengths in the areas of biochemistry and genetics, increasing emphasis will be placed on large-scale, global studies in systems biology, functional genomics and proteomics. There are also plans to continue the recent trend of integrating the Unit's traditional strengths with more chemistry and chemical biology and to expand its horizon towards synthetic biology and the development of nanotechnology in future. To embody its new, broadened scope, the Unit will be renamed the Genome Biology Unit. The new name stresses the focus on genome-wide studies and will in future facilitate the recruitment of interdisciplinary staff members who do not necessarily see themselves as working in the traditional area of gene expression.

All of the envisaged developments have been conceived in the broader context of developing a new approach to understanding biological systems in general, which is likely to dominate biomedical research in the future. This approach involves understanding the role of individual variation and its effect on biological responses, and will eventually be useful in medicine in developing preventative personalised treatments, as opposed to the reactive, en masse therapies currently offered. Such a change requires a thorough understanding of molecular mechanisms at the systems level derived from the use of a range of tools and technologies, both of which are derived from basic research and technology development in the area of genome biology. A more detailed outlook on the future plans for the Unit can be found on page 88 in the following scientific report.

One of the Gene Expression Unit research highlights of the past year, a collaboration between the groups of Lars Steinmetz and Wolfgang Huber, showcases the power of global, genome-wide studies. By investigating all transcripts found in a yeast cell, the researchers overthrew the long-standing textbook view that transcription happens only in one direction along the DNA strand. A better understanding of transcription mechanisms is important and could find applications in new technologies to tune gene regulation for both research-driven and medical purposes in the future.

Chemical biology

Chemistry-based methods are acquiring more and more importance in life science research and chemical biology has

EMBL Scientific Publications and Collaborations 2008

- Total number of peer-reviewed publications: 619
- Internal collaborations: publications co-authored by more than one EMBL group leader: 36
- External collaborations: 1104 in total of which 87 resulted in a publication

gained momentum across all EMBL Units over the past year. Currently 10 EMBL group and team leaders are either chemists or are interested in pursuing chemistry projects. In January this year, 19 group and team leaders, representing all Units and outstations, attended the first EMBL internal chemical biology retreat, which was organised by Carsten Schultz. The retreat gave those groups interested in chemical biology a chance to introduce themselves, identify like-minded colleagues and find out who to approach with their specific problems and where overlap of expertise and applications could foster new collaborations.

To encourage interdisciplinarity, chemistry groups are embedded in and spread around the different EMBL Units rather than organised as a separate chemistry Unit. This arrangement ensures that chemists keep in close touch with biological problems and also distributes chemistry expertise across the lab. The main areas of chemistry application at EMBL are probe development, inhibitor design, small molecule screening, chemoinformatics and interactions of synthetic molecules and metabolites with biological macromolecules.

Since July 2008, EMBL-EBI offers a new, powerful chemistry resource to support ongoing research efforts in chemical biology. With the financial support of the Wellcome Trust, EMBL-EBI was able to transfer a large collection of information on the properties and activities of drugs and a large set of drug-like small molecules from the publicly listed company Galapagos NV to the public domain. It will be incorporated into the EMBL-EBI collection of open-access data resources for biomedical research and will be maintained by a newly established team of scientists at EMBL-EBI. This will now allow researchers worldwide to make free use of knowledge essential for drug discovery and small molecule target identification.

The new High Field NMR magnet.

A project carried out in the group of Peer Bork illustrates how such information about drugs and their properties can benefit basic research. The team developed a computational method that compares the side effects of different drugs and uses side-effect similarity to predict potential new drug targets. The approach has the power to reveal the molecular basis of many side effects. It also shows promising therapeutic potential because it can hint at new uses for drugs that are already on the market.

Structural Biology

For structural biology at EMBL, the launch of the new High Field (800MHz) NMR spectrometer was a highlight of the past year. Many alumni attended the scientific symposium held in Heidelberg on 13 March 2009 to inaugurate the new

The Advanced Training Centre on the Heidelberg campus is close to completion.

NMR machine and to discuss recent advances and future directions of NMR in terms of technology and applications. Since the late 1980s NMR spectrometry has been an integral part of EMBL's research in structural biology and current NMR group leader Teresa Carlomagno heralds the third generation of NMR groups at EMBL. Her research will focus on the structure and dynamics of larger RNA-protein complexes, the analysis of which is made possible thanks to the new high field spectrometer.

More advances in technology development for structural biology have been made in Hamburg and Grenoble. The two outstations have pooled their expertise in a bilateral effort to develop technology for the new PETRA III beamlines and to install the first small angle X-ray scattering (SAXS) beamline in Grenoble. Thanks primarily to the advance in data analysis made by Dmitri Svergun in Hamburg, SAXS is becoming increasingly popular as a technique in structural biology and the SAXS beamline is EMBL Hamburg's bestseller in terms of services. In order to meet the increased demand, one of the X-ray crystallography beamlines at the ESRF in Grenoble will be converted into a SAXS beamline dedicated to the structural analysis of large molecular complexes in solution. Dmitri and members of his team are providing support and expertise to group leader Andrew McCarthy, who will operate the new beamline in Grenoble. As well as increasing the availability of the technique, developments in sample handling and delivery designed by Florent Cipriani and his team in Grenoble are making SAXS experiments more efficient and easier to perform and are improving the quality of the data they produce.

Under the leadership of Dirk Heinz from the Helmholtz-Institute for Infection Research in Braunschweig, Germany, several universities and research organisations in Northern Germany have developed a concept to establish a new Centre for Structural Systems Biology (CSSB) in close vicinity to EMBL Hamburg on the campus of the German Synchrotron Research Centre (DESY). The Head of EMBL Hamburg, Matthias Wilmanns, has been closely involved in the process. We welcome the opportunity to participate in this centre for biology research, which will enable optimal exploitation of the new facilities for synchrotron radiation at PETRA III and the laser facilities FLASH and XFEL. We hope that a decision to move ahead will be made by the German

federal and national funding organisations soon.

The cutting-edge infrastructure for high-throughput structural biology that already exists in Grenoble has led the groups of Stephen Cusack and Darren Hart to a scientific breakthrough this year. In collaboration with researchers at the Unit of Virus and Host Cell Interactions (UVHCI), which is operated jointly by EMBL, the CNRS and the University Joseph Fourier in Grenoble, they solved the structure of parts of the influenza virus polymerase, a protein that allows the virus to multiply in human cells. Scientists have tried to obtain such high-resolution images of the viral protein for more than 20 years, because it is a promising drug target. With the atomic structure at hand, structure-based drug design approaches can now identify small molecules that inhibit the function of the protein and may lead to new therapies.

Klaus Scheffzek in Heidelberg has also solved a longstanding scientific puzzle using structural biology approaches. His group was the first to uncover how eukaryotic cells make polyphosphates, long chains of phosphate molecules that serve as energy stores and have many other diverse functions in a cell. The findings have potential applications ranging from improving crops to fighting diseases such as sleeping sickness, which is caused by parasites that need polyphosphate chains to survive.

This year sees the retirement of Dietrich Suck, who has been at EMBL Heidelberg for 32 years. As well as leading his own research group since 1982, Dietrich also acted as Joint Head of the Structural Biology Unit for eight years. He led the Unit through a transition from a solely X-ray crystallography-based department to a well-rounded Structural Biology Unit including NMR, electron microscopy and biochemical methods. Dietrich Suck has been a tremendous asset for EMBL.

EMBL-EBI Research

EMBL-EBI researchers have made major contributions to improving the understanding of evolution this year. Nick Goldman and his group have developed a new computational tool that compares the genetic sequences of different species to learn about their evolutionary relationships. It provides the most accurate insights into the evolution of DNA and protein sequences to date. The group has also been working as scientific advisors in a new Wellcome Trust project to develop an interactive tree of life, which provided the graphical centerpiece of the BBC documentary 'Charles Darwin and the Tree of Life'. Further interesting insights into evolution were provided by Ewan Birney's group at EMBL-EBI. As part of an international consortium, the team analysed the newly sequenced genome of the platypus. The platypus combines features typical of mammals, reptiles and birds and its genome aids our understanding of how humans and other mammals first evolved.

Developmental Biology and Cell Biology and Biophysics

Evolution has also been a hot topic in Heidelberg over the past year. In a joint effort between the Developmental Biology and Cell Biology and Biophysics Units, Detlev Arendt, François Nédélec and Ernst Stelzer and their groups unravelled the first steps of eye evolution. They deciphered the molecular mechanism that likely underpinned the evolutionary precursors of today's eyes and visual systems. With a sophisticated combination of experimental and computational methods, they also provided the first mechanistic account of phototaxis, or swimming guided by light, in the zooplankton larva of the marine worm *Platynereis dumerilii.* This basic research has ecological implications, because to understand how climate change affects the oceans and the life in them, we first have to understand how plants and animals in the sea respond to changes in their environment.

In another collaboration between the Developmental Biology Unit and Cell Biology and Biophysics Unit, Jochen Wittbrodt and Ernst Stelzer achieved a remarkable breakthrough last year. Their groups generated a digital zebrafish embryo, the first complete developmental blueprint of a vertebrate. With a newly developed Digital Scanned Laser Sheet Microscope, they traced all cells of a developing zebrafish over the first 24 hours of its life and generated digital movies showing all cell positions, divisions and movements. The movies have been made publicly available for the scientific community and the general public; on YouTube the digital zebrafish movie has been viewed more than 100,000 times already.

In future, the Developmental Biology Unit will expand its horizons into the realm of plant biology. Marcus Heisler, the newest faculty addition to the Unit and the first group leader appointed as part of the joint Faculty Development Programme with associate member state Australia, will introduce *Arabidopsis thaliana* as a new model organism at EMBL. His lab will spend five years at EMBL and then move to a leading Australian research organisation for four further years. In this way, the Faculty Development Programme will lead to new inspiration and cross-pollination between European and Australian molecular biology.

Mouse Biology

The Mouse Biology Unit in Monterotondo has its ten-year anniversary this year, which was celebrated with a scientific symposium for alumni and the mouse biology community on 22 June 2009. Under the initial guidance of Klaus Rajewski and then, since 2000, Nadia Rosenthal, the Unit has grown continuously in size, research breadth, service provision and importance. Starting out in 1998 with two groups, EMBL

Monterotondo now hosts six and shares several jointly appointed group leaders with other EMBL Units. Since its early days, the services that EMBL Monterotondo offers its research staff and the European scientific community have continuously expanded and now include gene expression, histology, phenotyping and transgenic services as well as a core facility for the production of monoclonal antibodies. The past year has also seen the departure of EMBL Monterotondo founding member Walter Witke, who was replaced as Deputy Head of the outstation by senior scientist Cornelius Gross.

The mouse is one of biology's most powerful model organisms. Thanks to the many fundamental molecular and physiological similarities it shares with humans, it lends itself to the generation of disease models. For example, in the past year, Cornelius Gross's group developed a mouse model of sudden infant death syndrome. This revealed that an imbalance of the neuronal signal serotonin in the brainstem is sufficient to cause sudden death in mice and might also be involved in cot death in humans. In another study, Liliana Minichiello discovered that a surge of the chemical signal dopamine in the main olfactory bulb – one of the key brain areas for olfactory perception – of pregnant mice creates a barrier for male odours that otherwise would trigger miscarriage and reactivate the female ovulatory cycle.

In addition to being well integrated with the Consiglio Nazionale delle Ricerche (CNR) and the European Mutant Mouse Archive (EMMA) on the Monterotondo campus, the EMBL Mouse Biology Unit is a central hub in international research networks and participates in several EU-wide mouse research and information initiatives. Since 2008, Nadia Rosenthal has acted as the coordinator for CREATE, the EU network for the coordination of resources for conditional expression of mutated mouse alleles.

Services

One of EMBL's key missions is the provision of services to the scientific community in its member states and beyond. The main areas of service provision are: access to synchrotron radiation for structural biologists, access to molecular biology data and bioinformatics tools through EMBL-EBI's databases and access to various technologies and expertise as provided by the Core Facilities.

Across all Units, EMBL hosted 413 visitors during 2008. The visitor programme organises lab stays of varying periods for visiting scientists to carry out collaborative projects with EMBL research groups or to use the state-of-the-art equipment and expertise of the Core Facilities. Grenoble provided synchrotron access to 2,893 users in 2008. Despite a complete shutdown from January to October 2008 for construction works on the new PETRA III storage ring, Hamburg still welcomed 239 beamline users over the past year. The EMBL-EBI website, which is the common portal through which all databases can be accessed and which serves as a valuable source of information and updates about bioinformatics for the scientific community, received an average 3.5 million webpage hits per day in 2008.

Structural biology

Since 1 April 2009, EMBL has participated in a new initiative called PCUBE that promotes technology platforms and techniques for protein expression and biological sample preparation for structural biology. The €6 million project is funded by the 7th EU framework over four years. PCUBE is an international effort in which groups at EMBL Grenoble, Hamburg and Heidelberg and the unique technologies they have developed are playing a major role. These include Darren Hart's ESPRIT system for finding soluble protein fragments, Imre Berger's protein expression systems, the high-throughput crystallisation facilities coordinated at Hamburg by Jochen Müller-Dieckmann and at Grenoble by José Márquez, the expertise of the Advanced Light Microscopy Core Facility run by Rainer Pepperkok and the Protein Expression and Purification Core Facility of Hüseyin Besir at EMBL Heidelberg. Access to the Heidelberg-based facilities will be coordinated by Christoph Müller. More detailed information on PCUBE and EMBL's contribution can be found on page 14 in the scientific report.

For more than three decades, EMBL has provided the life sciences community with pan-European access to large infrastructures. Access for scientists from EMBL member states is supported by EMBL, while users from other countries have been provided access that is mainly funded by transnational access grants from the EU Framework Programme. This funding has now been greatly reduced despite the fact that the demand for more access funding is still increasing, especially from users in convergence regions. At the same time, the EU has started to fund access to new technology platforms such as PCUBE that are now being made available to external users for the first time. This is very positive, but it should not be done at the cost of less funding for access to synchrotron beamlines. This applies not only to the European Synchrotron Radiation Facility, which is an international organisation similar to EMBL, but also to the many national synchrotrons such as the one operated by DESY, our partner in Hamburg.

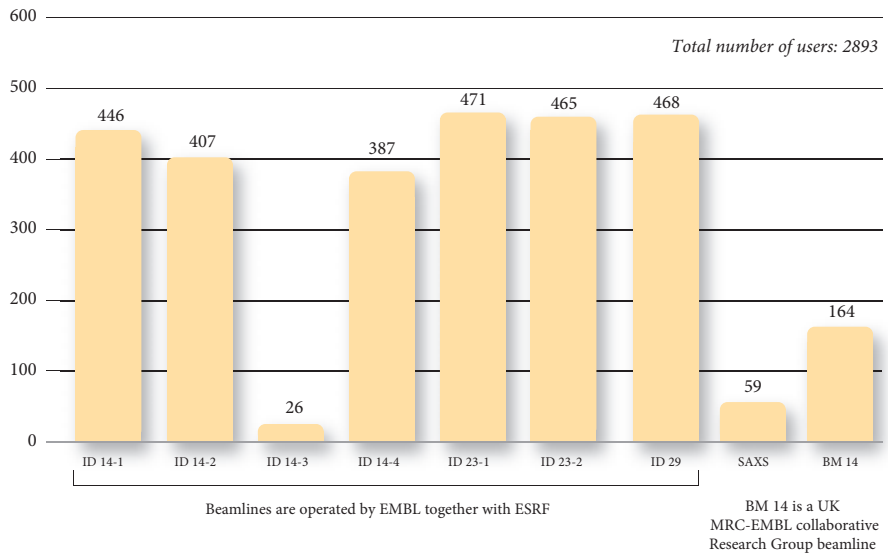
The European synchrotron landscape is going to change over the next 5-10 years and many current beamlines will be replaced by new third-generation beamlines at state-of-the art synchrotron facilities. The total number of beamlines will not increase but the quality of the radiation provided to the life sciences community will be greatly improved. This offers an unprecedented advantage for Europe and it is imperative that access funding should make these expensive resources accessible to all.

EMBL Hamburg's new integrated research facility for structural biology at the new PETRA III storage ring.

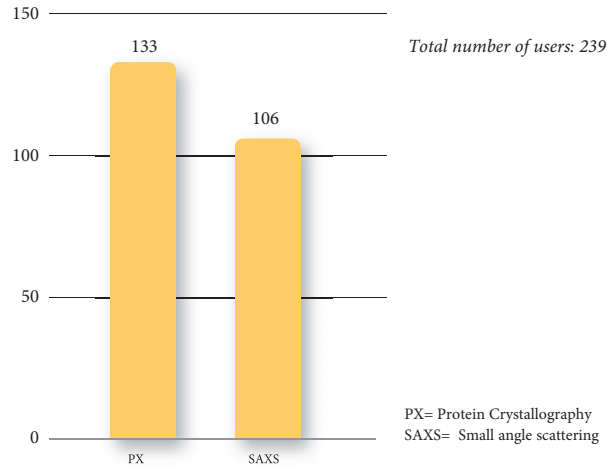
On 16 April 2009, the new PETRA III storage ring of DESY started operations in Hamburg. The construction of EMBL Hamburg's three new beamlines, two for protein crystallography and one for small-angle X-ray scattering, at the new state-of-the art synchrotron radiation facility are well underway. After a positive review of the plans by a Scientific Advisory Board of world-leading experts in the construction and operation of beamlines for SAXS and macromolecular X-ray crystallography, the procurement of the central components for the beamlines has started. The first instruments, which include a microdiffractometer developed jointly by EMBL Grenoble and the ESRF, are expected to arrive in Hamburg in May 2009, where they will initially be installed on the old DORIS ring for testing. In February 2009, less than 25 metres from the spot where the new beamlines will be installed, construction works have begun for a new building to host high-throughput protein crystallisation and sample preparation facilities. In order to tap PETRA III's full potential, EMBL Hamburg is also developing new technologies for sample handling in collaboration with EMBL Grenoble and the ESRF.

BM14 is a dedicated macromolecular crystallography beamline at the ESRF in France. It is widely acknowledged to be the most stable and successful multiple wavelength anomalous dispersion (MAD) beamline in Europe. It has been owned and operated by the UK Medical Research Council (MRC), and operated in partnership with EMBL as a Collaborative Research Group since 2001. The partnership aimed to provide access to a dedicated MAD beamline for use by the UK crystallographic community before completion of the new UK third-generation synchrotron, Diamond, and also by the EMBL member state community. The MRC will discontinue the operation of BM14 at the end of 2009. EMBL has expressed an interest in operating BM14 from 2010 onwards in collaboration with the ESRF and the National Institute of Immunology, an Indian institution under the Department of Biotechnology, which is part of the

Beamline Users EMBL Grenoble 2008



Beamline Users EMBL Hamburg 2008



Ministry of Science and Technology. Biomedical researchers from the EMBL, ESRF and Indian scientific communities will have access to the synchrotron beamline for macromolecular crystallography. This will require ESRF Council approval. However, the ESRF has already agreed to a transition phase in 2009 during which the MRC phases out its operations and new staff are hired by the ESRF-EMBL-India consortium to keep the beamline operational. We expect the new consortium to be established over the coming months.

Bioinformatics services

EMBL-EBI continues to host the major core biomolecular resources of Europe – collecting, archiving and distributing data throughout Europe and beyond. Its main databases include EMBL-Bank for nucleotide sequences, Ensembl for genomes, ArrayExpress for gene expression data, UniProt for proteins, Protein Data Bank Europe (PDBe) for macromolecular structures, and InterPro for protein motifs. The average number of requests for EMBL-EBI services from September 2007 to August 2008 was 2,709,000 per day, and 3,241,000 per day when including Ensembl (a collaboration between EMBL and the Sanger Institute).

One of EMBL-EBI's biggest challenges remains the increasing volumes of data being produced by ever-improving high-throughput technologies. These data need to be annotated, stored and curated in the databases. In the period from September 2007 to August 2008, all the core data resources grew significantly. More than 1.8 x 10^9 bases of DNA (compared with 1.56 x 10^9 in 2007) were received and bring the total number of nucleotides in EMBL Bank up to 1.17 x 10^{12}. In addition, 2.1 million UniParc entries, 115,000 microarray hybridisations, and 5,649 macromolecular structures were processed at EMBL-EBI and 12 new eukaryotic genomes were added to Ensembl during this time. The InterPro resource has grown to more than 16,500 entries, providing annotations for almost 80% of proteins in the UniProt Knowledgebase. The IntAct database of molecular interactions, which is of growing importance in the era of systems biology, has grown to more than 170,000 interactions.

In addition to these quantitative increases in the databases, the last year has also seen a range of qualitative improvements and a broadening of the spectrum of services offered by EMBL-EBI. Apart from expanding its services in the area of chemical biology with a new world-class data resource for drug discovery, EMBL-

The Google map shows the distribution of EMBL-EBI database users around the world.

EBI, together with the Sanger Institute, has also launched a new Ensembl genomes browser, which adds five new resources to the existing mammalian genome repository: Ensembl Plants, Ensembl Fungi, Ensembl Bacteria, Ensembl Metazoa and Ensembl Protists. These include many organisms of medical and agricultural importance.

The nucleotide- and protein-related service activities at EMBL-EBI were merged last year to form a single team, called PANDA, under the joint leadership of Rolf Apweiler and Ewan Birney. PANDA has more than 185 staff and two new levels of hierarchy were introduced in order to divide the responsibilities for subgroups among a larger number of senior-, middle- and lower-level managers. At the highest level, senior team leaders are responsible for several team leaders. At the lower management level, coordinators have leadership responsibility within large teams and might, for example, oversee a number of software developers or supervise an entire data curation effort.

EMBL Core Facilities

To enable it to offer its scientists and external users the most cutting-edge technology, EMBL has centralised important scientific infrastructures, such as complex microscopes, chemical screening facilities and genomics and proteomics robots, in nine Core Facilities. The Core Facilities collaborate closely with industry, which provides them with the newest, state-of-the-art instrumentation, and employ specialised technical staff to support the users.

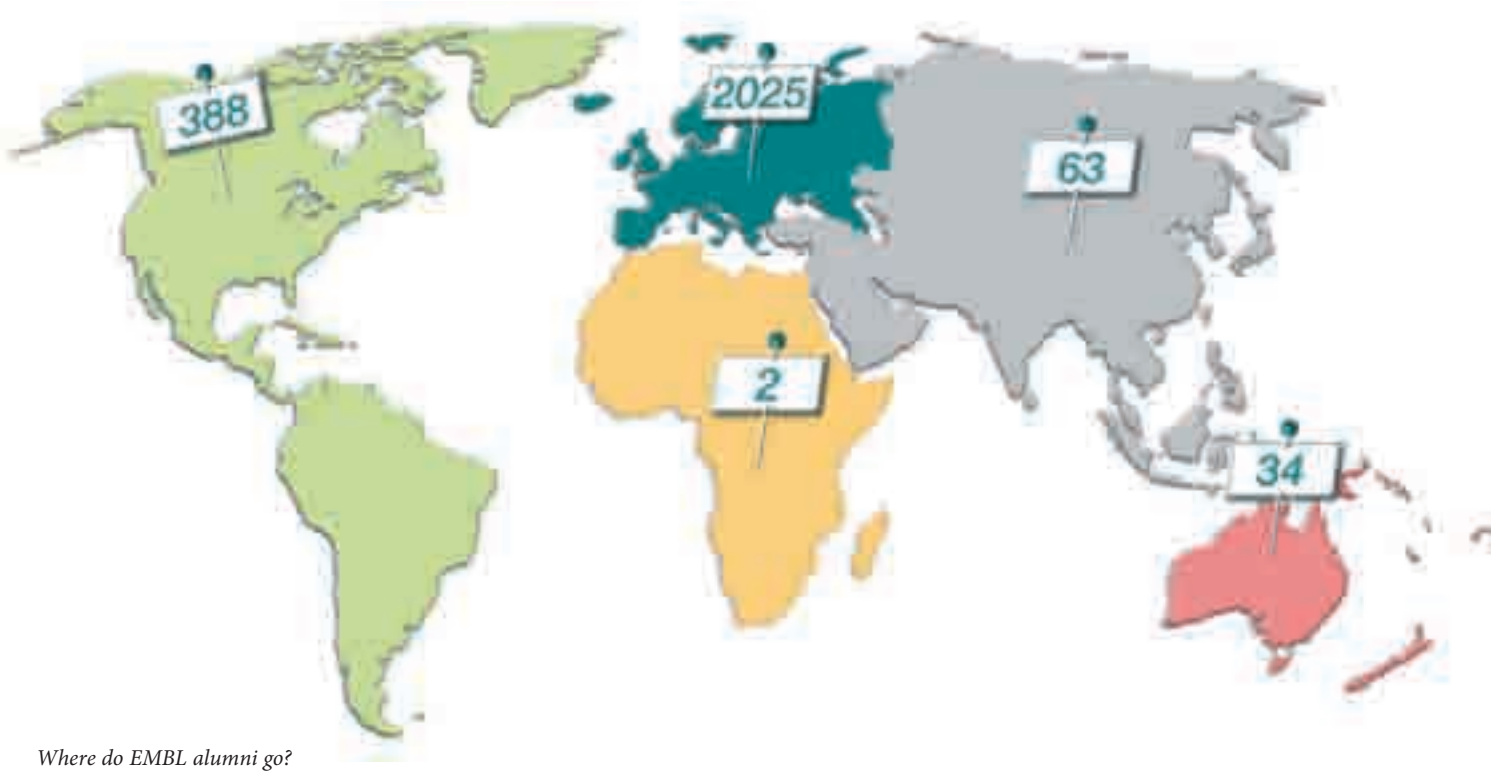
After nearly eight years, Thomas Franz, the first Head of the Proteomics Core Facility, left EMBL at the end of April 2009 to take up new challenges in Cologne, where he will become the Head of the Proteomics Facility at the newly created Max Planck Institute for the Biology of Ageing. We wish him success in his new position and hope he will continue to promote EMBL's concepts for efficient facilities serving the scientific community.

Jeroen Krijgsveld, who joined EMBL last October as a new team leader in the Gene Expression Unit, will take on future responsibility for the Proteomics Core Facility. During the past months, discussions have taken place with the major companies in the field of proteomics to partly re-equip both the research lab as well as the Core Facility with state-of-the-art instrumentation. This will allow both an increase in throughput and more refined characterisation of proteins, including the rapid analysis of post-translational modifications. At the same time we have relocated the Proteomics Core Facility to a contiguous area previously used by proteomics research activities. This will increase the synergies between the research component and the service aspects and will benefit the entire community. Currently, the newly acquired instrumentation is being installed and commissioned in the new space for proteomics. We expect to be able to offer the improved services during the coming months.

Training

The EMBL International Centre for Advanced Training (EICAT) provides a broad spectrum of training activities designed for scientists at different stages of their careers.

The EMBL International PhD Programme (EIPP) celebrated its 25th anniversary in 2008. Since its foundation in 1983, several hundred students have graduated from the Programme; 34 of them completed their degrees in 2008. Many of the former PhD students stay associated with EMBL through the Alumni Association. The EIPP has had the right to award its own PhD degrees since 1997, but to integrate the students into national academic networks in the member states, EMBL awards joint PhD degrees with universities in the students' home countries. For this purpose, we have established collaboration agreements with 29 universities in 19 countries. The EIPP currently has a steady-state student body of 200 with an annual intake of 50. The Programme is highly competitive, and received 900 applicants from all around the world in 2008. To ensure that EMBL continues to attract Europe's most talented students, we are continuously improving the EIPP, integrating the newest scientific developments and complementary and transferable skills training into its curriculum. The PhD Programme has been extremely successful. It has been copied by many other institutions, frequently with EMBL's help and advice.



Where do EMBL alumni go?

The EMBL Postdoctoral Programme is an umbrella structure for all postdoctoral fellows at EMBL. It provides training courses and opportunities for scientific exchange for postdocs, such as the annual postdoc retreat. The second call for the EMBL Interdisciplinary Postdoc Programme (EIPOD) took place in 2008, with an impressive turnout of 120 applications. The 15 successful candidates started in the beginning of 2009. They are working on interdisciplinary projects that are supported by at least two different labs, often spanning different Units. From late 2009 EIPOD will be co-funded by a Marie Curie Action of the European Commission, allowing us to increase the available EIPOD positions from 12 to 20 per year.

The EMBL Courses and Conference Office has undergone a structural reorganisation in the past year. EMBL scientists have organised 13 conferences and workshops and 18 practical courses in the past year. Many of the seminars and lectures held at EMBL are now made available to the wider scientific community as webcasts. Throughout 2008, EMBL-EBI organised three conferences, eight industry programme workshops and 11 user training courses in the training facilities of its new East Wing.

The European Learning Laboratory for the Life Sciences (ELLS) has organised eight hands-on courses for secondary-school teachers: four in Heidelberg, three in Monterotondo and one in Hamburg. ELLS has been running a new project aimed at developing inquiry-based molecular biology modules for schools since September 2008. The project, called iNEXT, is funded by the Robert Bosch Stiftung and will run for three years.

In 2008, EMBL successfully launched a Corporate Partnership Programme, which is designed to create and enhance long-term, effective relationships between EMBL and top-tier corporate partners and to sponsor training activities and conferences taking place in the new Advanced Training Centre. In return for partnership fees, partner companies receive a range of different benefits. Six companies have already joined the programme: Leica Microsystems, GE Healthcare, Perkin Elmer, Qiagen, Becton Dickinson and Eppendorf. Six more companies have pledged their participation and several more have expressed a strong interest.

EMBL Alumni

One of EMBL's biggest assets is its distributed alumni network. After their time at EMBL, more than 80% of alumni take up positions in academia and industry in the member states. In 2008-2009, eight faculty members left EMBL for positions in member and associate member states, notably one as a Max Planck Institute Director (making a total of 20 EMBL alumni Max-Planck-Institute Directors) and several others as university professors or department directors in Europe, South Africa and Australia.

The Alumni Association continues to grow and now has more than 1,500 members, of which 234 joined in the last year alone. As well as growing in size, the activities of the Association have also increased. Local chapters met last year in Madrid (Spain/Portugal chapter) and Dilofo (Greek chapter). Alumni in Sweden organised a highly successful event with the Royal Swedish Academy of Sciences (RSAS) and the Swedish Research Council, which was aimed at

encouraging young scientists to do research abroad and informing them of the benefits of working at EMBL.

In autumn 2008, the EMBL Alumni Association elected six new members to its board, bringing the total to 14 representatives: new members Anastasios Koutsos, Oscar Martin-Almendral (Treasurer), Cedric Notredame, Anastasia Politou, Giulio Superti-Furga (Chair), and Maria del Mar Vivanco join continuing members Oddmund Bakke, Colin Dingwall, Freddy Frischknecht, Bernard Hoflack, Claudia Koch-Brandt, Giovanni Paolella, Annalisa Pastore and Niovi Santama (Vice Chair). The Association would like to thank board members who stepped down in 2009 for their contribution: Antony Hyman, Fotis Kafatos, Daniel Louvard, Richard Morris, Konrad Müller, Albert Stegmüller and especially Angus Lamond for his chairmanship of the board since 2002.

The Alumni Association board selected Julius Brennecke as the 2009 John Kendrew Award winner for his remarkable contributions to science in the field of RNA silencing. The board also launched the first Association-wide initiative, the alumni wiki – a new online careers resource for EMBL staff and alumni.

Outreach

The goal of EMBL's outreach activities is to communicate the Laboratory's activities in research, training and services to a wide range of audiences and to engage in a dialogue with the public. With the help and participation of many scientists, a large number of events have been organised by the Office of Information and Public Affairs (OIPA), EMBL-EBI's Outreach and Training team, the Science and Society programme, EICAT and many others.

Last year, OIPA organised 13 individual visits for students from schools and universities from different countries, including Austria, Finland, Spain, Greece and the United States, as well as a Girls' Day for employees' children at EMBL Heidelberg.

The look and feel of the new EMBL website.

Highlights of outreach activities at EMBL-EBI in 2008 included its contribution to the 'Biology Zone' at the Cambridge Science Festival, which offered a range of hands-on and computer-based activities, attracting more than 2,500 visitors. To effectively support recruitment for the EMBL PhD programme and to demonstrate career opportunities, EMBL-EBI organised two masters open days in 2008.

For the Science and Society programme, 2008 was another typically rich and varied year featuring lectures and presentations by distinguished speakers from such diverse backgrounds as science writing and philosophy to astronomy and sociology. The first European Science and Society Summer School, focusing on 'Deconstructing and ordering living organisms' and 'Re-making life: new bioentities and their meanings', brought students and postdocs from the life sciences, humanities and social sciences to Heidelberg. Increasingly, Science and Society lectures and symposia also take place at outstations. EMBL Monterotondo, for example, hosted social scientists and neuroscientists at an interdisciplinary 'neuroschool', which focused on behavioural genetics. EMBL-EBI launched a series of mini-symposia, which covered topics such as 'The Personal Genome – Hopes, Facts and Fears' and 'Scientific and Social Aspects of Ageing'. The annual EMBL/EMBO Science and Society Conference remains the flagship of the programme; this year, the ninth Conference was entitled 'Systems and Synthetic Biology: Scientific and Social Implications'. For a more detailed report please refer to page 100.

Other public relations activities coordinated by OIPA encompass the production of publications, media work, providing graphic support, maintenance of the EMBL websites, hosting visits and representing EMBL at major conferences and career fairs. Thus, we reach out to political stakeholders, the media, future employees, the next generation of scientists, staff and alumni alike.

Highlights of last year's media work include stories on sudden infant death syndrome and the digital zebrafish embryo, with its impressive video material. Both stories yielded particularly wide international media coverage.

Five years after it last revamped its websites, EMBL has just completed the latest update. With a modern look, and based on a content management system for fast and easy content update, the new web pages offer easy access to key information about EMBL. EMBL staff can now find all relevant information on an intranet portal that is updated daily.

The EMBL *Etcetera* newsletter, a key publication to keep staff, alumni and friends informed about what's going on in the Laboratory, celebrated its 50th issue this year with a fresh design in four colours.

Administration

EMBL Council regularly reviews the terms and conditions of employment at EMBL. In the most recent review, which was concluded in November 2008, the Council Working Group (WG), established to carry out the review and chaired by Jeannette Ridder-Numan, recommended introducing social security for EMBL fellows. This has significant

financial implications and the earliest we will be able to introduce this change will be at the beginning of the next Indicative Scheme in 2012. The Council WG is still in place as it needs more time to complete a review of the EMBL pension scheme.

At the beginning of this year, we carried out a survey of our administration services involving all staff at EMBL. The development and evaluation of the survey was supported by a staff survey consultancy at the University of Bristol. The survey was available in English, German and French. Fifty percent of EMBL staff across all sites participated. This is a very high percentage for participation in such a voluntary survey, which shows that the staff responds to opportunities to provide feedback. The positive results reflect the hard work of the administration staff, who have been rated as consistently helpful across all work areas and locations. Purchasing functions was another area that received positive reviews, as well as the staff training and development programme, which had only been introduced in 2008. Many of those surveyed commented that EMBL was the best place that they had ever worked and that they enjoyed working here very much. As can be expected, there is still room for improvement and this includes the staff induction process, performance assessments, support with external grants and the functionality of the SAP system. With respect to the latter, some improvements have already been implemented and further work is planned to facilitate the administration of grants in future. We will also follow up on the other areas highlighted by the staff responses and consider how to further streamline work processes in EMBL's administration.

The work on the SAP system was linked to a change in our accounting procedures, which aims to introduce cash budgets at all EMBL sites and to meet changing requirements of the EC and other external funding organisations regarding depreciation and other aspects of cost charging.

ATC topping out ceremony

Our new head of personnel, Ulla Böhme, who has Swedish nationality, started in early 2009. Ulla has more than 15 years' experience in human resources management, which she developed in the private sector and in an international organisation in Sweden.

Facilities

On 25 September 2008, we celebrated the topping out ceremony and the completion of the helix structure of the Advanced Training Centre, which is being built on the Heidelberg campus. The construction works are proceeding largely according to plan, although there is some delay caused by the exceptionally

long period of freezing weather during the 2008/2009 winter months. The opening is planned for 11 March 2010, and ministers of our member states have been invited to the opening ceremony, where Nobel Laureate Tim Hunt will speak. The building will house all staff working in EICAT, the communications unit, and large parts of the administration. A total of 108 people will initially occupy the building.

The main laboratory building will be reorganised and partially refurbished over several years. The first projects are the conversion of the canteen into a cafeteria, and IT services and their server collection. This server facility refurbishment has become necessary in Heidelberg due to the increasing demand for high-performance computing continues, thanks to next-generation technologies in sequencing, high-throughput light microscopy screening, light and electron microscopy, bioinformatics, modeling and simulation. The present location of the server cluster in Heidelberg is inappropriate and unsuitable for the expansion that will need to take place over the next 2-5 years. We will soon reach the limit in terms of physical space to add more servers and have already reached the limits in terms of power and cooling capacity. A new transformer is needed, as is additional efficient cooling equipment, which will be housed behind the Annex building.

Integration of European Research

EMBL Partnerships

Unit of Virus Host Cell Interactions (UVHCI) in Grenoble

The Unit of Virus Host Cell Interactions (UVHCI) was created in January 2007 to formalise the collaboration between the University Joseph Fourier (UJF), EMBL Grenoble and the National Center for Scientific Research (CNRS) in the field of virus research. Its objective is to pursue high-quality research in structural and molecular biology, focused on, but not exclusively dedicated to, virus-host cell interactions. The Unit has been established for five years and Stephen Cusack, Head of EMBL Grenoble, has been appointed as Director, with Rob Ruigrok from UJF as Deputy Director.

Research themes include virus structure, assembly and maturation; host and virus gene-expression mechanisms; cell biology of infected cells; innate immunity; and anti-pathogen drug design. Viruses studied include: influenza, rabies, Epstein-Barr virus and HIV. In addition, methods and technical developments in high-throughput expression and crystallisation, synchrotron X-ray and neutron diffraction instrumentation and electron microscopy are pursued.

Partnership for Structural Biology

One of the four partners in the Partnership for Structural Biology (PSB), the Institute for Structural Biology (IBS), will relocate to the ILL/ESRF/EMBL campus in Grenoble. The IBS has also been nominated as a second partner for France in the INSTRUCT project for structural biology research infrastructures. The first partner is the Institute of Genetics and Molecular and Cellular Biology (IGBMC) in Strasbourg.

Under the title 'Presqu'île Scientifique', the partners on the ILL/ESRF/EMBL campus are planning to further develop the Polygone Scientifique campus, which is located on a peninsula created by the confluence of the rivers Isère and Drac. The plans are supported by local and central French government agencies, which are planning to invest €800 million (not including money for research infrastructures). Eight thousand researchers will work on-site in the future and the large research facilities are planning to welcome more than 20,000 visiting researchers every year.

Framework agreement with University Heidelberg

EMBL recently concluded a framework agreement with the Ruprecht-Karls-University Heidelberg to provide an umbrella for a broad range of successful cooperations that will be continued and expanded. These include the Molecular Medicine Partnership Unit and the Chemical Biology Core Facility as well as the Joint PhD Programme.

Collaboration with DESY

EMBL Hamburg is located on the campus of the German Synchrotron Research Centre (DESY) and is now building several new beamlines and a sample preparation facility to make PETRA III synchrotron radiation available to the life science community. The collaboration with DESY is going extremely well and I would like to thank the recently retired DESY Director, Albrecht Wagner, for his strong support for biology research on the DESY campus. I am confident that our good relations will continue under the new leadership of Helmut Dosch, whom I met recently during a visit to EMBL Hamburg.

A major part of the beamline project is funded by the German Federal Ministry of Education and Research (BMBF), and I would like to take this opportunity to thank them for their cooperation and support. We have been invited by DESY and BMBF to participate in a German-Russian research programme that will make use of the new research facilities at DESY.

European Research Infrastructures

ESFRI

The European Strategy Forum on Research Infrastructures (ESFRI) was set up by the EU member states as a platform to explore new initiatives for the development of European research infrastructures. Delegates nominated by the research ministers of the EU member states and associated countries represent the 33 countries in the forum. The European Commission (EC) is also represented. In 2004, the EU Council mandated ESFRI to develop a strategic roadmap for Europe in the field of research infrastructures. The first ESFRI roadmap was published in 2006, an updated roadmap at the end of 2008. Forty projects span a broad range of scientific areas including particle physics, astronomy, social sciences and humanities, biomedical sciences, and environmental and e-sciences. Ten projects are in the area of biomedical sciences.

EMBL participation in ESFRI biomedical science projects

EMBL is currently participating in seven of the ten biomedical science projects, four of which were on the 2006 ESFRI roadmap. These are now in an EU FP7-funded three-year preparatory phase with the aim to develop a detailed plan for construction and operation.

ELIXIR – European life sciences infrastructure for biological information

This is the largest and most important infrastructure for EMBL, because it concerns the longer-term funding of the European core data resources, many of which are located at EMBL-EBI. EMBL-EBI Director Janet Thornton is coordinating the ELIXIR preparatory project, in which 32 partners from 16 countries are participating. The project started in November 2007 and will end in 2010. Much progress has been made to define the scope of ELIXIR, the legal and governance model, and how it could be financed. A detailed plan to coordinate negotiations with the EU member states for funding is expected by the end of this year. ELIXIR will be a distributed infrastructure with EMBL-EBI serving as the scientific and technical hub. A number of nodes will be located in other EU member states. The goal is to build a sustainable European infrastructure that will be able to handle the huge amounts of data that are being collected in the life sciences and to make the information available broadly to the scientific user community in academia and industry. Even though there is broad consensus among the stakeholders that this is an essential infrastructure for Europe, the challenge will be to secure sufficient funding to deal with the increasing quantity and diversity of information. Life scientists are now generating petabytes of data on a daily basis. As a result, computational requirements are approaching those of large-scale physics infrastructures: for example, the large-scale DNA sequencing projects in 2008 produced 10% as much data as is expected from the Large Hadron Collider at CERN once it is functioning at full capacity. ELIXIR provides a link to all other biomedical science projects because they all produce data that need to be handled and stored properly. EMBL's future involvement in ELIXIR is being addressed by a Council Working Group chaired by Nigel Watts, who also chairs EMBL Council's Finance Committee. This has been reviewing potential risks and opportunities for EMBL's participation in ELIXIR and other biomedical research infrastructures.

INSTRUCT – Integrated Structural Biology Infrastructure

EMBL is one of six core partners in INSTRUCT. EMBL Grenoble, Hamburg and Heidelberg representatives are involved in carrying out the necessary work packages, which include user access and possible applications of new technologies such as X-ray imaging and free electron lasers for life sciences. The INSTRUCT preparatory phase started in April 2008 and will run for two years. The project is aiming to bring a new dimension to structural biology services, which are traditionally provided by synchrotrons, by offering integrated technology platforms, from sample preparation to data acquisition and analysis. The integration of technology platforms will also cross several levels of spatial resolution in order to address complex problems. Users will not be restricted to the structural biology community; INSTRUCT will also encourage cell and molecular biologists to apply structural biology to their scientific questions by providing technical support platforms and expert help. These are very ambitious goals, but they will offer a unique service in Europe, especially if they can be integrated well with access to existing large-scale synchrotron and NMR facilities.

EMBL-EBI is involved in data storage and IT infrastructure issues in two other ESFRI projects: INFRAFRONTIER (phenotyping and archiving of model mammalian genomes) and BBMRI (European Biobanking and Biomolecular Resources).

The following three projects are on the updated ESFRI roadmap published in December 2008, but have not yet entered the preparatory phase:

EURO-BioImaging (biomedical imaging) is jointly coordinated by the Head of the EMBL Cell Biology and Biophysics Unit, Jan Ellenberg, and Gabriel Krestin from Erasmus University, Rotterdam, the Netherlands.

EMBRC (European Marine Biology Resource Centre), coordinated by Roberto Di Lauro from the Stazione Zoologica, Naples, Italy, and EU-OPENSCREEN (screening platforms for chemical biology) coordinated by Ronald Frank, Leibniz-Institut für Molekulare Pharmakologie (FMP), Berlin, Germany.

A busy EIROforum stand at the 2008 European Science Open Forum in Barcelona.

EMBL's role in establishing biomedical research infrastructures in Europe

All European biomedical research infrastructures are distributed, which means that they are operating facilities providing important services to the scientific community in several countries. Ideally, it would be possible to establish an organisation similar to EMBL that can operate as one entity in several countries and freely move staff and resources from site to site. Setting up new intergovernmental organisations has been dismissed by the ESFRI member states as too complicated. Therefore different solutions are being investigated by all ESFRI projects that are currently in the preparatory phase. Each infrastructure will have to establish a legal entity to employ staff, handle money and enter into contracts. For most of the distributed infrastructures, this will be a relatively small central coordination unit. Because we think the biomedical science projects are very important for European biomedical research, EMBL would like to offer help. We have suggested using an existing organisation such as EMBL as an institutional umbrella to set up some of the biomedical research infrastructures. This has the advantage that it will benefit from EMBL's existing legal personality, as well as its experience and reputation in managing an international research infrastructure for more than three decades.

Such a solution would require the stakeholders in one the ESFRI projects forming a consortium and entering into an international agreement. The consortium agreement would establish its own governance structure that could include, for example, a supervisory board, a scientific advisory board, and a central coordination unit. The infrastructure would be spread over a number of nodes, which would be mainly national research organisations. The research infrastructure consortium would benefit from EMBL's experience handling money and employing staff at the central coordination unit. However, its governance structure would be independent of EMBL and its budget ring-fenced from other EMBL activities. By comparison to setting up a new international organisation, this would offer a straightforward and rapid way to realise the ESFRI biomedical science projects. This could also be used as an interim solution until the new EU regulation for a legal structure for research infrastructures becomes available.

EIROforum

EIROforum is a partnership of the seven largest intergovernmental research organisations in Europe (CERN, EFDA-JET, EMBL, ESA, ESO, ESRF, ILL). These have extensive expertise in the areas of basic research and the management of large, international infrastructures, facilities and research programmes. The mission of EIROforum is to support European science in reaching its full potential. EIROforum simplifies and facilitates interactions with the EC and other organs of the European Union, national governments, industry, science teachers, students and journalists. The last year has seen the departure of two Director Generals: William Stirling from ESRF, who is followed by Francesco Sette, and Robert Aymar from CERN, who retired and was replaced by Rolf-Dieter Heuer.

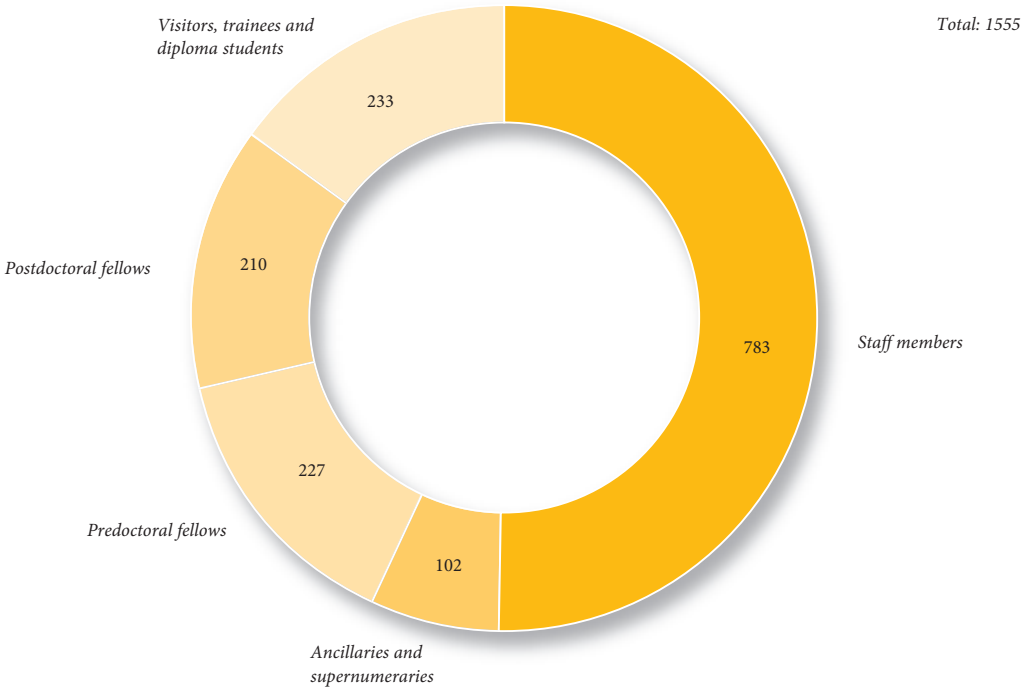
For the second time, EMBL will take over the chair of EIROforum in July 2009. Planned activities under its one-year chairmanship include the renewal of the Statement of Intent between EIROforum and EC that was signed in 2003 with former Commissioner Philippe Busquin. The current Commissioner, Janez Potočnik, proposed a review of the EC–EIROforum collaboration and an updated Statement of Intent to express the mutual interest in continuing the cooperation in the future.

In November 2009, EMBL and its EIROforum partners will organise a conference on technology transfer in Heidelberg to exchange knowledge and best practices across disciplines. EIROforum has a long tradition in outreach and education activities and we are planning to establish a series of teacher training courses and to continue the publication of Science in School, a European journal for science teachers that is reporting on new findings in the seven EIROforum organisations.

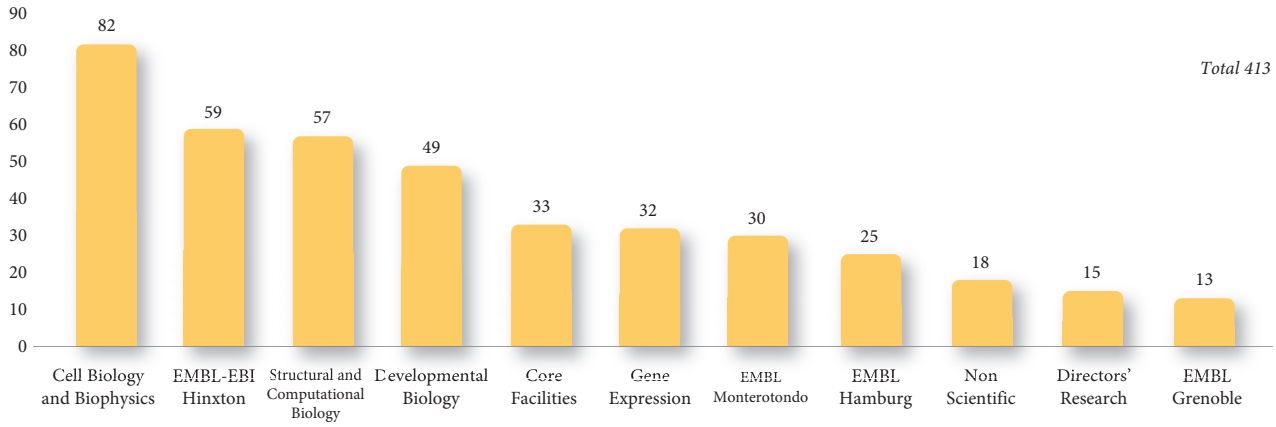
Personnel statistics

On 31 December 2008, 1555 people, including visitors, from more than 60 nations were employed by EMBL.

Personnel on 31 December 2008

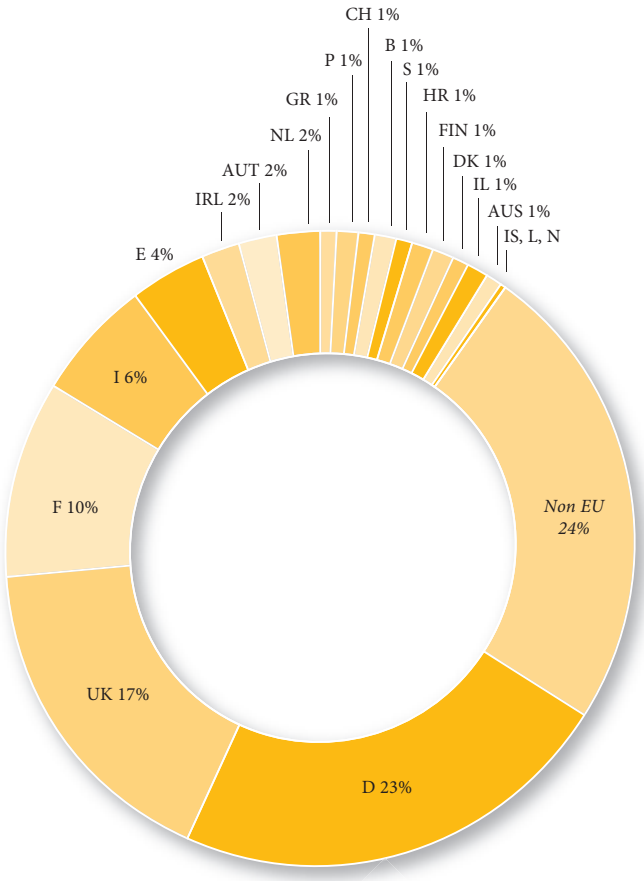


Visits to EMBL Units during 2008

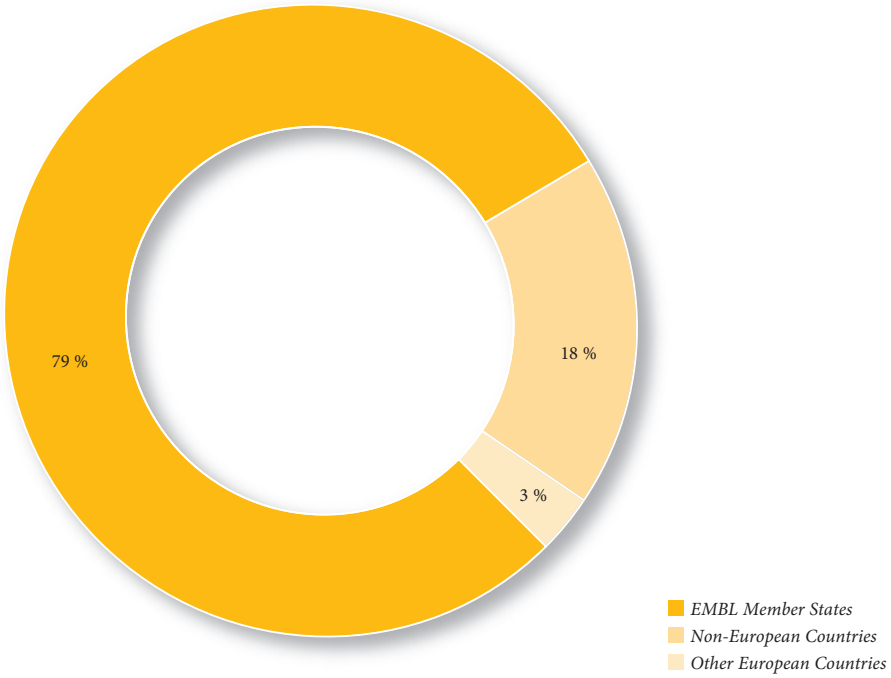


Staff Nationalities – Research

Please refer to CD for more information



Staff Nationalities – All



Financial report

External grant funding

	2008		2007	
	€ 000	%	€ 000	%
BBSRC	1,457	3.6	1,244	3.4
BMBF	3,257	8.1	2,436	6.7
DFG	1,461	3.7	1,090	3.0
EU	16,893	42.2	17,124	46.9
HFSPO	492	1.2	583	1.6
MRC	7	0.0	178	0.5
NIH	6,169	15.4	5,908	16.2
Swissprot	-	0.0	957	2.6
VW Foundation	294	0.7	241	0.7
Wellcome Trust	5,240	13.1	2,038	5.6
Others	4,735	11.8	4,749	13.0
TOTAL	**40,005**	**100.0**	**36,548**	**100.0**

Income/expenditure statement

INCOME	**2008**	**2007**
	€ 000	€ 000
Member states contributions	85,589	78,712
Internal Tax	18,734	18,139
External Funding	40,005	36,548
Other Receipts	18,867	13,786
Total Income Excluding pension contributions	**163,195**	**147,185**

EXPENDITURE	**2008**	**2007**
	€ 000	€ 000
Staff Costs	81,255	79,363
Operating Costs	49,101	40,107
Capital Expenditure	25,942	33,647
Total Costs	**156,298**	**153,117**
Surplus (deficit) transferred to reserves	6,897	(5,932)

EMBL budget 2008: € 163 million

Please refer to CD for more information

Member states contributions

	Ordinary and one-off contributions				Pension contribution	
	2008		2007		2008	2007
	€ 000	%	€ 000	%	€ 000	€ 000
Austria	1,739	2.2	1,573	2.2	27	26
Belgium	2,122	2.7	1,927	2.7	33	31
Croatia	56	0.1	-	0.0	1	-
Denmark	1,356	1.7	1,226	1.7	21	20
Finland	1,085	1.4	971	1.4	17	16
France	12,724	16.0	11,286	15.9	197	185
Germany	16,441	20.6	15,543	21.9	254	255
Greece	1,683	2.1	1,041	1.5	26	17
Iceland	80	0.1	-	0.0	1	-
Ireland	949	1.2	-	0.0	15	-
Israel	694	0.9	864	1.2	10	14
Italy	10,267	12.9	9,210	13.0	159	151
Netherlands	3,677	4.6	3,096	4.4	57	51
Norway	1,587	2.0	1,410	2.0	25	23
Portugal	973	1.2	822	1.2	15	13
Spain	6,174	7.7	4,945	7.0	95	81
Sweden	2,090	2.6	1,884	2.7	32	31
Switzerland	2,449	3.1	2,317	3.3	38	38
United Kingdom	13,625	17.1	12,731	18.0	211	209
SUBTOTAL	**79,771**	**100**	**70,846**	**100**	**1,234**	**1,161**
Ireland	-		730		-	12
Iceland	-		45		-	1
Special contribution Iceland	-		16		-	-
Croatia	-		67		-	1
Special contribution Croatia	23		23		-	-
Luxembourg	112		104		2	1
Special contribution Luxembourg	41		41		-	-
Australia associate member	3,000		-		-	-
TOTAL CONTRIBUTIONS	**82,947**		**71,872**		**1,236**	**1,176**

Additional one-off contributions

	2008	2007
	€ 000	€ 000
Germany – to ATC	2,642	4,062
UK (BBSRC & MRC) – to EBI	-	2,778
TOTAL	**2,642**	**6,840**

2008/2009 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 Gene Expression Unit Review Report

Heidelberg · 7 and 8 May 2008

1. EMBL is generally fortunate in regularly being able to rely on the assistance of world-leading scientists who participate in the SAC reviews although they receive no direct benefit from their participation. I however consider that this particular review panel was exceptional. First, as noted in the panel's report, the Gene Expression Unit has developed such that it pursues a considerable breadth of scientific approaches. In spite of this, the panel maintained an excellent balance between reviewing the individual group leaders and the collective achievements of the Unit as a whole, which meant that each panel member had to review both familiar and unfamiliar science. Second, I would like to emphasise the strong participation of SAC members in the review. In this case more than half the reviewers were from SAC. This new tradition of SAC participation has been fostered by the chair of SAC, Paul Nurse, who has participated in almost every Unit review held during his tenure on SAC and is of great value to the Laboratory as it means Units are not seen in isolation, but as part of all of EMBL, and can allow comparison with other EMBL Units. I thank the panel as a whole, particularly its chair, Christine Guthrie, for their excellent work. In addition, I extend my special thanks to the departing SAC Chair Paul Nurse for the commitment to EMBL that has been evident during his time on SAC and in particular for the help and advice he has given me in my first years as Director General.

2. Two of the Unit's group leaders are very recent recruits and are considered highly promising by the panel. It is however notable that all eight group leaders who have been at EMBL during the term of the review (2004-2008) received extremely positive evaluations. This is highly gratifying, particularly given the fact that the Unit has had three different Coordinators during these four years (Mattaj 2004-05; Izaurralde 2005-06; Ellenberg, 2006-08) and clearly, more than is usual for EMBL Unit reviews, the praise offered by the review panel reflects the quality of the efforts made by the individual group leaders. I congratulate them.

3. Jan Ellenberg receives deserved high praise for the quality, breadth and quantity of his scientific production over the review period. He is described as an international leader in his field, the cell biology of the nucleus, and as being of great value to EMBL and European science. His leadership and role in the choice of the last two recruits is also praised. I agree with these positive opinions of Jan and his science.

4. Although not formally part of Gene Expression, the Associate Director, Matthias Hentze, was also reviewed with the Unit because his research area logically fits within the area covered by with Gene Expression. Considering Matthias' many other commitments for EMBL (he is involved in the ATC project and EICAT and has primary responsibility for the Alumni Association, the Endowment Foundation and the EMBL side of the Molecular Medicine Partnership Unit) it is remarkable that the review panel evaluates his research activities as world-leading in both translation regulation and disorders of iron metabolism. I congratulate Matthias and thank him for his efforts on behalf of EMBL.

5. The panel felt that the Unit has reacted in a timely manner to the way science is changing by increasing its scientific breadth and interdisciplinarity. It was felt that this had been achieved without the Unit becoming diffuse. Two potential concerns were however raised. First, the Unit now has one position dedicated to mass spectrometry (that has just been re-filled) and two to chemical biology, including the most recent recruit present at the review. It was felt that, since these groups all link to several Units, it might be advantageous if one of the chemistry groups were administratively associated with another Unit, in order to free up a position in Gene Expression. Second, it was noted that the Unit had been, and at present remains, very strong in studies that aim to provide a mechanistic explanation of biological phenomena on the biochemical level. The panel urged EMBL not to lose its commitment to this area. We will act on this advice.

6. The panel notes the large investments in equipment, personnel and computational infrastructure that will be needed to maintain EMBL's position in pursuing high throughput studies. Recent technical advances mean that EMBL will need to divert more resource to this area. We note the concerns of the panel.

7. Finally, the minor but justified complaints of the PhD students and postdoctoral fellows concerning transport (a common issue at virtually all EMBL sites) and access to food during weekends and holidays will be discussed with the Administrative Director and Heads of Units.

Iain W. Mattaj
Director General
3 June 2008

Director General's Response to the EMBL-EBI Research Activities Review Report

Hinxton · 24 and 25 February 2009

1. This review report must be seen in its historical context. Research at the EBI had to be built up very gradually because of the difficulties in funding that marked much of the EBI' s first decade. Both the actions of EMBL Council in partly stabilising the funding situation with its agreement to the present Indicative Scheme and tireless efforts of the senior EBI staff in generating external funding, particularly for the EBI' s service activities, were highly appreciated by the panel. I am happy to join in this praise. The effect has been to allow the number of research groups at the EBI to grow from three at the last review to the current number of eight. Considering that several (currently six) of the "service" teams also spend some time on research and/or research and development activity the research aspect of the EBI is now, in my opinion, approaching a healthy steady state.

   However, as noted by the panel the future situation of EMBL-EBI is not assured. The flood of biomedical data production will continue to strain the EBI' s ability to cope, both technically and financially. Much further effort by EMBL, EMBL Council, external funders and member states involved in the ESFRI ELIXIR Project will be required.

2. The panel provides high praise for the EBI Director Janet Thornton who, apart from managing her other heavy responsibilities, has led an internationally top-class research group and supervised, with help from Nick Goldman, the build-up of a substantial, diverse and strong set of research groups and teams. I join the panel in congratulating Janet and in their admiration of her leadership.

3. Several of the other research group leaders were recognised by the panel for their achievements during the review period, notably Nick Goldman, Nicolas Le Novère and Wolfgang Huber. In addition, team leaders who excelled in combining research with heavy service responsibilities, Ewan Birney and Rolf Apweiler, received praise. The panel's view is that many of the EBI' s research activities have achieved international standing and recognition.

4. The panel noted the considerable increase in collaborative interactions of the EBI groups both locally with the Sanger Institute and Cambridge University with other EMBL Units and internationally. Given the EBI' s central role in European biomedical research, I am very pleased by these developments. In the EMBL context, I find that these interactions are having a very positive effect on a broad variety of research activities.

5. Nevertheless, the panel notes that some EBI activities would benefit from even more interaction with "wet biologists". We will intensify our mentoring efforts in this and other areas.

6. The panel comments positively on the strong interaction between the service and research activities at the EBI and points out that the co-location with the world's biggest collection of major life science data resources provides bioinformatics researchers with a unique opportunity.

7. The panel was happy to note that the PhD students are very content and I join them in commending both Nick Goldman for his efforts as graduate student mentor and the many people who, under the leadership of Lars Steinmetz, were responsible for the redesign of the introductory course for EMBL's PhD students.

8. The panel noted that the "postdoctoral fellows" at the EBI are rather diverse, ranging from fellows who have external project funding and work full time on research to staff members with a doctorate who carry out research for a proportion of their time. Their advice, which EMBL will act on, is that we should avoid confusion and the raising of false expectations in this group by providing more restricted definitions of these diverse categories.

Iain W. Mattaj
Director General
28 April 2009

Director General's Response to the EMBL Grenoble Review Report

Grenoble · 17 and 18 March 2009

1. I thank the review panel for their considerable efforts and attention to detail in evaluating the activities of the Grenoble Outstation in research, technology development and service provision to users. I am gratified by their very positive overall evaluation of the efforts of the EMBL Grenoble staff in all these areas.

2. No fewer than five of the nine group and team leaders under review were appointed during the four years since the last review of EMBL Grenoble. Although such periods of turnover are not without risk, the panel notes that the opportunity to redirect the activities of the Outstation towards tackling complex, ambitious problems in structural biology using new technologies has been well used by the Outstation leadership.

3. The panel praises the Head of Outstation, Stephen Cusack, for his major leadership role in redefining the Outstation direction, for his outstanding scientific productivity and for the critical part he played in the realisation of the Partnership for Structural Biology in Grenoble, which has both broadened and deepened collaboration between the various local institutions engaged in diverse aspects of structural biology. The panel points out that this collaboration has allowed the development of critical mass and shared platforms benefitting the whole local community. I congratulate Stephen Cusack on this very positive review.

4. The panel commends the excellent demand-driven beamline endstation technology developments realised under the creative leadership of Florent Cipriani. I share their very positive opinion of Florent's many contributions to improving the design and functionality of the beamlines at ESRF.

5. The panel notes the much-improved level of interaction and collaboration between EMBL Grenoble and EMBL Hamburg. I thank everyone involved in this very positive development at both Outstations and in particular the leadership of Stephen Cusack and Matthias Wilmanns in promoting joint activities.

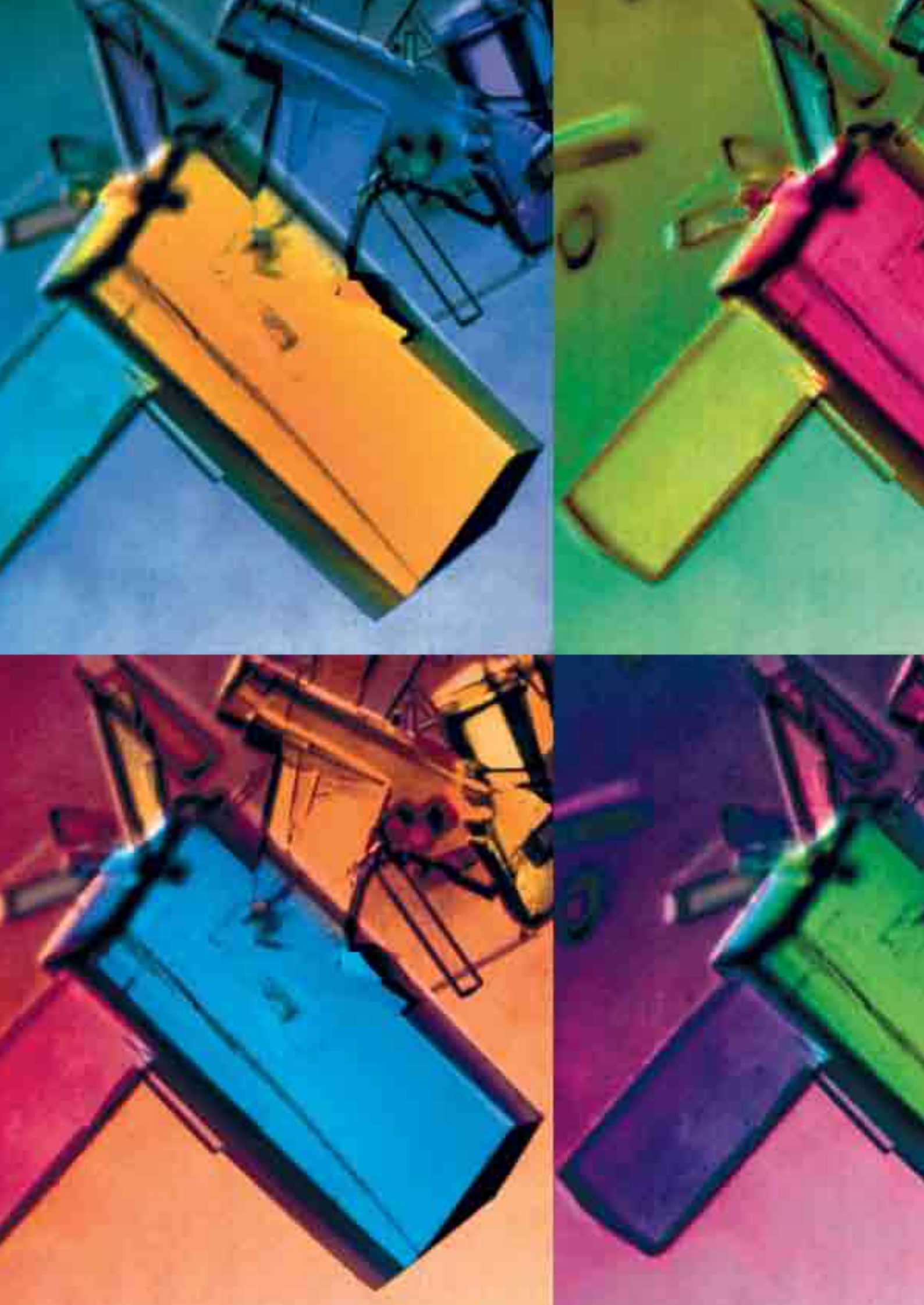
6. The panel also notes the high level of collaboration of the Outstation groups and teams with others in Grenoble as well as the maintenance of a good level of interaction with EMBL Heidelberg, EMBL Monterotondo, and EMBL – EBI.

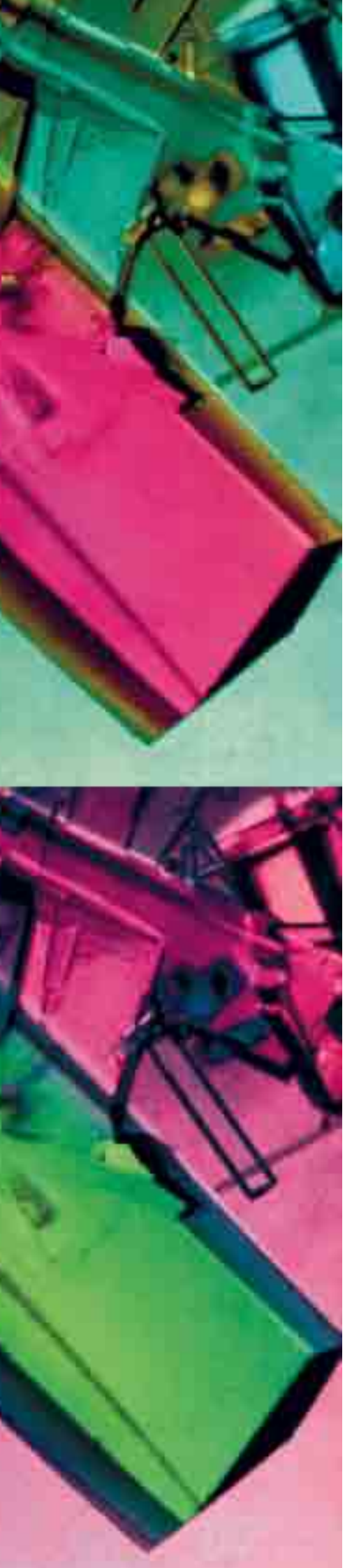
7. The panel is positive about the broadening of research themes at the EMBL Grenoble Outstation to encompass cell biology but recommends that recruitment in the near future should focus on the unique environment in Grenoble for structural biology research and methods development, in particular in relation to optimal exploitation of the available environment with the synchrotron and strength in physics. It is our intention to follow this advice.

8. The other main recommendation of the panel is that EMBL Grenoble continues to develop new beamlines and structural biology experimental pipelines in close collaboration with ESRF. It is our intention to continue our present policy of working closely together with ESRF in these areas.

Iain W. Mattaj
Director General
11 May 2009

Scientific Report





The chemistry of life

A little over a century ago, German embryologist Hans Driesch was struggling to interpret his experiments. At the time, the prevailing explanation of life held that organisms were simply machines. But Driesch had split an early sea urchin embryo into its constituent cells, and found that each cell could form an entire sea urchin, rather than fractions of a whole as he had expected. Convinced that no machine was capable of such a feat, Driesch rejected the idea that life could be explained in terms of physics and chemistry. Some kind of vital force, he thought, made living things distinct from lifeless matter.

This idea, called vitalism, was later disproved by biochemists and biologists. The information needed to create a living organism can be stored in the arrangement and behaviour of its molecules and atoms – in the chemistry of a cell. Discoveries such as these paved the way for a revolution in our understanding of the detailed molecular mechanisms that make life possible.

Today, chemistry plays a central role in our understanding of biology. It helps explain many aspects of life, such as how DNA encodes instructions for building proteins, or how signals are relayed between the neurons in our brains. This year, for example, a team at EMBL has shown that unusually low levels of a signalling molecule, serotonin, in an area of the brain called the brain stem might help explain cot death. Another group has uncovered a clue about how cells make a chemical called polyphosphate, which could shed new light on our understanding of basic biology.

Work like this also helps scientists design drugs to treat disease. Recent work at EMBL has yielded useful insights into how a key influenza enzyme works, and how charting the shared side-effects of existing drugs can help predict their mode of action. Recognising the importance of integrating chemistry with biology and bioinformatics, EMBL-EBI has acquired a new chemogenomics database, which promises to transform the pace and power of drug research.

Had he been alive today, Driesch may have been gratified to learn that researchers are now realising that organisms are much more than just the sum of their chemical parts. This field, called systems biology, aims to discover how the properties of life emerge from the synergy of all these molecular interactions. EMBL has recently launched new platforms and technologies for structural biology and protein expression to help scientists understand how these complex interactions take place. While it won't unveil a mysterious life force, such work will help reveal what brings intricate biological machines such as you or I alive.

Group leader Cornelius Gross with PhD students Rosa Chiara Paolicelli and Apar Jain

An unexpected turn

It is the nightmare of all parents. No warning signs, no conspicuous behaviour; nothing to indicate that their baby is at risk. Yet, in Europe every year, several hundred parents lose their children to a condition still so ill-defined that it is referred to by its only obvious symptom: sudden infant death syndrome (SIDS). Colloquially called cot or crib death, the syndrome affects 1 in 1000 children and is the most common killer of babies between a month and a year old in the developed world.

The sudden death strikes mostly at night, when babies are sound asleep. It approaches without warning and leaves without a trace, not giving doctors and scientists much to work with. That is why SIDS is still very poorly understood. Everything scientists know is based on post-mortem studies, which offer insights into anatomical abnormalities but say little about the physiological events that caused the death. One reoccurring finding of such studies are abnormalities in a specific population of nerve cells in the brainstem of SIDS victims. The brainstem is the lower part of the brain that forms the link to the spinal cord. Through this connection with the body, it coordinates many fundamental processes including cardiovascular and respiratory systems. Some of the nerve cells found in the brainstem communicate using serotonin – a signalling molecule with many different functions which is involved in psychopathologies such as mood disorders, aggression and anxiety. It is its role in the latter condition that got Cornelius Gross, group leader at EMBL Monterotondo's Mouse Biology Unit, interested in the messenger molecule. Cornelius and his team genetically altered the serotonin system of mice to see which effects it had on aggression and anxiety. "But sometimes science takes unexpected turns and suddenly you find yourself exploring something totally different than what you set out to do," says Cornelius.

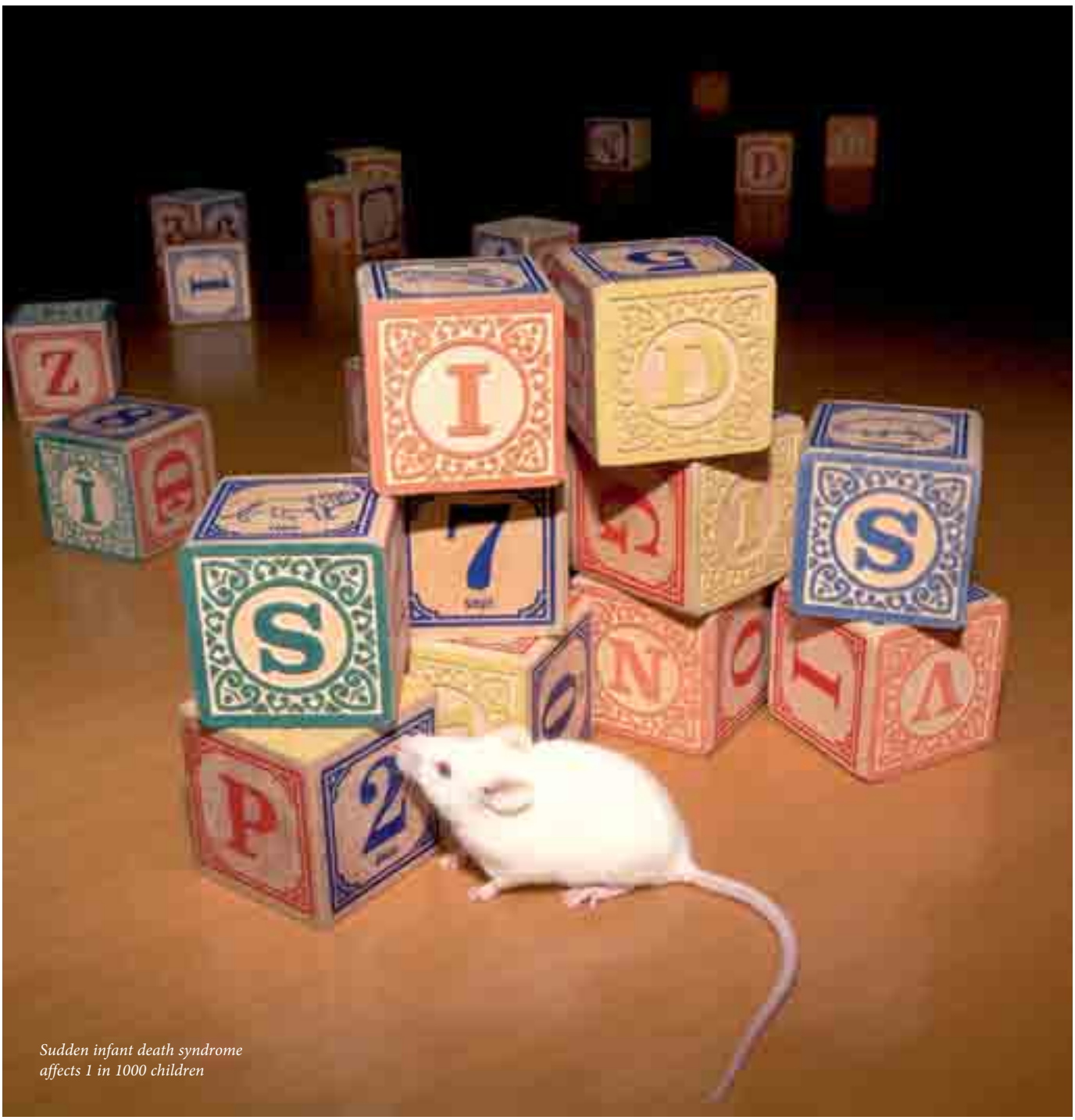
Together with postdoc Enrica Audero, Cornelius engineered mice to express more than the normal level of a particular serotonin receptor in the brainstem. The receptor, called serotonin receptor 1A, serves as a kind of thermostat for serotonin release. It sits in the membrane of serotonin-secreting cells and, when activated by serotonin, triggers a chain of events inside the cell to decrease any further release of the chemical messenger. This 'negative feedback loop' ensures that not too much of the signalling molecule is around at any time. For the mice engineered to have more of the autoreceptor, this means that the serotonin signals in their brainstem are dampened down compared with the signal levels in normal mice. Cornelius and his team expected this intervention to change the anxiety and aggressive behaviour of the animals. But much to their surprise, most of the animals died suddenly. "Most of the animals did not make it past two months of age. At first we tried to ignore the death because really we were interested in the behaviour. But when a colleague at a conference pointed out that we might have a model of SIDS, we started looking at it in a different way," explains Cornelius.

At first sight the animals grew, developed and behaved normally. However, before reaching adulthood, seven out of ten died suddenly and inexplicably. If they made it past a critical point of four months, however, they were safe and had a normal lifespan. "It was then that we became convinced that we might be looking at something relevant to SIDS, because crib death is also restricted to an early period of life," Cornelius says.

This finding led the researchers to look into the cause of the sudden deaths. They monitored the mice closely over the crit-

ical period from week three, which corresponds to human birth, to the onset of adulthood, using radiotelemetric implants that allowed them to monitor heart activity and body temperature without disturbing the mice. When studying the data, Enrica observed something very striking. Some of the animals showed dramatic and sudden drops in body temperature and heart rate. "We are talking about substantial differences here. The body temperature suddenly fell by more than 10 degrees from a normal 37°C to room temperature. Also the heartbeat sank from 600 beats per minute to only 300. This is something you never see in normal animals. The mice must have major deficits in controlling basic body functions," Enrica says. And so it came as no surprise that most mice eventually died, often after suffering several of these crises.

How a comparatively small defect in serotonin signalling could have such a drastic effect becomes clear by looking at the location and connectivity of the serotonin neurons in the brainstem. They are wired up to control many basic body functions through connections with spinal cord control centres of the so-called autonomic nervous system. The autonomic nervous system is a control system that establishes a



Sudden infant death syndrome affects 1 in 1000 children

EMBL Monterotondo

balance between all vital body functions. Among others, autonomous nerves innervate the heart, respiratory organs and tissues involved in temperature regulation. Scientists have proposed in the past that imbalances in serotonin signalling in the brainstem could upset feedback from the brain to these crucial body systems, but so far no one had shown that this system can suddenly go wrong – as appeared to be happening in these mice.

To test whether mis-regulation of the serotonin feedback could be the reason for the sudden death of the mice, Enrica observed their behaviour in a cold room. Normal mice would generate heat by activating brown fat tissue under these conditions, but the modified mice were unable to do so. Activation of brown fat tissue is one of the proposed targets of the serotonin feedback, suggesting it is indeed this mechanism that is affected in the mice.

Could something similar be causing death in SIDS babies? According to Cornelius it is unlikely that SIDS victims are dying by exactly the same mechanism. For example, the brains of babies who have died from SIDS seem to have fewer, rather than too many, copies of serotonin receptor 1A. But several other changes in the serotonin system – such as a failure of serotonin cells to mature properly – have been observed. So, sporadic failure to maintain normal serotonin feedback of autonomic control might still play an important role in SIDS. It could be caused genetically like in the mouse, or by environmental influences. The evidence argues for a combination of both. In the past, factors such as the baby's sleeping position and the smoking habits of parents have been found to affect the risk of SIDS, and putting newborns to sleep on their backs instead of their bellies has reduced the number of sudden deaths. While the EMBL scientists have not yet identified any environmental factors that accelerate death in their mouse model, they consider it likely that external triggers could act on a genetic predisposition and boost the serotonin system. One possible trigger could be sleep and Cornelius' lab is currently investigating if there is any unusual relationship between sleep and death in their mice.

"This research is a great example of how chance observations, often initially resisted by the researcher, can lead to important new findings," says Cornelius. He now hopes the model will provide a better understanding of the role of serotonin in autonomic feedback. Why does this system seem to be particularly important during a brief developmental period? Can diet affect the risk for death and potentially be used to decrease risk for SIDS? Are there signs in newborn mice that might indicate which mice will die later? "We hope that in future our model will help identify risk factors and give new ideas to doctors about how to diagnose babies at risk for SIDS," says Enrica. And Cornelius already has some more specific ideas as to how the model could push clinical research in new directions. "Traditionally the field has focused strongly on respiratory patterns as markers for SIDS. Our findings suggest that body temperature and heart rate might be equally important to study and informative for diagnosis."

The new mouse model opens new avenues for SIDS research, but Cornelius will leave it up to other scientists to explore them. "This is where we hand over work to clinical researchers with a lot more expertise in the field. It would be wonderful if other people could put the model to good medical use." For Cornelius himself, other burning scientific questions are waiting. For example, questions about anxiety and aggression – after all, the problems he set out to investigate in the first place are still not solved. But here he also has an interesting new lead: those mice that survive the increased SIDS risk are much more aggressive.

Audero E, Coppi E, Mlinar B, Rossetti T, Caprioli A, Banchaabouchi MA, Corradetti, R, Gross C (2008) Sporadic autonomic dysregulation and death associated with excessive serotonin autoinhibition. *Science* **321**: 130-3

Esther Lenherr, Andreas Ladurner, Jeannette Seiler and Klaus Scheffzek and from afar Michael Hothorn, Vladimir Rybin and Andreas Mayer.

Finding the missing link

It was Christmas time, 1938, and the fishing boat *Nerine* had just returned to port after trawling the Chalumna River in South Africa. Hauling their catch on board, the crew found they had captured an enormous blue fish with bizarrely fleshy fins. Only later, when a naturalist saw the catch, did they realise they had reeled in one of the living dead from the deep: a fish believed to have gone extinct millions of years before, a living fossil. A fossil, what's more, that was alive and well and playing its role in the local ecosystem.

This fish, called a coelacanth or *Latimeria chalumnae*, is one of a set of rare examples of relics from the past that have suddenly revealed themselves to biologists. Now Klaus Scheffzek and his team at EMBL Heidelberg in collaboration with the groups of Andreas Mayer (Département de Biochimie at the Université de Lausanne, Switzerland), Andreas Ladurner (EMBL Heidelberg) and Christian Herrmann (Ruhr University Bochum) have uncovered an intriguing new lead in another blast from the past. The relic in this case is not a fish or a fossil, but a molecule called polyphosphate that is found in almost every cell. Once overlooked as an evolutionary vestige of the pre-biotic world, it is now becoming clear that polyphosphate plays a key role in a vast range of cellular processes, from the survival and virulence of disease-causing bacteria to the proliferation of breast cancer cells.

Polyphosphate is, as its name suggests, a long string of phosphate molecules, linked together as a polymer. It exists in all species tested to date and is involved in many different aspects of cell metabolism. These findings, together with various peculiarities of polyphosphate's chemistry, have led biologists to suggest that the molecule's role in biology is an ancient one, possibly predating the emergence of cellular life. Its long, flexible structure and pattern of electrical charge could have acted as a scaffold for other large molecules to orient themselves or assemble together. Given its widespread occurrence in cells today (up to 20 per cent of a yeast cell's dry weight can consist of stored polyphosphate), biologists are now taking a keen interest in finding out more about it.

Most of our understanding of what polyphosphate does and how it is made has come from studies of bacterial, or prokaryotic, cells. Biologists had identified an enzyme called polyP kinase 1, or PPK1, that was responsible for making polyphosphate in many bacteria. Unfortunately, no matter how hard scientists had looked, they had been unable to find an equivalent enzyme in more complex, eukaryotic organisms, such as yeast, plants, algae and multi-cellular animals such as humans. Now, Klaus, his team and their colleagues have discovered the first polyphosphate-synthesising enzyme in eukaryotic cells, a bold step forward that should help researchers start to explore the role of these molecules in the biology of complex organisms.

Klaus and his lab weren't actually looking for the enzyme at first. Instead, they were investigating the structure of the so-called VTC (vacuolar transporter chaperone) protein complex found in the membranes surrounding the storage depots of a yeast cell, its vacuoles. Biochemical studies in the group of Andreas Mayer, who has been studying these proteins for quite a while, had suggested

LOOKING INTO PROTEINS

Crystals are sets of objects – such as molecules or atoms – that are arranged in an orderly fashion in three dimensions. That orderly arrangement is at the core of the defining property of crystals: periodicity, which means that the objects that make up a crystal are located at regular intervals. X-ray crystallography is a method of determining how those molecules or atoms are arranged within a particular crystal, by exposing the crystal to X-rays and studying how it scatters them.

When X-rays strike the atoms within a crystal, they make those atoms jitter. This jittering eventually leads to each atom emitting X-rays with the same energy in all directions, in a process called elastic scattering. The waves travelling outward from each atom interfere with those coming from other atoms, reinforcing or cancelling each other out depending on the angle at which they meet. The resulting pattern of diffracted waves, called a diffraction pattern (see image on page 29), can be recorded by placing a detector in front of the crystal. Every substance has its own unique diffraction pattern, depending on the identity and the position of the atoms within its crystals, so by analysing that pattern scientists can determine what atoms make up a particular crystal, and how they are positioned in relation to each other.

This is the principle behind X-ray crystallography: scientists expose a crystal to an X-ray beam, and record the angles and intensities of the X-rays that emanate from it. This allows them to produce a 3-Dimensional model of the arrangement of atoms within the crystal. Finally, they use computer software to refine that model: the software calculates the diffraction pattern which the model structure would generate, compares it to the one scientists obtained in their experiments, and alters the model until it fits those observations.

This technique was initially used to study minerals, but since it was first employed to determine – or `solve' – the structure of a protein in the late 1950s, it has become a regular part of the molecular biologist's toolkit. To date, more than 50,000 protein structures have been solved through X-ray crystallography, and, with techniques for producing and analysing protein crystals being constantly enhanced (see pages 14 and 27), that number is steadily increasing.

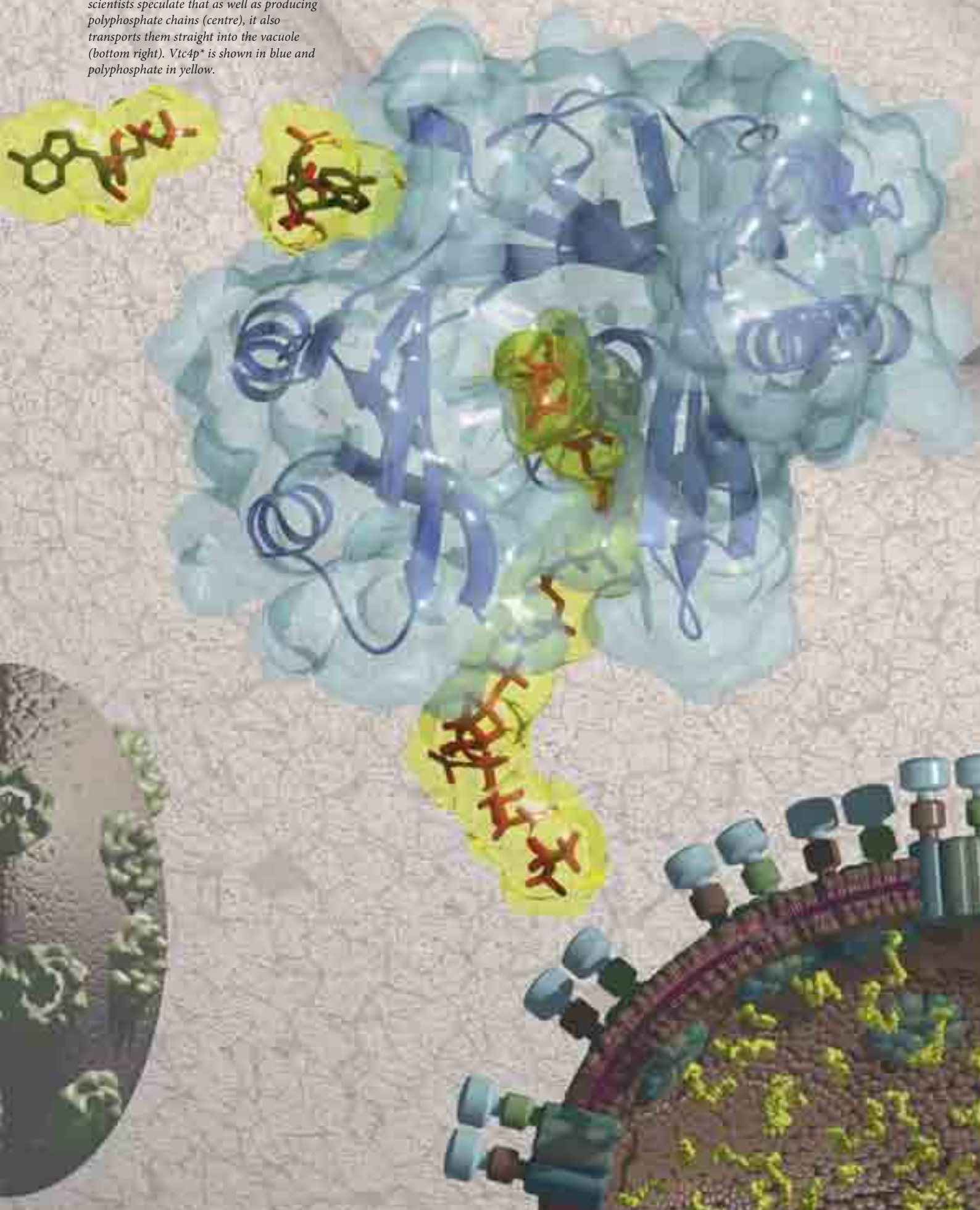
that the complex might be involved in some aspect of lipid metabolism. To find out more, they suggested looking at the structure in order to uncover some clues about the function of this complex. One of Klaus' predocs, Michael Hothorn (currently at Salk Institute for Biological Studies) crystallised one of the components of the complex, a protein called Vtc4p, together with manganese, a metal needed for enzyme function, and adenosine triphosphate (ATP), a compound that functions as the cell's energy source, which the Mayer group had shown binds the protein.

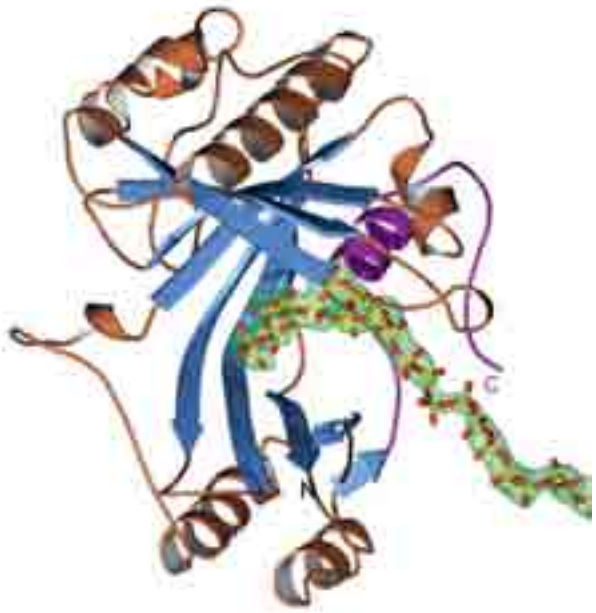
The team expected to see the protein simply bound to ATP. Instead, they saw that its tunnel-like structure contained a long chain of phosphate molecules. This immediately suggested that Vtc4p had somehow taken some phosphate molecules from ATP and linked them all together to form the polyphosphate polymer. Indeed, inspired by this key observation, the group of Andreas Ladurner at EMBL Heidelberg was able to demonstrate that Vtc4p and ATP readily generate polyphosphate not just in the crystal but also in solution. "A couple of pennies dropped," recalls Klaus. Could this be the discovery of the first-ever eukaryotic polyphosphate-making enzyme? "At the beginning of the project, this idea was just not on our radar," says Klaus, who has been working on enzymatic phosphotransfer in signalling for several years. "Solving the structure gave such a twist to the story that we were now directly looking at polyphosphate metabolism."

To find out more, the team started a detailed biochemical analysis involving the biophysical expertise of Vladimir Rybin from EMBL's Protein Expression and Purification Core Facility and of Christian Herrmann's group at Ruhr University Bochum. They altered, or mutated, the amino acids in Vtc4p that made contact with the polyphosphate, and monitored the resulting protein's consumption of ATP to see whether the mutations had any effect on its ability to form the polymer. Sure enough, mutating two of the amino acids that contact two of the three phosphate components of ATP stopped the enzyme from working in the test tube, thus giving insight into how this enzyme carries out its remarkable job.

To find out how the enzyme operated in living cells, the Mayer group studied yeast cells that had Vtc4p removed. Adding back Vtc4p restored the ability of such cells to grow

Since Vtc4p is part of a protein complex that straddles the membrane of the vacuole, scientists speculate that as well as producing polyphosphate chains (centre), it also transports them straight into the vacuole (bottom right). Vtc4p* is shown in blue and polyphosphate in yellow.*





The 3-D structure of Vtc4p (in brown, purple and blue) forms a tunnel in which polyphosphate (green, red and yellow) is produced.*

in an environment with low levels of phosphate. However, adding back Vtc4p versions in which amino acids that are key for enzyme function were mutated resulted in sickly cells: their growth was stunted in the same way as cells lacking Vtc4p completely, confirming that these amino acids were key to the enzyme's activity. Further structural studies suggest how the enzyme holds a phosphate molecule in its reaction area, or active site, and then catalyses a reaction that allows it to steal a phosphate from an ATP molecule and join it to the phosphate it is already holding. In this way, it may build up a chain of polyphosphate, gradually feeding it down a tunnel in the enzyme as the polymer grows. The mechanism also suggests a way in which the protein might deliver polyphosphate to its final destination inside the yeast cell's vacuole.

But the vacuole is not the only place to which polyphosphate is transported in fungal cells such as yeast. Surprisingly, some of it ends up outside the cell. To find out more, the team labelled proteins known to associate with Vtc4p and studied their behaviour in living cells under the microscope. They found that one combination of proteins localises to the membrane surrounding the vacuole, while another, different, combination is found in the plasma membrane, the membrane that surrounds the whole cell.

This finding is rather intriguing, as it suggests a basis for a symbiotic function of polyphosphate and has relevance in modern agriculture. Although Vtc4p isn't present in plants, it is present in certain fungi that associate with plant roots. Plants need polyphosphate to grow, and Vtc4p in the fungi could thus play an important role in making it available to plants, by shuttling it from their vacuoles through the plasma membrane into the space surrounding the plant's roots. Understanding more about this process could pave the way for new fertilisers and high-yield crops.

The work has implications for human health too. Scientists already knew that a number of disease-causing bacteria lose their virulence if they lose their ability to make polyphosphate, making the PPK1 enzyme a possible drug target. Now they can also turn their focus on eukaryotic parasites that cause diseases such as sleeping sickness and Chagas disease, and see whether disabling their Vtc4p enzyme could offer new avenues for treatment.

For Klaus, one of the most satisfying aspects of this entire project was how structural biology almost serendipitously led to the discovery of the enzyme's function. Classically, biochemical or genetic studies of a protein or gene uncover or strongly hint at its workings, only to be explored later by structural studies. "This study emphasises the importance of structural biology to show not just what molecules look like and how they work but also what that function is," says Michael.

This is not the end of this explorer's tale, of course. "There are still a lot of things to explore," says Klaus. Of the many studies that are now possible, immediate questions concern how the enzyme gets hold of its starting phosphate molecule, and the roles of the subunits that associate with Vtc4p. In addition, the features of a potentially novel polymer translocating channel need to be explored. And, given that Vtc4p doesn't seem to exist in animals or plants, but humans walk around with polyphosphate stores in our cells, there must be other polyphosphate-synthesising enzymes waiting to be discovered. The coelacanth may have been a unique discovery. For Klaus and his collaborators, at least, it seems there are plenty more fish in the sea.

Hothorn M, Neumann H, Lenherr E, Wehner M, Rybin V, Hassa P, Uttenweiler A, Reinhardt M, Schmidt A, Seiler J, Ladurner A, Herrmann C, Scheffzek K, Mayer A (2009) Catalytic Core of a Membrane-Associated Eukaryotic Polyphosphate Polymerase. *Science* **324**: 513-516

Clues in disguise

The side effects listed on many medicine's leaflets may hold clues to new cures.

Most of us tend to think of side effects as the downside of medicines: a nuisance and a source of concern. Researchers at EMBL Heidelberg saw them as a resource: clues to other conditions that the drug might treat. They were not the first to consider the good side of side effects. The drug marketed as Viagra®, for example, was initially developed to treat angina, but its side effect of prolonged penile erection led to a change in its therapeutic use. The EMBL scientists have gone a step further. They have been able to predict which drugs may have additional, previously unknown therapeutic uses and what those uses may be.

Side effects are most commonly caused by a drug interacting with a protein other than the one it was intended to target – a so-called additional target. Drugs that are similar, or target similar molecules, are likely to interact with the same additional targets, thereby causing similar side effects. But drugs that are dissimilar or target dissimilar molecules can also have similar side effects. This implies that such drugs, which are often used to treat entirely different conditions, may in fact target the same protein. "Such a correlation not only reveals the molecular basis of many side effects, but also bears a powerful therapeutic potential. It hints at new uses of marketed drugs in the treatment of diseases they were not specifically developed for," says Peer Bork, Joint Head of EMBL's Structural and Computational Biology Unit.

With this in mind, his group collaborated with Anne-Claude Gavin's team to develop a computational method that predicts the likelihood of two drugs acting on the same molecule, by comparing their side effects. This method allows scientists not only to predict which drugs may have additional targets, but also what those targets are likely to be. And it is self-improving: the more targets that are known, the more accurate the predictions can be.

The scientists used this new method to compare over 700 drugs already available in the market, based on the side effects listed in their information leaflets. This gave them a shortlist of drugs which were likely to have additional, unexpected targets. Researchers subsequently tested nine of those drugs in cellular assays, and they all bound to the additional targets the scientists had predicted, producing the desired effect on the cell. For instance, donepezil, a brain enhancer used to treat Alzheimer disease, was proven to share a target with the anti-depressant venlafaxine, indicating that donepezil could also be used to treat depression.

According to Peer, "with some more tests and refinement, our method could in future be applied on a bigger scale. New drugs could routinely be checked in the computer for additional hidden targets and potential use in different therapeutic areas." And, since marketed drugs have already been tested and approved for safe use in patients, "this will save a lot of money and would speed up drug development tremendously," he concludes.

Campillos M, Kuhn M, Gavin A-C, Jensen LJ, Bork P (2008) Drug target identification using side-effect similarity. *Science* **321**: 263 -6

Most things in life boil down to mathematics, and biology is no exception. It is certainly the inspiration behind a new EU Framework 7 project that aims to bring state-of-the-art protein preparation techniques to structural biologists in Europe. Dubbed 'PCUBE', the project's name stands for Protein Production Platforms (PPP, or P3) and conveys a key attribute of the project: by combining state-of-the-art technologies together under one umbrella, it will multiply their impact on the international research community.

PCUBE is an international effort, involving groups at EMBL Heidelberg, Grenoble and Hamburg, together with scientists at Oxford and Zurich, with the latter coordinating the programme. The initiative started on 1 April 2009 and has €6 million of funding for four years. Although EMBL is not coordinating PCUBE, its scientists and the unique technologies they have

The power of three

Express yourself

The new frontier of structural biology is now about determining the structures of protein complexes, rather than just looking at individual proteins. Thanks to two new technologies pioneered by Imre Berger's group at EMBL Grenoble, this has now become a whole lot easier. The first innovation is MultiBac, an advanced eukaryotic expression system based on a designer baculovirus, which lets researchers easily create constructs and produce their proteins in large quantity by using insect cell cultures. The second innovation is ACEMBL, the first fully automated pipeline for rapid protein complex production and engineering (see page 27). ACEMBL is still being tested, but Imre plans to bring it online later in the PCUBE programme.

Picking the right pieces

The first port of call for many researchers grappling with tricky proteins will be Darren Hart's lab in EMBL Grenoble. One of the main stumbling blocks for structural biologists is producing their protein fragments in a suitable soluble form for making crystals. ESPRIT is a high-throughput, automated system that allows researchers to quickly and accurately identify the soluble fragments they need.

"When I first came up with the technology, a few people were brave enough to come and use it," recalls Darren. Now that a number of successful outcomes have been published, more and more biologists want to use ESPRIT and set up collaborations with him. The major limitation to high throughput is resources, which is where PCUBE will help.

"What we have with PCUBE is a series of methods to address difficult projects," explains Darren. His team plans to use ESPRIT in projects that have been defeated by applying classical methods. Applications to use the platform will be considered by a transnational access board, which will review prospective projects in a transparent way. Once selected, a project will be fully funded to cover the costs of researchers' visits, materials and so on.

developed are playing a major role. These include Darren Hart's ESPRIT system for finding soluble protein fragments, Imre Berger's protein expression systems, the high-throughput crystallisation facilities at Hamburg, coordinated by Jochen Müller-Dieckmann and Grenoble by José Márquez, expertise of the advanced light microscopy platform run by Rainer Pepperkok and the protein expression and purification facility of Hüseyin Besir at EMBL Heidelberg.

Broadening access, however, is just one of three main threads to the programme. The second is to provide training, networking and on-site expertise to users, while the third supports joint research activities between the groups. The aim is to develop new methods that will improve the scientific scope, capacity, throughput and robustness of the platforms.

Crystal clear

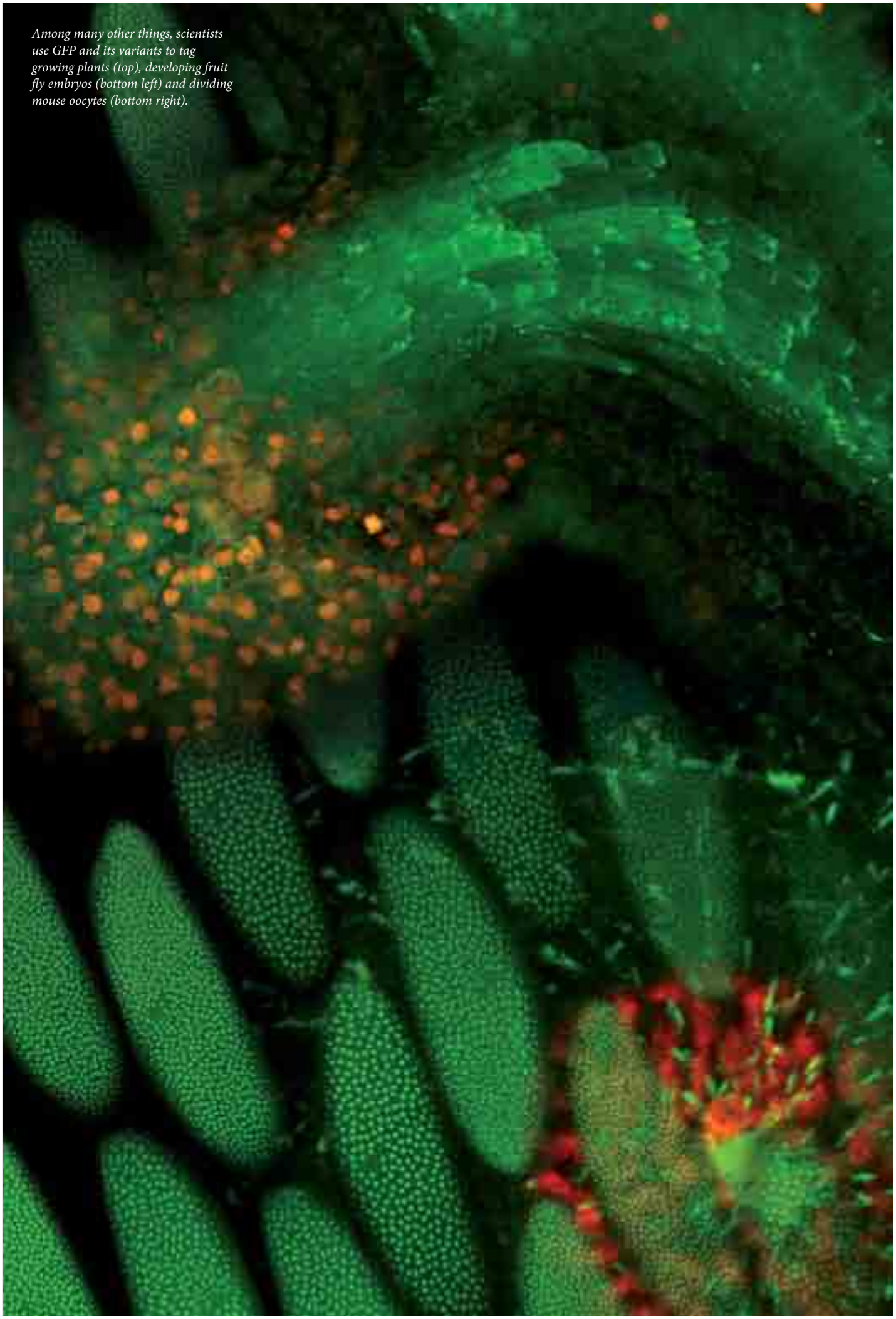
Even if you have soluble protein or protein fragments, obtaining high-quality crystals is no easy task. This is where EMBL's high-throughput crystallisation platforms in Hamburg and Grenoble can save the day. Thanks to the automated setup of these platforms, researchers can rapidly screen hundreds of different protein fragments to find those that make good crystals far more quickly and easily than they could ever do by hand.

Jochen Müller-Dieckmann and his team at EMBL Hamburg already have extensive experience of running their platform for the benefit of the international scientific community. They will be using the research component of PCUBE to develop new technologies that will allow users to screen the fragments produced by ESPRIT for their ability to crystallise. Jochen hopes to deploy microfluidics chips: glass slides containing many tiny wells that hold a drop of liquid, each containing a different soluble protein fragment. Multiple constructs can be rapidly screened in parallel for their suitability in crystallisation experiments using very small amounts of sample. To save even more time, researchers would run this directly after their ESPRIT experiments, selecting validated constructs before proceeding with tedious scale-up steps, as they do at the moment. José Márquez's equivalent platform at the Grenoble outstation will also increase crystallisation capacity.

The light fantastic

The study of protein complexes is a large trend; another is the merging of cell biology and structural biology. This means that structural biologists are increasingly seeking to determine how proteins behave in living systems, as opposed to the test-tube *in vitro* environment. "Traditionally, many structural biologists have used X-ray crystallography, NMR or single particle electron microscopy," says Christoph Müller, Joint Head of the Structural and Computational Biology Unit at EMBL Heidelberg, who in the PCUBE project coordinates access to the advanced light microscopy and protein expression platforms. "Increasingly, they want to look at proteins in their cellular context."

There are several advantages to using light microscopy techniques to do this, says Christoph. First, biologists are able to study protein dynamics using fluorescently labelled proteins. Second, they can follow proteins or protein complexes in living cells, and third, they can also correlate *in vitro* and *in vivo* findings. As part of the PCUBE project, EMBL scientists can offer visiting scientists advice on how to label their proteins with fluorescent molecules and also provide expert help with data interpretation. As part of the research element of the programme, Christoph's team will test and develop labels, especially adapted for the needs of structural biologists. Christoph also plans, in collaboration with Rainer Pepperkok, Imre Berger and the PCUBE collaborators in Oxford, to develop ways to use fluorescence techniques to monitor the expression of a protein within cells, to optimise the expression systems that biologists use.



Among many other things, scientists use GFP and its variants to tag growing plants (top), developing fruit fly embryos (bottom left) and dividing mouse oocytes (bottom right).

Painting life green

It all started with an agitated jellyfish. The transparent *Aequoria victoria* has spots around its rim that glow green when it is agitated; a behaviour that was first described by scientists in 1955. Studying this 'oddity' led to what has been hailed as a scientific revolution, and, for three scientists, a Nobel Prize. All because of a single protein, called green fluorescent protein (GFP), which is responsible for the jellyfish's fluorescence.

In the early 1960s, Japanese scientist Osamu Shimomura discovered that this jellyfish has a protein which glows in the presence of calcium. He surmised that the protein must be involved in *A. victoria*'s ability to produce light, and named it aequorin after the jellyfish. However, aequorin glows blue, while the jellyfish glows green, so something must transform aequorin's blue light into the jellyfish's green light. Shimomura discovered that something is another protein: GFP, which absorbs blue and ultra-violet light and emits green light. So, in the jellyfish, aequorin emits blue light, and GFP absorbs that blue light and emits green light, giving the jellyfish its glow.

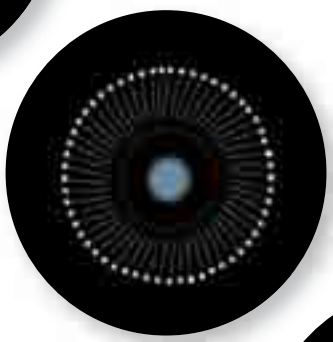
Martin Chalfie, who was working on the roundworm *Caenorhabditis elegans*, realised this jellyfish protein's potential as a biological marker. Chalfie reasoned that model organisms like *C. elegans* could be genetically engineered by attaching the gene for GFP to the specific gene that the scientists were interested in studying. In such a situation, when the gene of interest was expressed, i.e. when the protein it encodes was produced, it would have GFP attached. This would allow scientists to know when and where a particular gene is turned on: they would just have to look for the green glow.

At the time, two things were apparently missing before Chalfie's vision could become a reality. The gene for GFP had to be identified, and the mechanism behind GFP's fluorescence had to be unveiled. Scientists knew that GFP glows because the chain of amino acids comprising it folds into the shape of a cylinder, with three of those amino acids suspended in the middle to form a fluorophore, a chemical group that absorbs and emits light. They assumed that, like most naturally fluorescing molecules known at the time, other proteins called enzymes would be needed to fold GFP into this shape, and thought only *A. victoria* would produce them.

So when Douglas Prasher identified the GFP gene in 1992, the general consensus was that introducing this gene into other organisms would result in the production of a non-fluorescent version of GFP, since such 'receptor' organisms would not have the enzymes required to fold GFP into its fluorescent shape. However, when Chalfie and his team attached the newly found GFP gene to a bacteria's DNA, the bacteria glowed green!

It turns out that, unlike most other naturally fluorescent proteins, GFP doesn't need enzymes to make it glow. Instead, it spontaneously folds into the fluorescent shape, and, biochemist Roger Tsien discovered, the reaction between the amino acids in the fluorophore requires only oxygen, which is readily available in most living cells. Having established exactly how GFP's fluorophore is formed, Tsien was then able to manipulate this protein. By exchanging different amino acids in different parts of the chain, he developed new versions of GFP, which were brighter, absorbed light of different wavelengths, and

GFP was first discovered in the jellyfish Aequoria victoria.



Seen from below, the transparent jellyfish's glowing rim is clearly visible.

GFP's amino acid chain folds into the shape of a drinks can, with the fluorophore at its centre.

GFP in A. victoria's *rim makes it glow under blue and ultra-violet light.*

glowed in different colours: cyan, blue and yellow. And, once a red fluorescent protein was found in coral, Tsien and his colleagues used their knowledge of GFP to make it useable as a biological marker too.

Shimomura, Chalfie and Tsien were awarded the Nobel Prize in Chemistry in 2008, "for the discovery and development of the green fluorescent protein", and scientists all over the world have continued to develop variants of GFP, which are now available in virtually all colours of the rainbow.

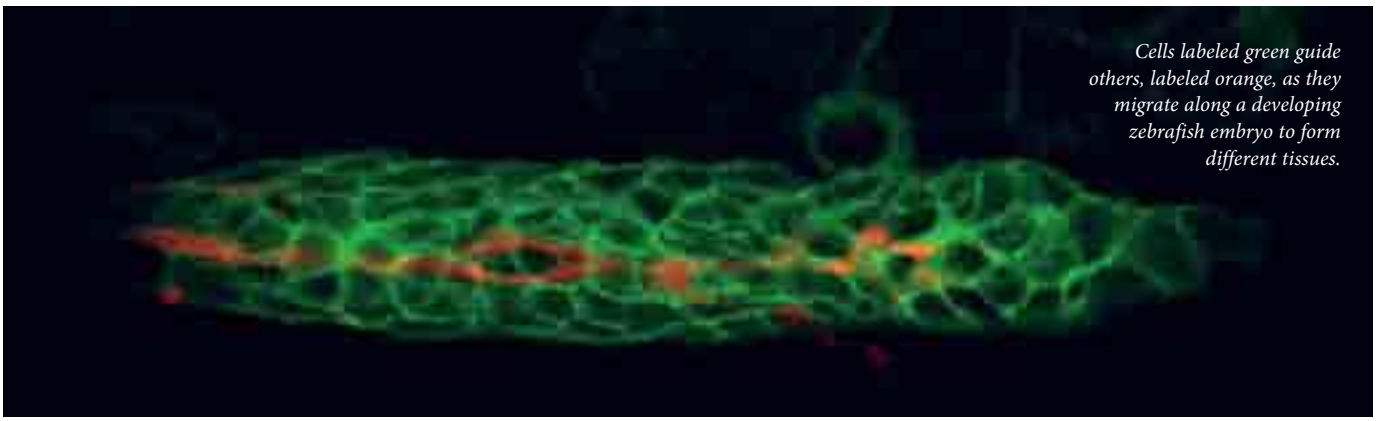
And, over 50 years after *A. victoria*'s green light was first described in a scientific publication, GFP has become an invaluable tool for scientists all over the world, including those at EMBL, many of whom still use it every day. Some even have trouble imagining life without it.

"I'd say almost everyone here at EMBL uses GFP in some way or another," remarks Jan Ellenberg, Joint Head of the Cell Biology and Biophysics Unit. And even though everyone uses it as a marker, by attaching it to a specific protein, different researchers use GFP to study different processes occurring on completely different scales.

"That's the beauty of GFP," says Darren Gilmour, group leader in the Cell Biology and Biophysics Unit. "With it, you can look at all these different scales – you can paint them all with the same paint, you don't need to change brush." In their work on zebrafish embryos, Darren and his group use GFP to tag groups of cells, which they can then follow as the embryo develops, watching how they behave, where they go, and what tissues and organs they ultimately give rise to. The zebrafish embryos that Darren studies are transparent, so you'd think it would be easy to see what was happening in them. The problem, Darren says, is that there is too much happening. "It's an overload. You just can't focus, you can't pick out one thing. But with GFP," he adds, "you can turn out the lights and just focus on a group of cells, or even on a single cell."

Alongside advances in microscopy techniques, the discovery and development of GFP enabled scientists to watch such dynamic processes unfold "in living cells, using a very simple method which people have been using ever since photography was invented: time-lapse imaging," explains Rainer Pepperkok, head of the EMBL Advanced Light Microscopy Facility and team leader in the Cell Biology and Biophysics Unit.

This ability to follow dynamic processes is also crucial to Francesca Peri, group leader in the Developmental Biology Unit, as it allows her to follow the development of her labelled zebrafish embryos under a microscope for days. The alternative would be to sacrifice the animal, slice it and take still pic-

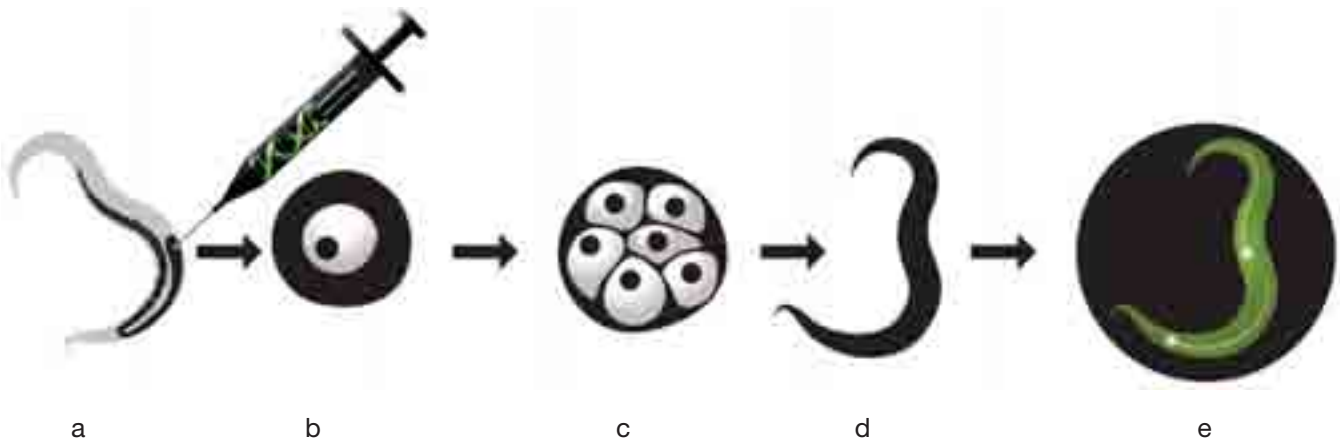


Cells labeled green guide others, labeled orange, as they migrate along a developing zebrafish embryo to form different tissues.

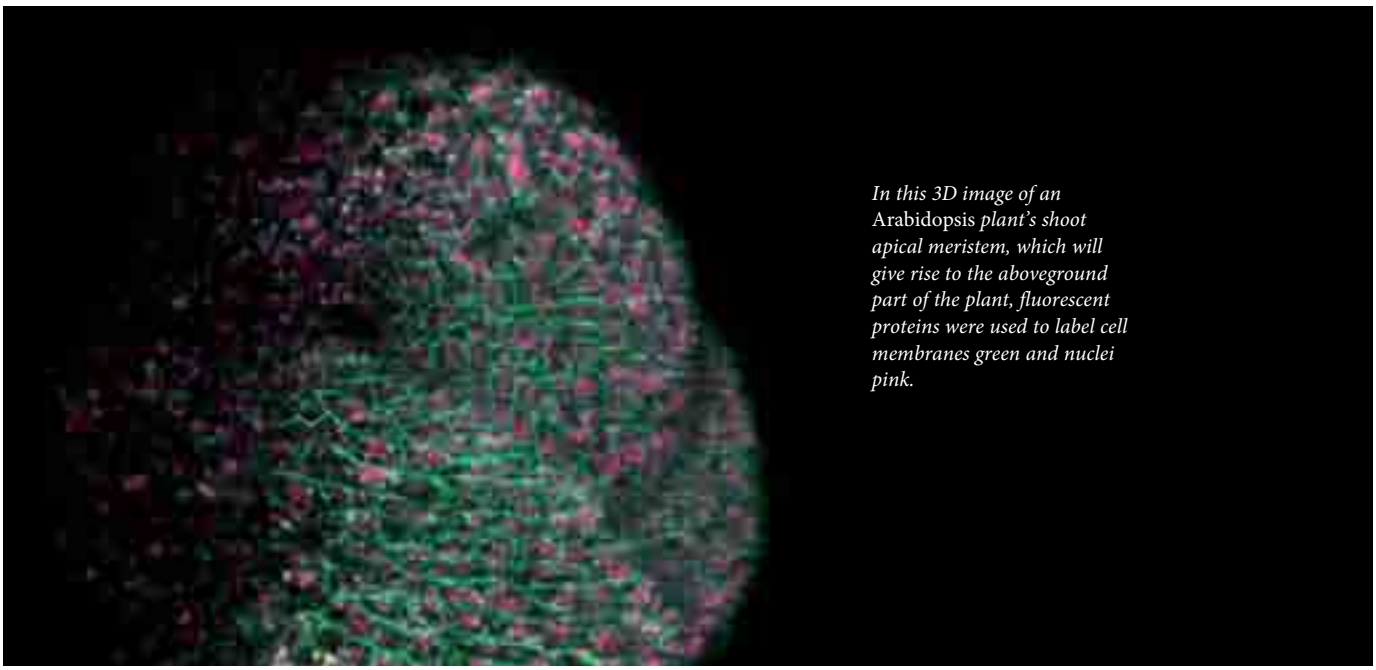
tures. "It would be like trying to understand a football match based on just half a dozen photographs," says Francesca. "You'd never get the whole picture." She and her group study microglia, cells which are able to eat dying or damaged neurons. "Using GFP, we can colour-code the different cell types, so microglia will be labelled green, for example, and neurons red. If we see a red cell inside a green one, we know that a neuron has been eaten, to prevent it damaging the rest of the brain tissue."

Stefano de Renzis uses GFP to study a different embryo, that of the fruit fly *Drosophila*. He and his group in the Developmental Biology Unit use GFP not only as a tag but also to help them establish a timeline. They label the nuclei of all the cells in an embryo, and see when they divide. "This allows us to pick embryos at specific stages – say after 11, 12 and 13 divisions," he says, "and then compare what genes are expressed in early stages and which are expressed later." This gives them an idea of what genes, and consequently what proteins, may be involved in key processes at these different stages. "We can then use GFP to mark these proteins and follow them inside the cells," Stefano concludes.

Another group leader in the Developmental Biology Unit, Marcus Heisler, uses GFP and its variants to study plants. He and his group are interested in how a single tissue, the shoot apical meristem, gives rise to the whole above-ground part of the plant. They focus on a plant hormone called auxin, which is transported to the outside of the cell by a carrier that sits on the cell membrane. This carrier can move around the cell, changing the direction in which it sends the hormone. "GFP allows us to follow that very dynamic process in living plants," Marcus says. He and his group also use GFP to look at where and when different genes are expressed. "In fact," he adds, "one of the key advantages of GFP is that it gives us great spatial and temporal resolution, so we can look at a gene expression pattern, then stimulate the system and see how it changes."



Chalfie's experiment: DNA with the gene for GFP attached was injected into the gonads of a C. elegans *worm (a). The worm is a hermaphrodite, so it can fertilise itself, and the GFP gene was present in many of the eggs it then laid (b). The eggs divided (c), forming new individuals (d) whose touch receptor neurons glowed green in UV light (e).*



In this 3D image of an Arabidopsis *plant's shoot apical meristem, which will give rise to the aboveground part of the plant, fluorescent proteins were used to label cell membranes green and nuclei pink.*

In their work on starfish and mouse oocytes (the cells that will mature into eggs), Jan's group also put GFP to two different uses. They use it to study cellular architecture, by marking different structures within a cell and observing their movements, and also to understand the molecular dynamics of single proteins, which they tag and follow in living cells. "For both our uses," Jan asserts, "there's not yet anything like GFP in terms of ease of use, robustness, reliability and also being tolerated very well."

GFP does much less damage to cells than chemical fluorescent markers do. After being illuminated for a certain period of time, a fluorophore releases an electron, after which it will never fluoresce again: it is bleached. The electrons released in this bleaching process very quickly react with oxygen, forming highly toxic oxygen radicals, which damage cellular components, eventually causing the cells to die. But GFP's structure acts as a shield, protecting the cell. When the fluorophore releases an electron, the radicals that are formed react within GFP, damaging GFP but not the cell. As Jan puts it, "at least it only commits suicide, it doesn't hurt the rest of the cell in the process!"

For Ernst Stelzer, whose group focuses on developing technologies for 3D imaging over time, GFP's reliability is also very important. "Because we work with thick specimens, getting the dye into a specimen is always a very serious problem," he says. Markers injected into the specimens tend not to penetrate very well, so the labelling can be uneven: outer layers will be well labelled, but ones near the middle are apt to be badly labelled, if at all. "With GFP," Ernst points out, "we can be really sure that the whole specimen will be labelled, because the dye is produced inside the cells."

Marko Kaksonen also takes advantage of the fact that, as he puts it, "with GFP the cells do the work for us." By creating and using strains of budding yeast in which the genes for GFP and other fluorescent proteins have been knocked-in, i.e. have been integrated in the yeast's DNA, Marko and his group in the Cell Biology and Biophysics Unit can study how vesicles are formed to bring in materials from outside the cell. "If one protein is marked green, and another is marked red," Marko explains, "and if we watch the sites where vesicles are formed turn green, then yellow, then red, we know the green protein arrives there first, then the red one arrives and the green one disappears." They can compare strains that highlight different pairs of proteins, to establish the sequence in which these proteins act, and they can look at mutants which lack one of the proteins and see how that affects the behaviour of others.

Rainer and his team in the Cell Biology and Biophysics Unit also use GFP to study proteins involved in transporting other molecules, but they focus on transport between certain structures within the cell, in the so-called secretory pathway. They also study how proteins interact with each other. Rainer says, "We can really study molecular biology in cells, while things are moving, instead of in a test tube."

He and other scientists take advantage of the fact that GFP now comes in many different colours, and exploit a physical phenomenon called fluorescent resonance energy transfer (FRET). This phenomenon occurs when two fluorescent molecules of different colours – classically red and green – come close to each other. If the green molecule then receives ultra-violet or blue light, it will absorb and then transfer some of the light's energy to the red molecule, which will then emit red light, in a

manner similar to GFP glowing green in the jellyfish thanks to the blue light emitted by aequorin. However in FRET only energy is transferred, not light. "So if you have a protein tagged with green GFP that interacts with another tagged with red GFP," Rainer explains, "the red will be brighter and the green will be dimmer." These changes in brightness are often not very great, however, so such techniques require state-of-the-art equipment and, Rainer stresses, experience.

Rainer also emphasises GFP's ease of use. "It is so easy," he says, "that you can even automate procedures, like we did in a collaboration with Stefan Wiemann from the German Cancer Research Centre (DKFZ) in Heidelberg." This automated approach allowed them to track thousands of different proteins encoded by newly sequenced human genes, enabling them to be the first to determine where those proteins went and what they did in living cells.

As head of the Flow Cytometry Core Facility, Andrew Riddell also sees GFP on a daily basis. "About 80 per cent of our users use GFP," he says. The facility can help users who have transferred a GFP-tagged gene into another organism to see whether that transfer worked. Andrew's team can rank and sort the cells according to the amount of GFP in them: "What flow cytometers refer to as GTTR – green to the right," he comments. But GFP is also put to more complex uses at the facility, such as quantifying how fast a particular enzyme breaks up an association between two tagged proteins, or tracking exactly which part of an animal's body a white blood cell has ended up in. "And what's more, we can retrieve the cells and return them to the scientists for further experiments," Andrew adds.

Asifa Akhtar and her group in the Gene Expression Unit use other fluorescent proteins to study how male fruit flies compensate for having only one copy of all X chromosome genes. "We stain the chromosomes one colour and proteins that could help regulate this process another, and look to see where they coincide," says Thomas Conrad, a pre-doctoral fellow in the group.

Despite all GFP's different uses, or perhaps because of them, scientists are still not satisfied. A common request would be GFPs that glow under red or even infra-red light, as this penetrates biological tissues better. "It would also expand the spectrum of available colours," Jan points out, "which would allow us to tag more proteins and follow them at once." He thinks it may take a new discovery to make this wish come true, though, as he believes the standard way of making GFPs which glow at longer wavelengths – stacking more amino acids onto the fluorophore – is pretty much exhausted.

Michael Knop, whose group in the Cell Biology and Biophysics Unit has tested many GFPs and even developed GFP variants specifically for their own use, believes that GFP still has room to improve. "There's still a huge potential for developing fluorescent proteins with new spectral properties, for example ones that fluoresce at wavelengths in the near infra-red," he says. "This should be reached in the near future and will enhance *in vivo* imaging, especially in animal models. Also," he adds, "GFP technology and microscopy techniques have driven each other's development, and that will surely continue".

Darren agrees that microscopy techniques now need to catch up. "At the moment, we're at a stage where we could make a zebrafish with five colours, but then we wouldn't be able to tell them apart with most of the available microscopes." As for Marcus, the organisms he studies dictate his request: "I wish there were commercially available plant-optimised versions of GFP," he says. "As it is, we have to get the DNA sequence for the GFP variant we want and then pay another company to produce a version that we can use on plants."

Nevertheless, GFP remains such a valuable tool that Claude Antony, head of the Electron Microscopy Core Facility, wishes he had something like it, as none of the markers currently used in electron microscopy can be produced by the cells themselves. In this case, GFP itself is no use, since it is not visible under electron microscopes. Nevertheless, GFP is expanding beyond the realm of science. It is employed in some glow-in-the-dark toys, in glow-in-the-dark fish being sold as pets, and even in bacteria that have been genetically modified to detect arsenic, TNT and heavy metals.

In spite of all these advances, however, the initial mystery remains unsolved: we still don't know why the jellyfish evolved the ability to glow green in the first place.

Exposed to ultra-violet light, the GFP in these tubes glows fluorescent green.

Reaction time

In 2009, EMBL-EBI unveils its new chemogenomics database, ChEMBL, which will allow open access to data that could revolutionise the way both academic scientists and those in the pharmaceutical industry develop new drugs to treat many different diseases. The data in ChEMBL were originally owned by a private-sector biotechnology company called Galapagos, and the rights to them were recently acquired by EMBL-EBI with the help of the Wellcome Trust and EMBL. EMBL-EBI Director Janet Thornton, and John Overington, who joined EMBL-EBI from Galapagos, explain the significance of the new database and how it will multiply the value of existing public resources at the outstation.

What is chemogenomics and why is it so important?

John: Chemogenomics refers to chemical annotation of the genome: the mapping of interactions of small molecules and other potential drugs through to the underlying genes. I think there are a large number of advantages to seeing these interactions in a genomic context. For example, it makes comparisons between organisms a lot more straightforward.

Janet: Another key point is that a gene is more than a protein. So rather than just cataloguing interactions between small molecules and proteins, chemogenomics also deals with information about how small molecules might interact with DNA or other factors that control gene activity, for example. As well as producing a bigger picture of how small molecules map to the genome, the data provide an integrated view, involving genes, proteins and small molecules. Often, a lot of the integration efforts in biology push back to the genome and gene loci, so that's a useful tag to share across all of these sources.

Does this relate to what is often called personalised medicine?

John: Absolutely. There are a couple of areas of hot interest to us at the moment. The first of these is how cancer cells and disease-causing microbes develop resistance to the drugs normally used to treat them. Often, this is caused by variations in the genes or proteins targeted by those drugs. The second is how genetic differences between people affect their disease susceptibility and response to drug therapy. The data structures that we have allow us to handle that kind of data, and find ways of predicting drug response or resistance.

How did the new database come about?

John: It was started by a biotech company called Inpharmatica, which was spun out from academic work at University College London. We ended up focusing one of our activities on chemogenomics, and were acquired by a larger biotech company, Galapagos, at the end of 2006. We worked inside Galapagos for two years before being 'spun out' to EMBL-EBI. We were interested in moving back into the public domain, having become increasingly involved with not-for-profit organisations and the World Health Organization's Tropical Disease Research group. We also had a long-term relationship

with many of the resources and people at EMBL-EBI and were aware of Janet's strategic interest in adding chemistry and pharmacology data to the existing resources.

Janet: I realised that these data provided unique information which would complement the data that we already had at EMBL-EBI. I also felt that this was the way into the small molecule world and ultimately the world of small molecule therapeutics.

Moving the database out of the commercial world into the public domain can't have been straightforward. How did you manage it?

John: We are very grateful to the Wellcome Trust for their support and vision in this. They were absolutely fantastic in terms of getting the interactions between EMBL-EBI and Galapagos going.

They also processed our grant application incredibly quickly, and dealt with a lot of the inevitable complications that come with bringing something out of the private sector into the public domain. The EMBL member states have also been very generous in the infrastructure investment to the group and we've been made to feel very welcome here.

John Overington, team leader of the ChEMBL group

Janet Thornton, Director of EMBL-EBI

There are a number of databases out there. What's so special about this one?

John: We're very conservative in terms of what we consider to be drug-like compounds. All the molecules within our database have some defined biological activity. It's a hand-curated dataset – that's the big difference between it and other databases such as PubChem: this combination of careful manual curation of large-scale medicinal structure activity relationship (SAR) data, which isn't currently in any other public database.

Why is this especially useful?

John: If you know about the pharmacology and biological effects of a drug, you can then make predictions about how it might interact with other proteins. This means you could predict harmful side effects – or, even more exciting, useful side effects, and so use already-developed drugs to treat new conditions. It could also provide a way to improve existing or discover potential new drugs. In drug discovery, it's often about coming up with a better chemical structure that does the same or slightly modified thing. Our data is organised so that

you can track changes in small molecule structure and document these effects. Importantly, we also track the other side of the equation: what happens when you have changes in a target protein. These could be different versions from the same organism, or the same protein but in different species. So if you are, for example, using mouse to validate your potential new drug compound, you need to be sure you can predict human pharmacology or human response, and this is where our database could help.

What's the state of play of the database at the moment?

John: It's up and running within EMBL-EBI – we've not released it externally yet. We need to recruit the team for deployment of the data at EMBL-EBI – that's well underway. We anticipate full public access this summer, and the Wellcome grant will support this for five years.

How will this fit in with the other databases and projects at EMBL-EBI?

Janet: There's a very obvious close link to ChEBI, which enumerates the different small molecules of biological interest. ChEMBL also links very closely to the protein structure database because many of the interactions that John's database describes are interactions with proteins for which we might know the structure. Then, there are links with all the gene expression data we have at EMBL-EBI. Many of these expression studies look at gene activity both with and without the presence of a given chemical. So you need to know what the chemical is and what its targets are. It then links back to the genomes because of the genome annotations, and also forward to the data on biochemical pathways. It's multi-arrow; I can't think of a single EMBL-EBI resource to which there is not a connection.

Do you see EMBL-EBI's resources, including this new database, synergising in some way?

Janet: A major goal within EMBL-EBI over the next two to three years is the deep integration of our data. I think this will be driven in part by the data in these new resources because these data provide the experimental biological links between small molecules and their targets.

John: A lot of the previous research focused on getting the toolkit or components in place and catalogued. Now there's a major phase in starting to understand the relationships between them; to understand the processes as opposed to just the components.

THE CHEMBL DATABASE

The newly acquired data from Galapagos will be housed in a database known as ChEMBL, and maintained by a new team brought together for this purpose. At the moment, ChEMBL comprises three separate databases, although these will eventually be merged into one.

The first database, StARLite, stores data on historical medicinal chemistry programs, and can help researchers identify chemical structures that might interact with a given target. The second database, CandiStore, follows drugs as they go through clinical development. "This is really important for understanding why compounds fail in the clinic," says John. "Did they fail because they didn't work against their target or the mechanism was wrong, or did they not work because there was something reactive or unsuitable in the compound structure?" CandiStore holds data on the chemical structure of drugs, their targets, modes of action, stage of clinical development plus other information that should help researchers answer these questions. The third database, DrugStore, stores information on existing drugs, both small molecule and biological, and includes data on their structure and chemistry, biological targets and mode of action, together with medical information on how the drug is used, what side effects have been reported and so on.

The ChEMBL databases should be available to outside users from mid-2009, and interested parties can keep track of their progress on the group's blog: http://chembl.blogspot.com/.

Why is open access so important?

John: Open access can radically reduce the costs of accessing this sort of data. What's more, it's just not accessible to people such as research students and academic research groups at the moment – and I think these are the people who will be doing an increasing amount of basic drug discovery in future. Pharmaceutical companies are moving increasingly towards more validated targets and more in-licensed clinical-stage products. EMBL-EBI's resources will really enable academic scientists to perform that all-important 'blue skies' drug discovery work.

EMBL-EBI in Hinxton

What are the challenges facing the project, and where do you see it going in the future?

John: At the moment, the key challenge is recruiting and training the staff we need. But that is well underway now. As for the future: hopefully during the period of the grant we'll be able to double the number of compounds in the database. So we'll get more diversity in chemistry, more diversity in biology as well. I think, much as Janet said before, it's the integration and synergy with the other data resources here, and elsewhere in the world.

One key vision is to start thinking about annotating small molecules on to genomes as soon as the sequences come off the sequencing machines. Imagine, for example, a new, deadly bacterial infection emerges and starts spreading through the population, and imagine being able to try, *in silico*, all previously existing therapies against that to prioritise them or identify new potential targets.

What collaborative work do you do?

John: We already have collaborations between groups here at EMBL-EBI and the Sanger Centre on the Hinxton Campus. We're also starting to network worldwide with scientists at the National Center for Biotechnology Information and the PubChem database, both in the USA. In addition, we have our industry programme, which played an important role in emphasising the importance of these data to us and other groups at EMBL-EBI. The reaction from the industry partners has been really positive – they are delighted that we now have these resources.

Imre Berger

Robots get a grip on complexes

Necessity is the mother of invention, or so the saying goes. And it was necessity that prompted Imre Berger, a group leader at EMBL Grenoble, together with his colleagues, to step outside his usual sphere of expertise and pioneer a revolutionary method of making proteins in the test tube. Thanks to the innovation, biologists can now make and manipulate the large protein complexes, so central to the biology of our cells, that once took them years to produce. "It blew me away," says Imre, recalling the first time his team used their technology to crack a huge membrane protein complex that had long resisted biologists. "It opened up a whole new field."

Imre's new technology involves robots, but as a structural biologist, robotics was far from his mind when he first started out. His team was trying to tackle a problem facing modern structural biology, namely, how to make enough of these protein complexes to study. "Most of the vital functions in cells are controlled by these large molecular machines, made up of several proteins," he explains. "Until recently, most people have only been able to study small pieces of these machines." But these isolated pieces often do not really reflect how the proteins behave in real life.

Unfortunately, it is very difficult to extract most naturally occurring complexes from cells in large enough quantities. Genetically engineering cells to make more of them is a laborious, time-consuming, and often frustrating process. Researchers had to stitch together the many genes encoding the proteins present in a complex and insert them, using DNA rings known as vectors, into bacteria or eukaryotic cells – a process that easily took months to complete. Worse, if the researchers then wanted to alter any of the proteins to find out more about how the complex worked, they had to go right back to the beginning of the process.

Imre's innovation, called ACEMBL, builds on a set of molecular biology techniques that his team developed in the course of their basic research. These techniques allow scientists to rapidly create vectors that could shuffle the genes of interest into any combination they want as well as alter them to produce different variants of the proteins. So they could create whatever complexes they liked without having to start from scratch each time, to study in detail their function and architecture.

Even so, doing this all by hand still involved a lot of time. So Imre got in touch with some friends who were experts in robotics at the Paul Scherrer Institut in Switzerland, and together they set out to translate the procedures of his approach into automated routines that can be carried out by a robot.

The result, ACEMBL, is the world's first fully automated pipeline for making and re-engineering large and complicated protein complexes. Almost everything, from building the vectors, to shuffling the genes and re-engineering the proteins, is performed by the robots. Crucially, the robotics routines can be used on any kind of cell, not just the bacteria Imre used. The platform has already helped other EMBL scientists and outside users to obtain the proteins they need.

Bieniossek C, et al. (2009) Automated unrestricted multigene recombineering for multiprotein complex preproduction. *Nature methods*, 3 May 2009; DOI:10.1038

Group photo of the scientists at the ESRF beamline ID 23 where the measurement of the atomic structure was made. From left to right: Alexandre Dias, Stephen Cusack, Rob Ruigrok, Denis Bouvier and Thibault Crépin.

Watching our backs

Visit a cash machine to withdraw some cash, and you're likely to see a notice warning you to shield your PIN. The caution is to protect you from 'shoulder surfers': fraudsters who watch to see what number you enter so they can steal your card and empty you bank account. Most people will probably tell you that shoulder surfing is a thoroughly 21st-century crime. But it seems as though one of humankind's viral adversaries – the influenza virus – has been at it for far longer than that. Researchers at EMBL Grenoble have now laid influenza's criminal tactics bare, and in so doing, revealed new details that could be critical for finding new drugs to treat the disease.

A very promising target for combating influenza is its polymerase, the enzyme that the virus uses to copy its genetic material and make more viruses. As well as being vital to the virus's life cycle, its biochemistry is probably different from that of our proteins. Until recently, however, scientists have been unable to study the polymerase's structure, because they couldn't get it to form crystals for their X-ray crystallography studies.

That all changed when Stephen Cusack, Head of EMBL Grenoble, and Darren Hart, a team leader at the outstation, and their colleagues used a new technology called ESPRIT to crack the problem. Developed by Darren, ESPRIT allowed the team to identify compact parts of the three proteins, or subunits, that make up the polymerase and to crystallise them. Last year, they showed how one of the subunits, called PB2, is involved in an

influenza version of shoulder surfing known as 'cap snatching'.

The PIN in this case is a short stretch of chemically altered RNA called a cap. Human cells add caps to their RNA copies of genes during the process of making a protein. The cap tells molecular machines called ribosomes how to start making the protein, and if an RNA doesn't have a cap, the ribosomes can't use it.

Flu RNAs don't have caps, so the virus needs to steal them from its host cell's RNAs. Last year, Stephen, Darren and their colleagues showed that PB2 grabs hold of the cap. But nobody was sure how the virus cut the cap free from the host cell RNA; the prevalent view in the literature was that it was done by a region in the so-called PB1 subunit.

In fact, this turned out to be incorrect: the role falls to the polymerase's third subunit, PA. Stephen, together with Rob Ruigrok and his team at the neighbouring Unit of Virus Host Cell Interactions, performed a series of biochemical studies on a small part of PA, and showed that it had the properties of an enzyme that could cut off the caps. The region was crystallised and the atomic structure determined using the intense X-ray beamlines of the European Synchrotron Radiation Facility (ESRF) next door. The structural studies showed how bound metal ions are important to enable the enzyme to work, and also that certain amino acids which are vital to its function are the same across all flu strains. This, together with the fact that biologists already have a compound that blocks PA's cutting activity, makes the subunit an ideal target for further anti-viral drug development. "We have now precisely defined the region of PA involved in cap snatching, thus making it much easier to optimise existing or find new compounds that specifically inhibit this activity," says Stephen**.** So perhaps it won't be too long before we can shield our cellular PINs from prying flu viruses.

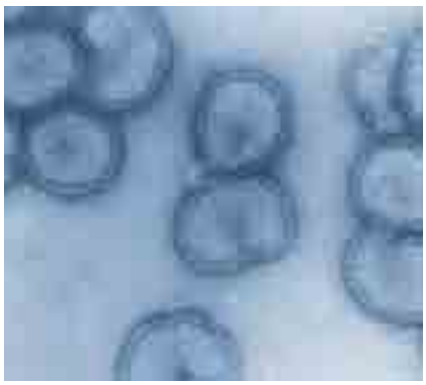
Dias A, Bouvier D, Crépin T, McCarthy A, Hart D, Baudin F, Cusack S and Ruigrok R (2009) The cap-snatching endonuclease of influenza virus polymerase resides in the PA subunit. *Nature* **458:** 914-918

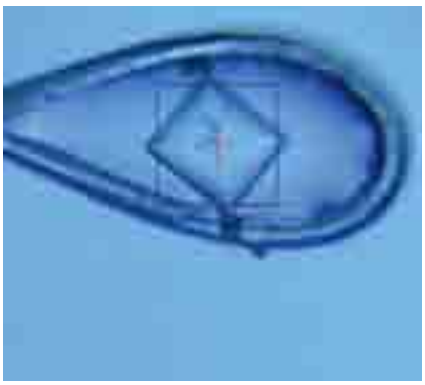
Top left: An electron microscopy image of the flu virus.

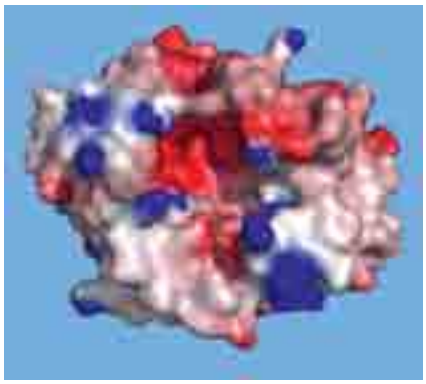
Bottom left: 3-D image of the PA protein domain where the cleaving of human genetic code by the virus takes place. The canyon is in the centre. The colours denote the electrostatic charge at the surface of the protein complex.

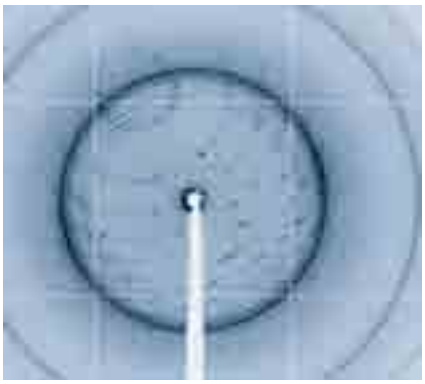
Top right: Photo of one of the crystals used to determine the atomic structure of the PA protein domain.

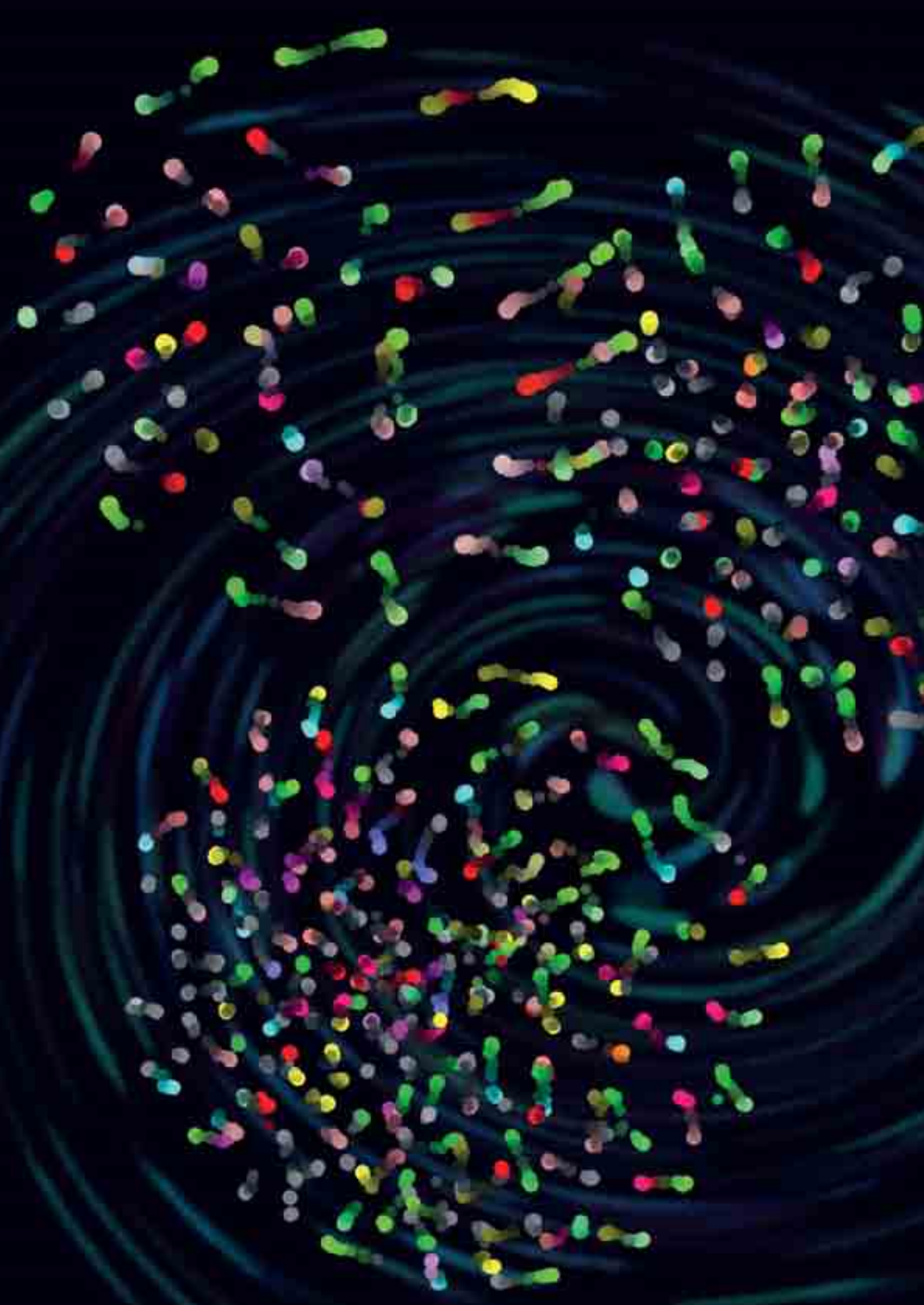
Bottom right: Diffraction pattern of the crystal, obtained at the ESRF.











Biology in space

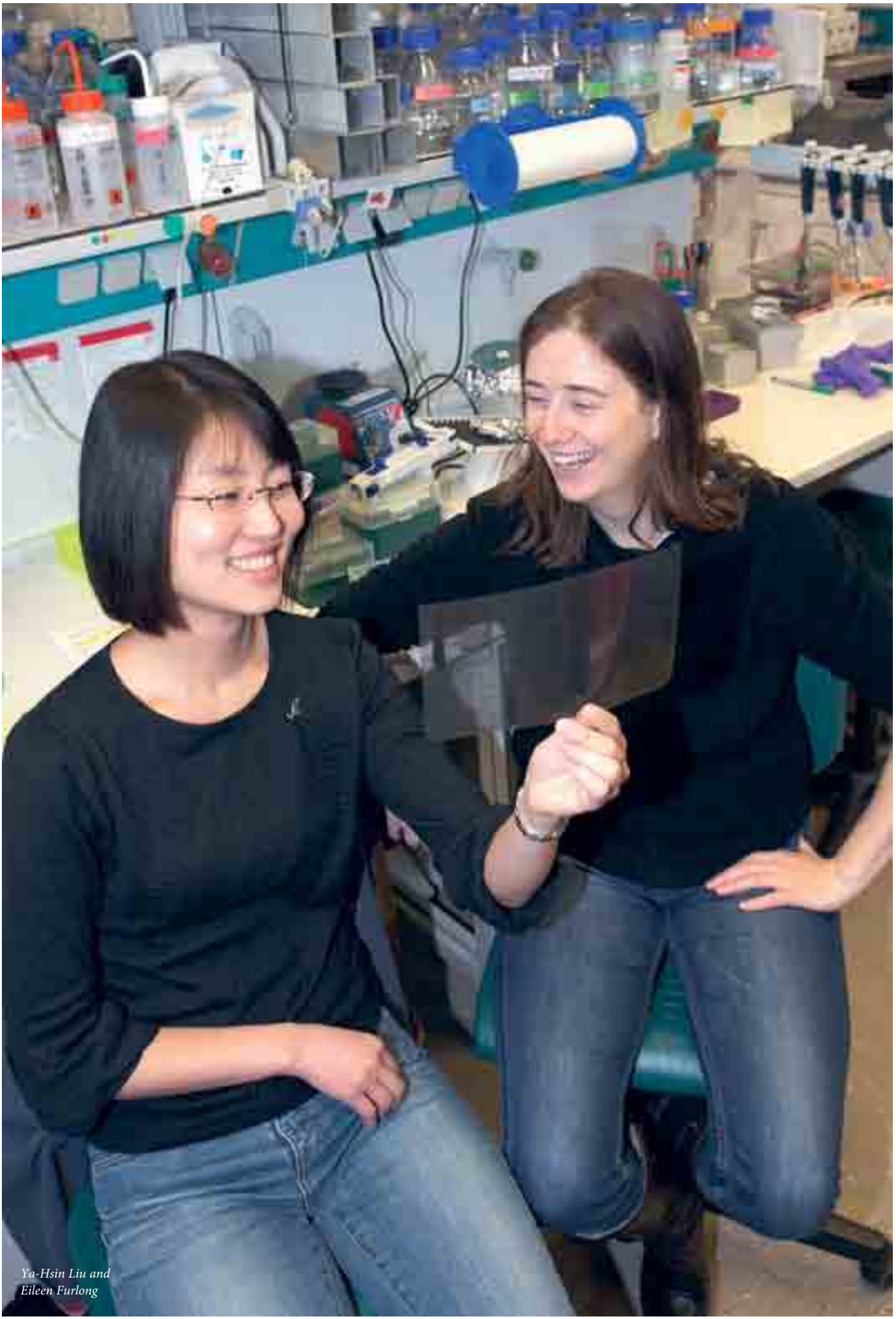
Ever since the 'space race' of the 1960s and 70s, the simple mention of the word space is enough to conjure up images of astronauts, comets, distant galaxies… and alien life forms. So much so that we almost forget that, here on Earth, life also happens in – and is constrained by – space.

On one level, the cells that make up an organism are arranged in space to form functional tissues. On another level, each cell is itself a confined space in which structures such as the nucleus are arranged. And there's space between cells, where molecules can be taken from or excreted to, and where cells can communicate with each other.

Space is also what allows – and limits – movement. Many of the fundamental processes of life rely on the fact that molecules, structures and cells have the ability to move, to occupy a different space. As an embryo develops, cells move and rearrange themselves to form tissues, organs and limbs. Within cells, too, essential molecules must be carried to the places where they are needed, and waste products must be removed from where they are unwanted. And in order for one cell to become two, the structures inside it must be rearranged and moved to the right place. That right place may vary from species to species, or according to the situation the cell is in, including its own place in a multi-cellular organism, which can also influence which of the cell's genes are turned on at a given time.

What's more, all this takes place in a three-dimensional space, which must be taken into account if we are to truly understand such processes. Scientists at EMBL have taken on this challenge, and developed a technique which enables them to follow the movements of all cells throughout the first day of an embryo's development. This digital embryo has been hailed as one of the scientific breakthroughs of the year, and its creators believe it has plenty of room for growth: they hope it will be the start of a new approach to storing and managing molecular and genetic data. At the same time, other EMBL groups are exploring life in space at different levels. Some are shedding new light on how direction and location influence the translation of genes into proteins, while others are investigating how the cell's inner scaffolding grows and shrinks, and how it helps transport molecules around the cell and relocate larger structures such as chromosomes.

This multitude of approaches seems set to ensure that, in molecular biology at least, space will not be 'the final frontier'.



Ya-Hsin Liu and Eileen Furlong

The fly with no heart

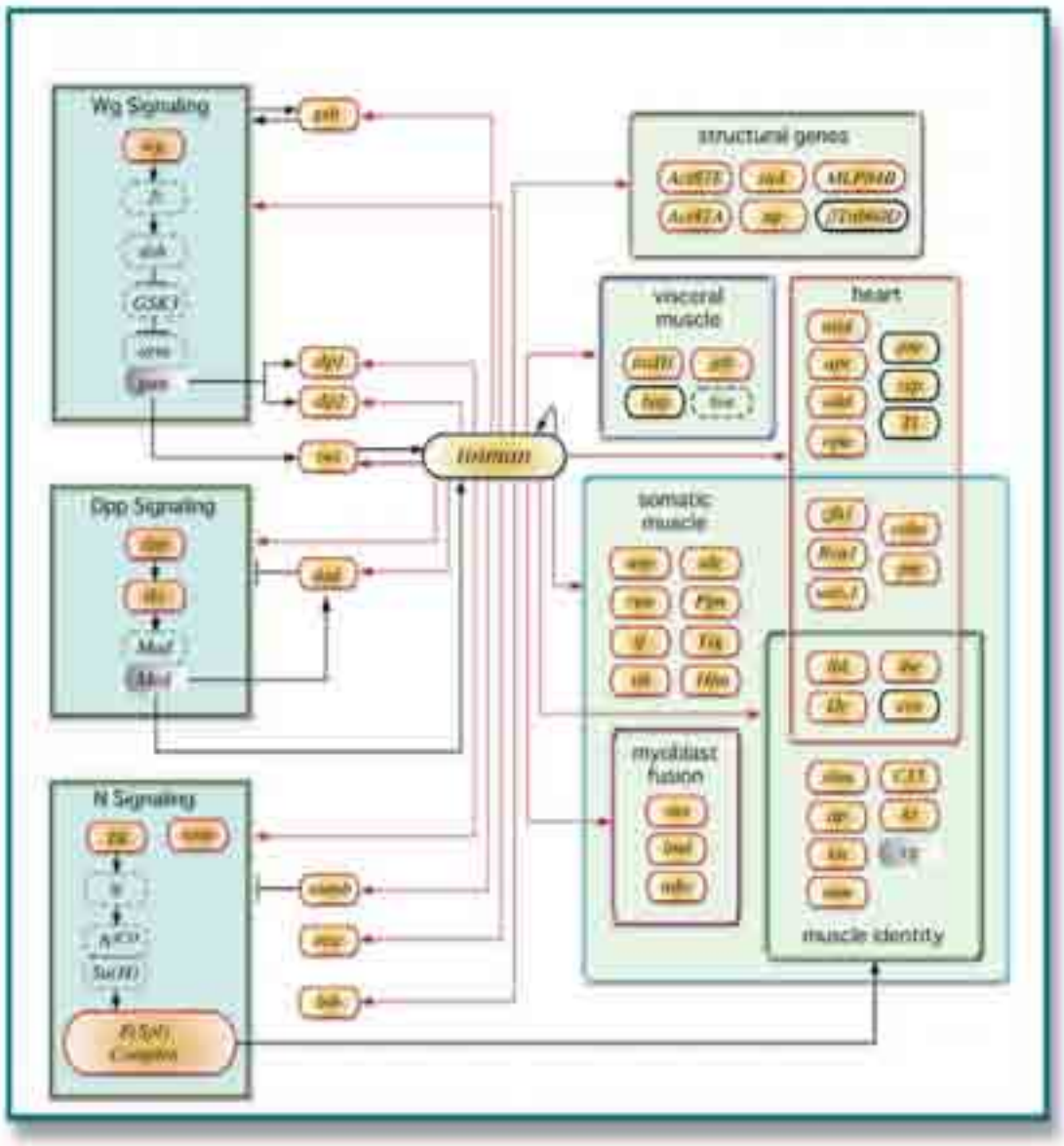
Some of the most imaginative – and sometimes gruesome – nomenclature in science is to be found among the mutants of the fruit fly *Drosophila*. Take the *tinman* mutant, so-called because, like its namesake in the film *The Wizard of Oz*, it lacks a heart. Unfortunately for *tinman*, that's not all it lacks. "It also has no gut muscle, and is missing many muscles along the length of its body," explains Eileen Furlong, Joint Head of EMBL's Gene Expression Unit. "All three of these tissues come from one domain of the mesoderm, the dorsal mesoderm."

The mesoderm is one of three germ layers or cell groups into which an embryo is initially divided, under the control of a transcription factor named Twist. A few years ago, Eileen's group elucidated the underlying transcriptional network driven by Twist during the early patterning of the embryo. Downstream of Twist, another transcription factor called Tinman governs the patterning of the dorsal mesoderm. Twist and Tinman belong to a vast and complex regulatory network that changes over developmental time, and which Eileen's group is attempting to reconstruct. "The ultimate goal is to use our global view of the network to make predictions about gene expression, and therefore to make predictions about cell fate choices," she says.

Transcription factors are proteins that determine which genes are active at any moment; they do so by binding to regulatory regions near genes, called cis-regulatory modules (CRMs), thus enabling them to be transcribed. The *tinman* gene has generally been associated with heart development, because when its counterpart is mutated in mice and humans, the result is heart defects. But the observation that it has a broader role in development, at least in the fly, spurred Eileen's group to try to capture a more global, molecular view of its myriad interactions.

Using a technique called ChIP-on-chip [see box page 35], PhD student Ya-Hsin Liu in the group identified 400 CRMs to which the Tinman protein binds. "We expected heart-related genes to account for most of those," says Eileen, "but we found a much longer list of known genes that are involved in body wall muscle development, which is analogous to skeletal muscle in vertebrates." The role of Tinman in this muscle-generating programme has been under-appreciated, she says, but that finding gave rise to a new question: if Tinman is important in the development of muscle in flies, why do mice and humans lacking Tinman show no obvious skeletal muscle defects?

To investigate this apparent vertebrate-invertebrate discrepancy, Eileen collaborated with developmental biologist Darren Gilmour at EMBL Heidelberg, and his PhD student Guillaume Valentin, who both work on another vertebrate, zebrafish. Ya-Hsin's and Eileen's analysis had shown that, in the fly, one of Tinman's many roles is to regulate the expression in the mesoderm of another transcription factor, STAT, which is the target of incoming signalling cascades that influence patterning. Zebrafish have a number of STAT genes, but the one most related to the *Drosophila* gene showed a beautiful expression pattern in the somites (segmental units in the embryo that give rise to skeletal muscle). When the researchers knocked the gene out, the mutant showed abnormal muscle development.



This transcriptional network map shows the many genes tinman targets that are required for somatic muscle specification.

Eileen believes that these species differences shed light on a fundamental mechanism of evolution. Many of the same molecules turn up again and again in transcriptional networks governing development, however their upstream regulators can differ dramatically from species to species. "Although a lot of the connectivity or wiring within developmental networks can diverge significantly," she says, "in the end what matters is that the downstream effector molecules, and their immediate regulators, are expressed in the appropriate tissue." This, she suggests, gives evolution a lot of room for tinkering.

Liu YH, Jakobsen JS, Valentin G, Amarantos I, Gilmour DT, Furlong EE (2009) A systematic analysis of Tinman function reveals Eya and JAK-STAT signaling as essential regulators of muscle development. *Dev Cell* **16**: 280-91

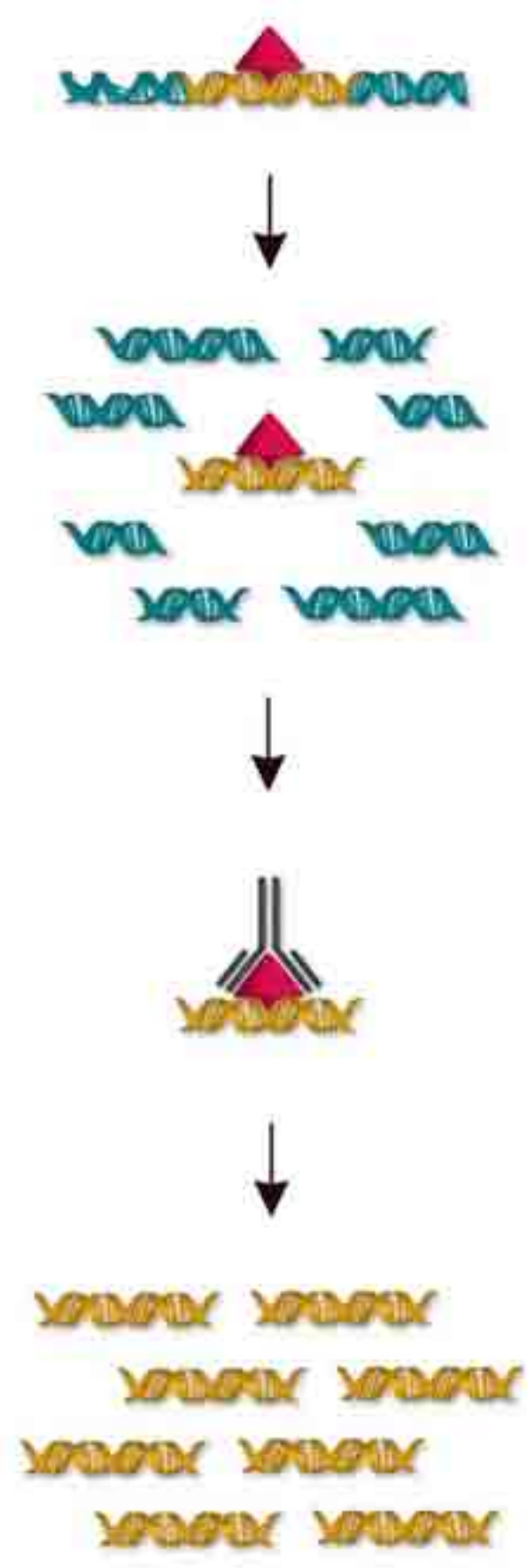
CHIP, CHIPS AND SEQUENCERS

Transcription factors are proteins that bind to DNA, determining when and where a gene is active. They therefore play a central role in the genetic regulation of biological processes, in both health and disease. Researchers would like to understand the protein-DNA interactions at the heart of that regulation.

ChIP, or chromatin immunoprecipitation, is a method of identifying the DNA sequences that transcription factors bind to, in intact cells. DNA extracted from those cells is first cut into pieces by a method that does not interfere with any proteins that might be bound to it. Then, using antibodies as bait, researchers 'fish' for the proteins they are interested in, extracting them along with the DNA fragments to which they are bound. To identify what genes they have 'caught' is then a simple matter of comparing those DNA sequences with the genome of the species in question.

ChIP is an elegant and useful technique, says Eileen Furlong, Joint Head of the Gene Expression Unit at EMBL Heidelberg, but it has one potential drawback. "Because you are looking at individual genes, you never know if you are seeing the norm or an unusual example," she says. "In order to be able to say, this is really how it works, you need to be able to look at the whole genome."

About five years ago, researchers gained access to that bigger picture by combining ChIP with microarray technology, in an approach dubbed ChIP-on-chip, or ChIP-chip. The use of a microarray, which comprises DNA probes designed to cover representative regions of the genome, enabled researchers to search for matches to the DNA extracted by ChIP across the whole genome. More recently, ChIP has been combined with tiling arrays, in which the probes either overlap or are very close together on the genome, providing a more unbiased analysis and a much higher resolution.



ChIP enables scientists to discover what parts of the genome specific transcription factors bind to, by 'fishing' out those transcription factors along with the DNA fragments they are attached to.

Like a microtubule spanning a cell, EMBL connections reached halfway across the globe, enabling Pernille Rørth, now in Singapore, to call upon her old colleagues in Heidelberg for help.

Lateral thinking

Until 2008, Pernille Rørth and Anne Ephrussi were neighbours. Their labs in the Developmental Biology Unit at EMBL Heidelberg were next door to each other, and though they were interested in different questions, they addressed them in the same biological system: the egg chamber of the fruit fly *Drosophila*. Then, one day, PhD student Hsin-Ho Sung in Pernille's lab stumbled on a puzzle that was relevant to both groups, and that would require their combined ingenuity to solve. With Thomas Surrey in the Cell Biology and Biophysics Unit, a fruitful collaboration was born.

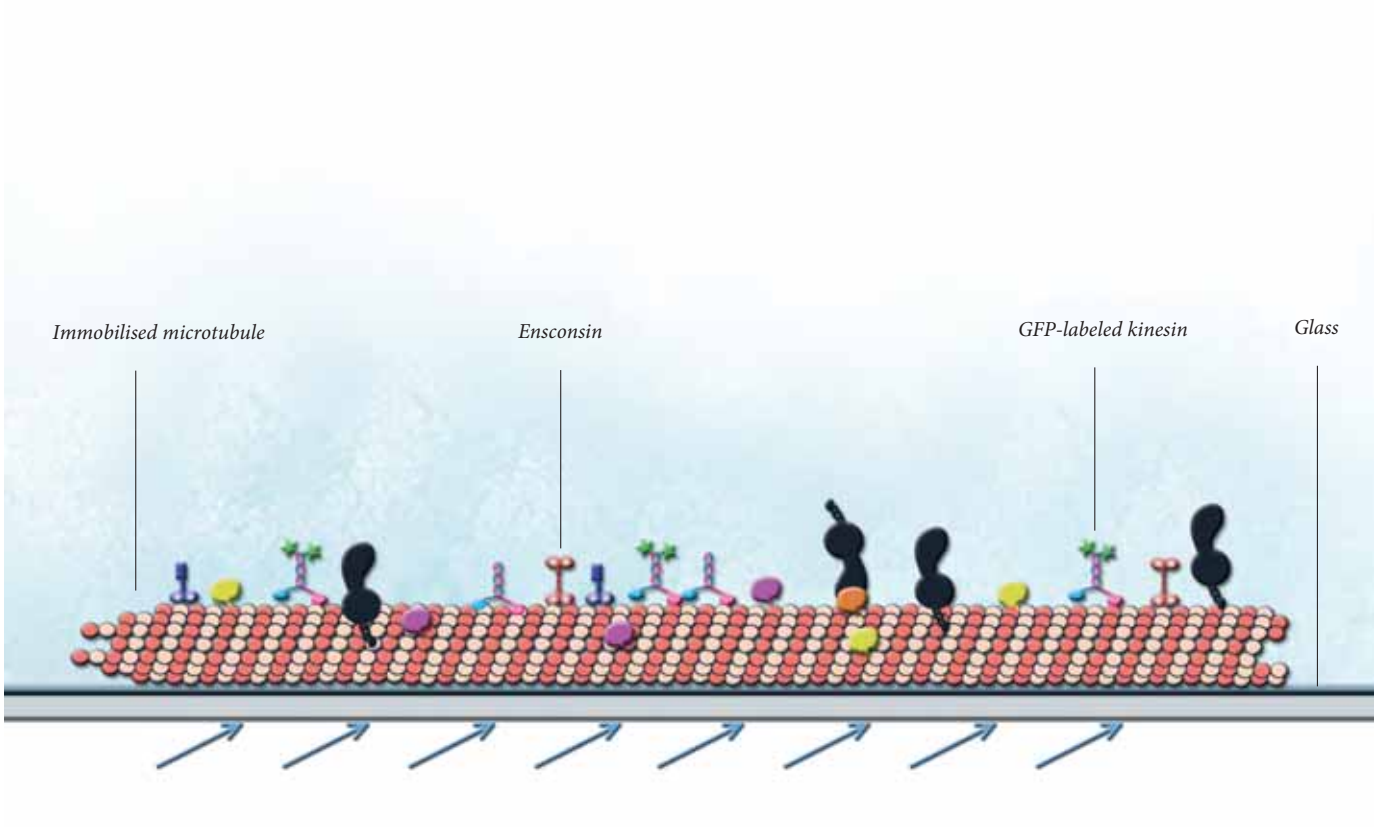
Anne's group studies how cells develop polarity – the asymmetric distribution of infrastructure, organelles and molecules that underpins their ability to function and survive – and the role of microtubules, a kind of dynamic cellular scaffolding, in bringing it about. Pernille's group is interested in cell migration, a very basic biological process which she studies in a specific population of cells: border cells. An egg chamber consists of several different cell populations, besides the egg cell or oocyte, and the role of the border cells is to move through the chamber, opening up a corridor that will allow a male fly's sperm access to the egg, and depositing chemical signals that will direct the egg's growth.

While conducting a screen to look for genes that affect the migration of border cells, Hsin noticed something that, although not directly relevant to his question, was intriguing nevertheless: when one of the genes his screen had revealed, *ensconsin*, was knocked out, the oocytes of the resulting mutant appeared abnormal. Molecular signals ended up in the wrong places, and in some cases, so did the nucleus. Most intriguingly – from Anne's point of view – it disrupted the characteristically asymmetric distribution in the cell of a type of messenger RNA called *oskar*, which directs the patterning of the abdomen and germ cells in flies. Hsin seemed to have stumbled on a mutation that was crucial for cell polarity.

Microtubules interact with many proteins that regulate their function, and the product of *ensconsin* turns out to be one such microtubule-associated protein (MAP). Originally named for its appearance of being 'ensconced' or permanently present on microtubules, ensconsin turns out to have been misnamed in mammalian cells, thanks to an artefact in the way the protein was initially isolated in mammals. In fact, says Pernille, it appears on, and disappears from, microtubules in a highly dynamic way. But the name stuck, and came to refer to a closely related protein in the fly too.

Meanwhile, says Anne, "We had been studying a kinase called Par-1 which is required for correct microtubule polarity in the oocyte, and consequently for the transportation of *oskar* RNA to its proper location at the oocyte posterior pole." Par-1 phosphorylates, or adds a phosphate group to, MAPs in mammalian cells, modifying those proteins in a way that affects microtubule dynamics. Exactly how it does so is not clear, but Hsin's discovery raised an interesting question: could ensconsin be a direct target of Par-1?

"This was how our part of the collaboration evolved," says Anne, "because we had a very good assay that we had worked hard over the years to put in place, for identifying



Experimental setup of the assay that allows the study of molecular motors: Microtubules are immobilised on a glass surface and the effect of ensconsin on the motility of kinesin is observed under a fluorescence microscope.

targets of Par-1 kinase." The assay, which was adapted from one developed by Kevin Shokat at the University of California, San Francisco, involves modifying the ability of Par-1 to bind to ATP. ATP is a key molecule in cells, and kinases bind to it in order to remove a phosphate group from it, which they then donate to other molecules.

Using a version of Par-1 in which the ATP-binding pocket had been artificially enlarged, postdoc Piyi Papadaki in Anne's group showed that this engineered Par-1 could bind to bulky, synthetic analogues of ATP that would not fit into any other kinase. She then showed that in its presence, ensconsin purified from extracts of *Drosophila* ovaries was still phosphorylated. "Since no other kinase can use these analogues, it was clear that ensconsin was a direct target of Par-1 *in vitro*," says Anne.

But what is the function of ensconsin *in vivo*? To answer that question, Pernille turned to biophysicist Thomas Surrey, whose group had been working for a long time to optimise an assay that would allow them to study molecular motors under the microscope. Derived from a technique that was developed to study the role of motors in the assembly of the mitotic spindle – a structure without which cell division cannot take place – the assay exploits the surface chemistry of glass to immobilise microtubules on a slide. Having done this, Hsin and postdoc Ivo Telley from Thomas' group added extracts from fly ovaries, in which molecules called kinesins had been fluorescently labelled.

Kinesins are the motors that travel along microtubules, transporting molecular cargo around the cell. By observing their behaviour under a fluorescence microscope, both in the presence and in the absence of ensconsin – using ovary extracts from wild-type and ensconsin-mutant flies, respectively – the researchers were able to see how ensconsin affects those motors.

"The major difference was that the landing rate went down in the absence of ensconsin," says Thomas. "That is, the probability that the kinesin in the extract would jump onto the microtubules and start to move was lower." Were the motors no longer working properly, in the absence of ensconsin, or were they not interacting with their cargo as they should? When Ivo looked more closely at the data, he found that ensconsin had a direct effect on the motors' ability to move.

The use of their assay with extracts from *Drosophila* ovaries marked a new departure for Thomas's group. "For biophysicists, it's interesting to look at motors in the presence of other molecules, because we wonder how they regulate them," he

"It was just a case of putting two cultures and skill sets together, and being lucky that it worked," Thomas says.

says. But whereas developmental biologists are used to working with complex biological mixtures, such as extract of ovary, biophysicists are generally loath to, because it is hard to disentangle the effects of the various components of those mixtures. They also expected that the autofluorescence of the fly egg yolk would interfere with the fluorescent signal they were trying to read. Surprisingly, however, that didn't happen. "It was just a case of putting two cultures and skill sets together, and being lucky that it worked," Thomas says.

Anne and Thomas tried to address similar questions about the mechanics of cell polarity about six years ago, but as Anne says, they didn't have the right methods then. Pernille's and Hsin's question about ensconsin has revived their attempt to collaborate. "The fact that one could observe the kinesins really moving in Thomas' system, when those kinesins were taken from extracts, was proof-of-principle that it could be done," says Anne.

Working together, the two groups will now try to answer some of the new questions their findings have raised, not least of which concerns the mechanism that ensconsin uses to recruit kinesins to microtubules. Pernille, in the meantime, has satisfied her curiosity as far as ensconsin is concerned. "To take this to the next level," she says, "you need to be able to do the kind of experiments that Thomas is doing." At the Temasek Life Sciences Laboratory in Singapore where she now heads a group, Pernille has returned to the main focus of her lab: cell migration.

Sung HH, Telley IA, Papadaki P, Ephrussi A, Surrey T, Rørth P (2008) *Drosophila* ensconsin promotes productive recruitment of Kinesin-1 to microtubules. *Dev Cell* 15: 866-76

Teleconference with Singapore: Thomas Surrey and Anne Ephrussi discuss ideas with Pernille Rørth (screen).

Follow the leader

Darren Gilmour with an image of lateral line migration.

Like pioneers in the American West heading out in convoy to form new settlements, cells in developing tissues organize themselves and migrate in groups. In zebrafish embryos, a group of cells known as the lateral line primordium migrates along each flank, from head to tail. At regular intervals along the way, subsets of those cells form rosette-shaped structures, which will eventually develop into the fish's lateral line – a line of sensory organs that detect vibrations in the water. Researchers at EMBL Heidelberg have uncovered the mechanism that regulates this process, along with some surprises.

Cells at the front line of migration (its leading edge) are followed by cells which group together to form rosettes. The cell at the centre of each rosette retains its original shape, remaining identical to cells at the leading edge, but the cells around it change. They turn into epithelial-like cells, pointed at one end, and bring their pointed ends together over the unchanged middle cell – like cloves in a bulb of garlic – to form the rosette.

Darren Gilmour and his group in EMBL's Cell Biology and Biophysics Unit discovered that this change in follower cells' characteristics occurs because fibrolast growth factor (Fgf) signaling is activated in those cells. Such results were unexpected, Darren says. "This is essentially the opposite effect from what has been described in other contexts. For example, elegant studies have demonstrated that during chick development, a gradient of Fgf signaling actually drives this transition in the opposite direction."

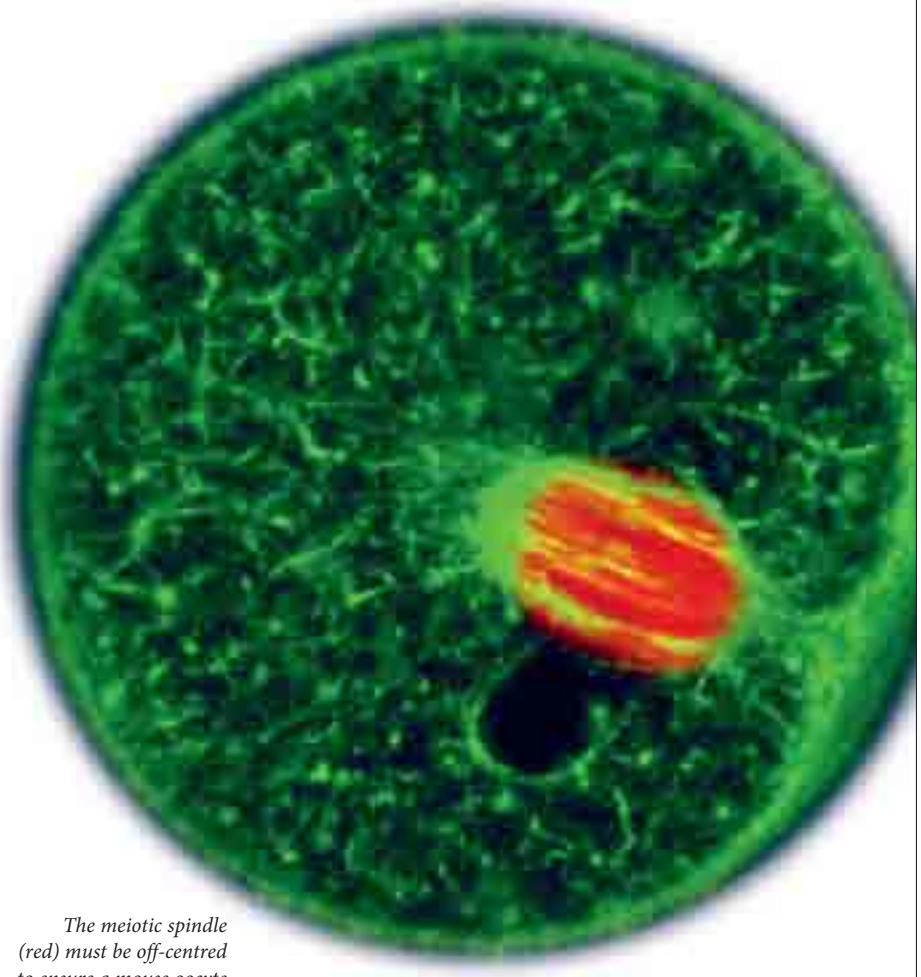
When the EMBL researchers inactivated Fgf signaling, none of the migrating cells took on epithelial characteristics, and therefore no rosettes were formed. As all cells, both in the leading edge and behind it, remained leader-like, one might expect cell migration itself to continue unhindered, but it didn't. Instead of advancing smoothly and simultaneously as they normally do, cells advanced in a stretch-and-pull fashion: the leading-edge cells advanced first (stretching) and then the others caught up. So when rosettes were not formed, migration became less coordinated and then stopped.

Darren and his group believe this could be because the set of migrating cells has its own internal checkpoint, which stops migration if there is a problem with rosette assembly. "Of course," he remarks, "a mechanism that couples migration behaviour to organ assembly makes biological sense, as a migrating primordium that does not deposit organs is of little use."

The actual mechanism that halts migration could also involve signaling, or it could be that rosettes simply make migration easier. In the same way that ensuring a convoy of two or three wagons migrates at the same pace is easier than making all their occupants march in tandem, collective cell migration could be made easier by reducing a large number of cells to two or three rosettes which can be controlled as single units. But then again, maybe the answer will turn out to be yet another surprise!

Lecaudey V, Cakan-Akdogan G, Norton WH, Gilmour D (2008) Dynamic Fgf signaling couples morphogenesis and migration in the zebrafish lateral line primordium. *Development* **135**: 2695-705

Pulling the right strings



The meiotic spindle (red) must be off-centred to ensure a mouse oocyte matures into an egg.

Imagine you are sitting in a boat in a lake, and can't see the shore. The boat has no motor, sails or oars, but is fitted with pulleys at the bow and stern – which is handy, as the boat is surrounded by pieces of rope lying in the water. To get ashore, all you have to do is pull yourself along the ropes. Unfortunately, you don't know if it is the bow or the stern that's closer to shore, and none of the pieces of rope reaches all the way to it. Instead, each piece of rope is part of a net that keeps coming undone and re-connecting in different ways. Short of abandoning ship and attempting to swim, how do you get out of this predicament?

When a mouse oocyte matures into an egg, a cellular structure called the spindle faces a similar problem. To ensure the egg is properly formed, the spindle must move from the middle of the cell to its periphery. Like the boat surrounded by lengths of rope, the spindle is surrounded by a string-like protein, filamentous actin (F-actin). Scientists knew this protein was essential for spindle relocation in several different mammals, including mice and humans, but they didn't know why. By employing time-lapse imaging to observe F-actin during spindle relocation, "we saw that a highly dynamic network of actin filaments fills the oocyte cytoplasm, bridging the gap between spindle and cell surface," says Jan Ellenberg, Joint Head of the Cell Biology and Biophysics Unit at EMBL Heidelberg.

Jan and postdoc Melina Schuh discovered that the moving spindle knows the answer to the boat riddle: pull at both ends. It moves by pulling on the actin filaments near both its poles, using a motor protein called myosin-2 as a pulley. Because the spindle is unlikely to be in the exact centre of the cell, one of its poles is closer to the cell's edge than the other. Since more of the ever-changing connections link that pole all the way to the edge of the cell, it is better 'anchored' to the edge; when the spindle pulls on actin filaments at both poles, it tends to pull itself in the direction of the 'anchored' pole. This brings that same pole even closer to the edge; as the spindle continues pulling, that pole is even better connected, which in turn brings it closer still. In short, by pulling at both poles the spindle effectively ends up travelling in one direction: towards the pole that was initially nearest to the cell's periphery. In Jan's words, "by pulling at the actin network with both of its poles, the spindle is able to reinforce its initial randomly asymmetric position, to move itself more and more off-centre until it reaches the edge of the cell."

Similar F-actin networks exist in the oocytes of fruit flies, starfish and frogs, but they don't appear to be involved in spindle relocation in these species, implying that evolution seems to have put the F-actin network to a new use in mammals. Human oocytes, on the other hand, mature in much the same fashion as mouse oocytes do, so the scientists would now like to know if the human spindle relocates through a similar process, especially since failures in spindle relocation in oocytes are linked to infertility.

Schuh M, Ellenberg J (2008) A new model for asymmetric spindle positioning in mouse oocytes. *Curr Biol* **18**: 1986-92

François Nédélec and Eric Karsenti

Mitosis in three colours

In development, the patterning of the body is governed by signalling molecules which diffuse through tissues, acting directly on cells to influence their differentiation decisions and ultimately, their identity. Those cells' responses may vary, depending on where they are in relation to the source of that signal – in other words, where they fall on its diffusion gradient. But the gradient itself is always the same: the laws of physics dictate that it fits an exponentially decaying curve. How does nature exploit that ubiquitous curve to elicit different kinds of cellular response? The answer is by adding in another step, to convert the exponentially decaying signal into a two-way, three-way or n-way switch.

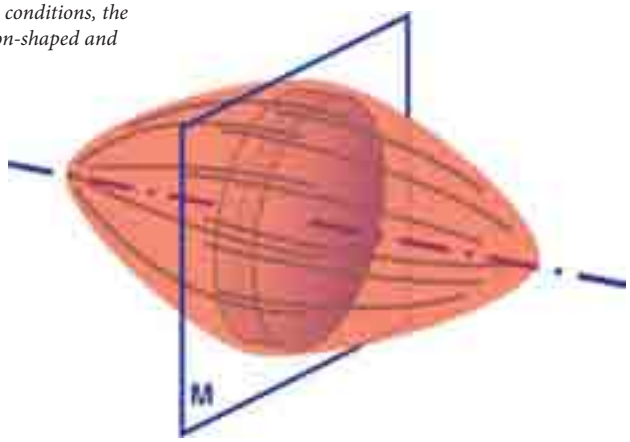
This was the insight of biologist Lewis Wolpert in the 1960s, who described the concept by analogy to the French flag: those cells receiving high concentrations of the signal turn on a 'blue' gene, those exposed to lower concentrations turn on a 'white' gene, while those receiving less than a certain threshold maintain a default 'red' state. Two Frenchmen working at EMBL Heidelberg now suspect that this basic principle holds much more widely across biology. In particular, François Nédélec and Eric Karsenti think that a similar switch may be at work during mitosis, the process by which a eukaryotic cell divides the chromosomes in its nucleus into two identical daughter nuclei, just before the cell itself divides in two.

During mitosis, microtubules or cellular 'ropes' form a symmetrical spindle which invades the nuclear space, dragging a single, condensed and duplicated chromosome in opposite directions to produce two sister chromatids. Two pathways of spindle assembly have been identified to date. In the centrosomal pathway, microtubules are nucleated, or born, at structures near the nucleus called centrosomes, from which they radiate out in star-shaped 'asters'. However, mitosis can also take place in the absence of centrosomes. "For example, in plants there are no centrosomes and no asters," says Eric, "and yet there is a spindle and chromosomes are segregated on the spindle."

A second pathway seems to be at work, both when centrosomes and asters are present and when they are absent, and researchers already know quite a lot about it. Chromatin, the substance of the chromosome, activates a protein called Ran that helps coordinate the microtubules. When they are born, microtubules are highly dynamic and unstable, constantly growing and shrinking. To form a spindle, they must be stabilised to increase their average length. An early observation, which brought this 'chromatin pathway' to Eric's attention, was that microtubules are more stable the closer they are to the chromosome. In this study, the researchers set out to answer two basic questions: at what distance do the microtubules become stable, and what is the nature of the switch that governs their transformation?

It seemed to François and Eric that the microtubules' behaviour fitted an on-off pattern, something that is not true of the initiating Ran signal. If so, how does the diffusing Ran bring about that on-off behaviour? "We propose that there is an exponentially decaying signal, but it's coupled downstream to a bistable switch," François says.

MACHINE OVERLOAD



Under normal conditions, the spindle is lemon-shaped and symmetrical.

The mitotic spindle was named for its resemblance to a device that was once used to spin fibres into thread. But since that device has fallen out of use, it is probably more useful to compare it to a lemon: an elongated sphere with two pointed ends.

"The spindle is a machine, if you like, and the role of the machine is to segregate the sister chromatids," says François Nédélec. But if you think of it as a machine, then a natural question to ask is, how much chromatin is too much for it to segregate? At what point does the machine break down?

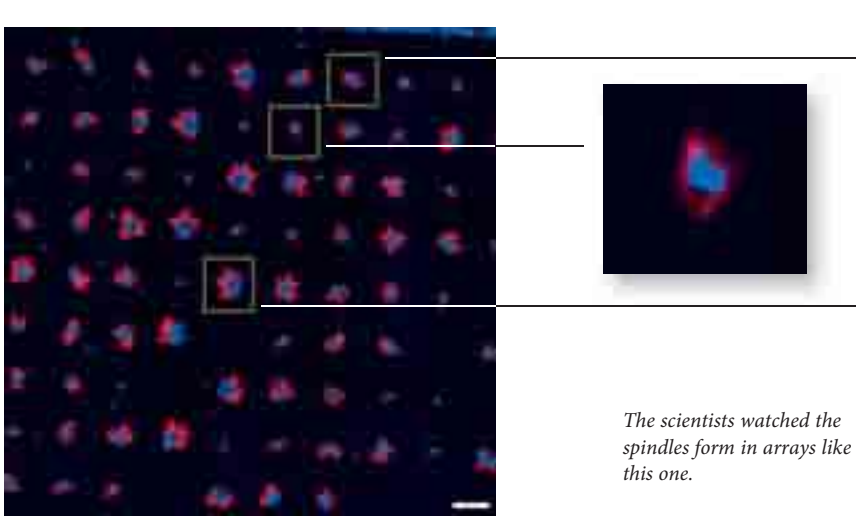
These questions are relevant for an important economic reason: thanks to millennia of crossbreeding, farmers have developed strains of edible plants such as wheat and barley whose cell nuclei contain larger complements of chromatin than those of their ancestors. "We have duplicated the number of chromosomes and yet the spindles in these plants are still able to function, or the plants would not grow," explains François.

Ana Dinarina, a former postdoc in François' group, set about trying to overload the machine. The basic method involved printing chromatin on glass slides, and incubating it with extracts from frog eggs of the *Xenopus* genus. With the help of the Advanced Light Microscopy Facility at EMBL Heidelberg, the researchers captured time-lapse images of the spindle that subsequently formed. Gradually, Ana added more and more chromatin until she saw the spindle break down.

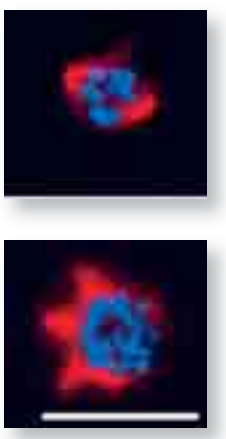
"If you put too much chromatin, instead of two poles of the spindle, you get three or four," says François. "That's broken, because the spindle no longer has the proper organisation to separate the chromatin." Ana was able to increase the chromatin complement by a factor of four before this happened, and she also found that there was a lower limit to the machine's performance: without sufficient chromatin, the spindle couldn't form in the first place.

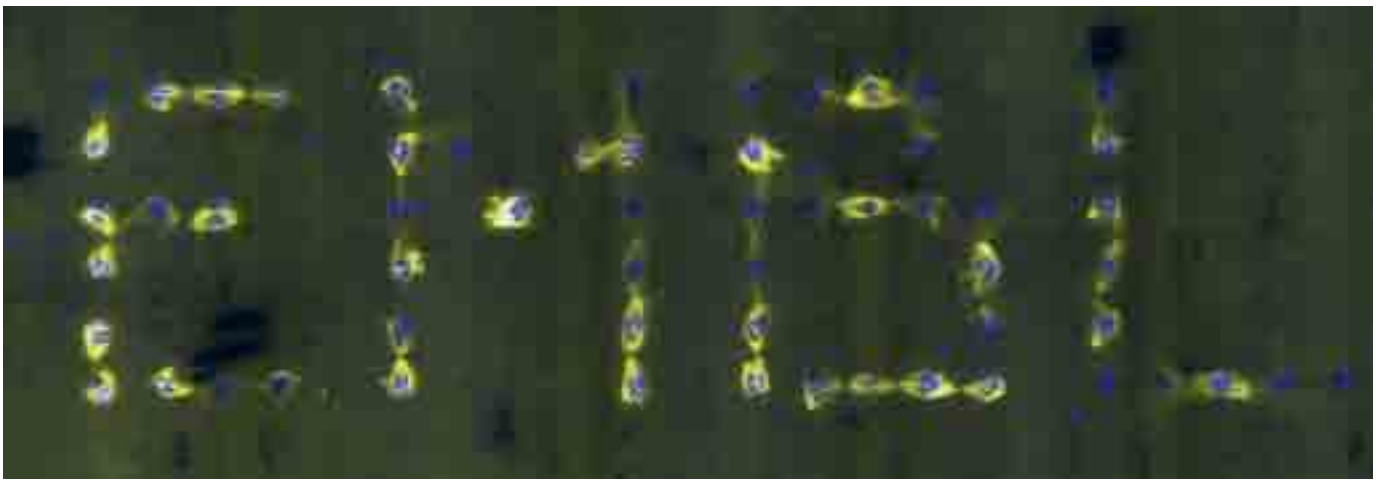
These limits vary depending on the cell and the species it comes from, says Eric Karsenti, but the fact that they exist at all provides an interesting new insight into evolution. "It shows that there must be co-evolution of chromatin geometry and biochemistry," he says. "You cannot have one without the other."

Dinarina A, et al (2009) Chromatin shapes the mitotic spindle. ***Cell***, in press



The scientists watched the spindles form in arrays like this one.





Alongside more serious endeavours, manipulating spindles enabled François' group to use them to spell out EMBL.

With Chaitanya Athale, a postdoc in Eric's group, and others, the pair took a novel systems biology approach to testing their hunch, combining experiments with computer simulation. On the experimental side, they printed chromatin on glass slides to immobilise it in various patterns, then incubated it with purified human centrosomes in egg extracts taken from the frog genus *Xenopus*. Asters formed at the centrosomes, sending out microtubules which grew preferentially towards the chromatin. By analysing microscopic images of this growth, they were able to measure the length of the microtubules as a function of the distance of the aster from the chromatin. In other words, they could describe mathematically the asymmetry of the microtubules in the presence of the Ran gradient.

On the computer simulation side, the researchers turned to a software application that they have been developing over the years, with the help of funding from the Centre for Modelling and Simulation in the Biosciences in Heidelberg, which allows them to simulate the collective behaviour of microtubules and associated proteins. First, they used computers working with differential equations to describe the shape of the Ran diffusion gradient – that exponential curve. By feeding this information into the simulation program, Cytosim, they were able to test various hypotheses of how that input signal might be modulated to produce an outcome, in terms of microtubule dynamics, that most closely matched the experimental data.

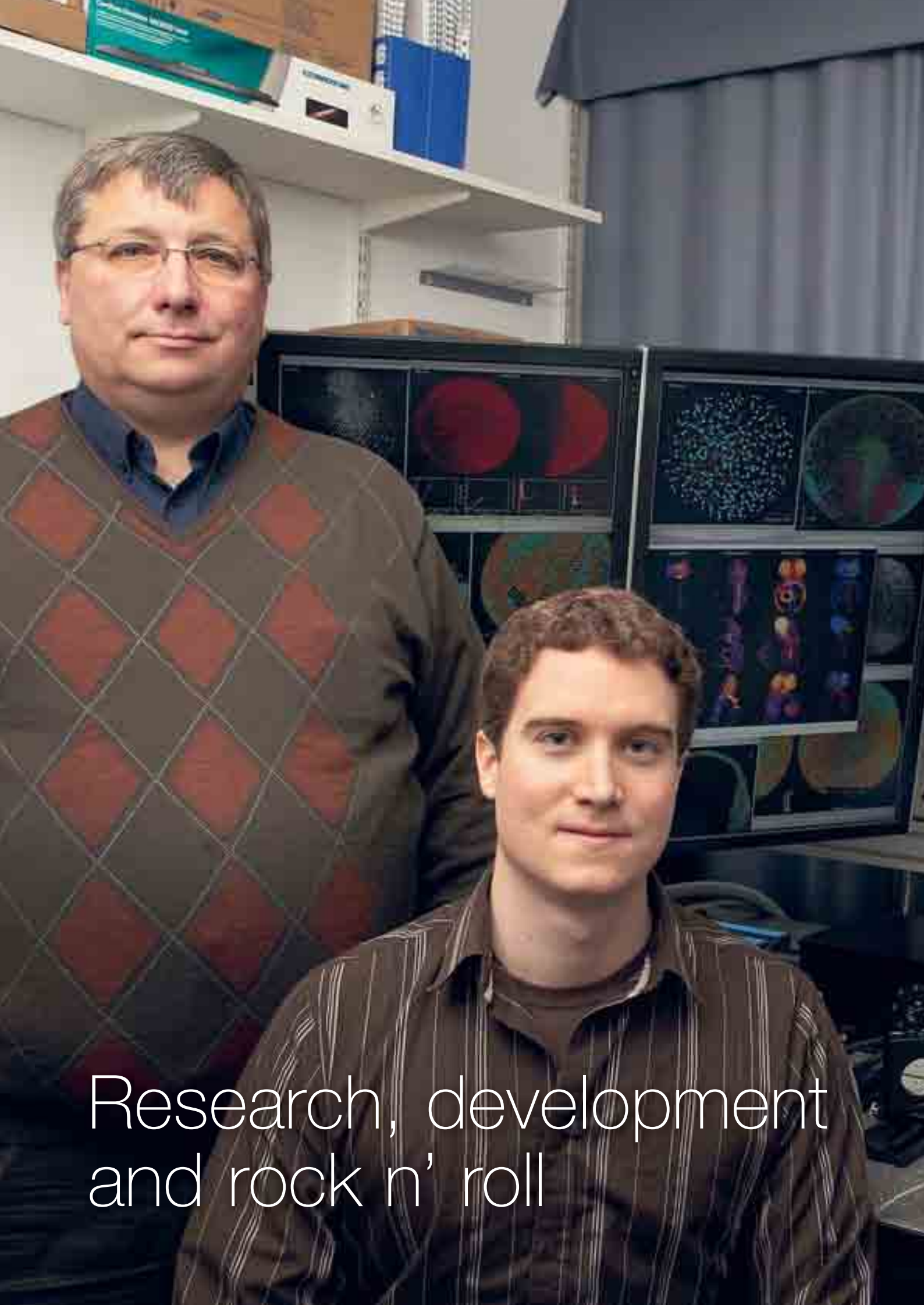
Sure enough, the model that best described the data involved a bistable, on-off switch, but whereas in the French flag model the switch operates through signal concentration, here the key parameter was space. "What is interesting is that what generates the extra step, the bistable switch, is the topology of the enzymatic network," says Eric.

Essentially, that network involves a protein called a kinase, activated by Ran, which phosphorylates (adds a phosphate group to) a substrate and is in turn dephosphorylated (has a phosphate group removed) by a phosphatase. The kinase signal is high around the chromatin, fading exponentially with distance from it. But the system is such that as soon as the substrate saturates the kinase – that is, there is not enough kinase to convert it all – it behaves in an all-or-nothing fashion: either all the substrate is phosphorylated, or all of it is dephosphorylated. *Voilà*: a bistable switch that shuts off at a certain distance from the signal source.

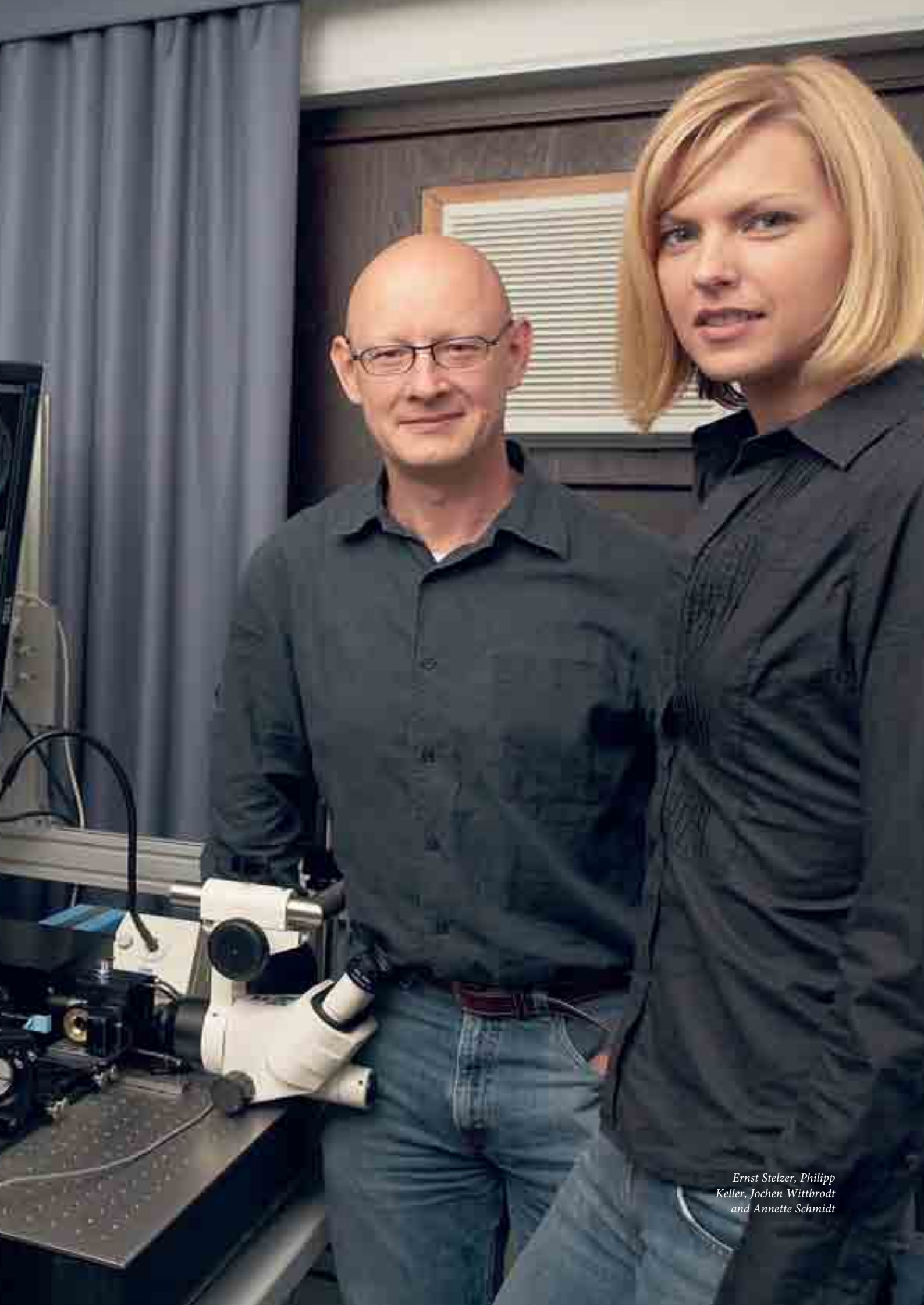
The beauty of Cytosim, Eric explains, is that the best-fit model makes predictions that can then be tested experimentally. In this case, for example, the researchers know the identity of the kinase, but not of the substrate or the phosphatase, but because they know their properties, they now have a good idea of what to look for. Thus they will be able to slot a few more important pieces of the chromatin pathway into place.

What François and Eric have described, in this bistable switch, is a nonlinear system in which inputs only produce an output – microtubule stability, in this case – when they reach a certain threshold. That is what makes them think that the fundamental concept behind the French flag model might have wider relevance. "Given that nonlinear conditions prevail inside the cell, we may find this same principle at work everywhere," says Eric.

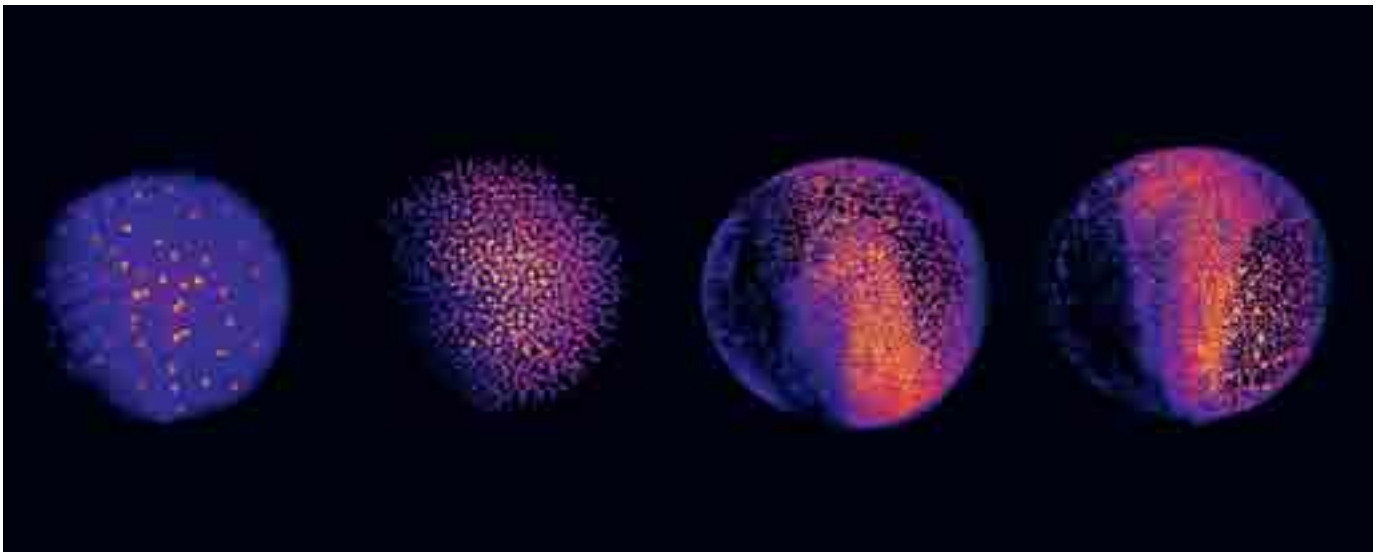
Athale CA, Dinarina A, Mora-Coral M, Pugieux C, Nédélec F, Karsenti E (2008) Regulation of microtubule dynamics by reaction cascades around chromosomes. *Science* **322**: 1243-7



Research, development and rock n' roll



Ernst Stelzer, Philipp Keller, Jochen Wittbrodt and Annette Schmidt



DSLM recording of zebrafish embryogenesis from early cell divisions to gastrulation.

Imagine Google Earth™ took new satellite photos at regular intervals, so that as well as viewing the whole planet and zooming in on different countries or cities, you could play back the sequence of photos and see what had changed over 10 or 20 years. Replace Earth with a developing zebrafish embryo, collect a few hundred thousand snapshots recorded over 24 hours, and you get what EMBL scientists have dubbed 'the digital embryo', commended by *Science* as one of the top ten scientific breakthroughs of 2008.

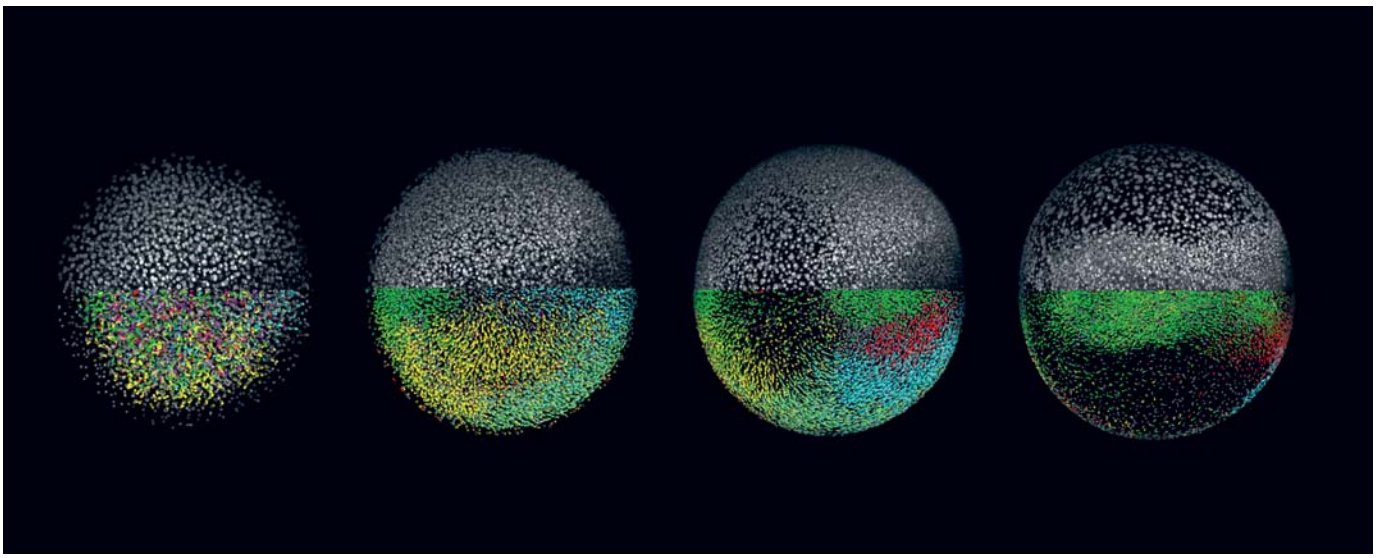
In true EMBL spirit, biophysicist Philipp Keller, biologists Annette Schmidt and Jochen Wittbrodt, and physicist Ernst Stelzer teamed up to develop a new technique which allowed them to be the first researchers to obtain a 3D representation of the first 24 hours of a zebrafish embryo's development. This novel approach has already enabled the scientists to make new discoveries and solve old controversies, but they are by no means planning to let it rest at that.

"It all started in the cafeteria, in early 2006," says Philipp. He was talking to fellow EMBL scientist Michael Knop, "and Michael asked me 'why don't you simply put an embryo in the microscope and track all the cells?' That got me thinking." Prompted by this conversation, Philipp wrote an outline for a new project in which he took a fresh look at single-plane illumination microscopy (SPIM), a 3D imaging technique developed by Ernst's group and featured in last year's Annual Report. In this project, Philipp and Ernst took the basic principles of SPIM and refined them in order to be able to record embryonic development; the result was digital scanned laser light sheet fluorescent microscopy (DSLM).

Because cells in a developing embryo are constantly dividing and moving, high-quality snapshots must be taken at very short intervals to track such changes. This meant scientists needed a microscope which combined high imaging speed with high image quality, whilst maintaining the ability to distinguish between cells sitting very close to each other. And, of course, in order to follow the embryo's development, the cells must be kept alive and behaving normally over a period of one or two days. So the microscope must not damage cells or interfere with developmental processes, and must maintain its properties over an extended period. The scientists had to be able to observe the cells throughout that period, so the microscope also had to avoid damaging the stain that scientists used to mark the embryonic cells.

The solution they found was to shine a very thin laser beam through the embryo, line by line, horizontally and vertically. By focussing the laser light into a single line, they minimised the damage to both the stain and the embryo itself. And by moving that beam along the embryo, line by line, it's as if they were cutting the embryo into slices thousandths of a millimetre thin, and identifying the cells in each slice. Because the scientists slice the embryo with light only, it can keep on living and developing, which means the 'slices' can be repeated periodically on the same embryo and compared to track changes. The EMBL scientists 'sliced' their zebrafish embryos every 60 or 90 seconds over a 24-hour period, obtaining around 400,000 images per embryo.

Having successfully developed a way to obtain the desired data, the next challenge was to devise a way to analyse it – all three terabytes' worth for each embryo! As Philipp notes, "three terabytes is not really accessible, so we needed an automated approach." They used clusters of computers working in parallel, both at EMBL Heidelberg and at the Karlsruhe



The montage shows the zebrafish digital embryo, in colour, and the microscopy data at different time points in zebrafish development.

Institute of Technology. "Each computer is given one snapshot of the embryo, and told to look for nuclei in that image," Philipp explains. Each nucleus represents a cell, so by combining all the responses from all the computers, scientists are able to generate their digital embryo: a visual representation of all the embryo's cells, where they are at a given point in time, where they move to next, and when and where they divide. The result is a 3D video of the developing embryo.

This ability to monitor the whole embryo for extended periods enabled Philipp, Jochen and Annette to shed new light on different stages of zebrafishes' embryonic development.

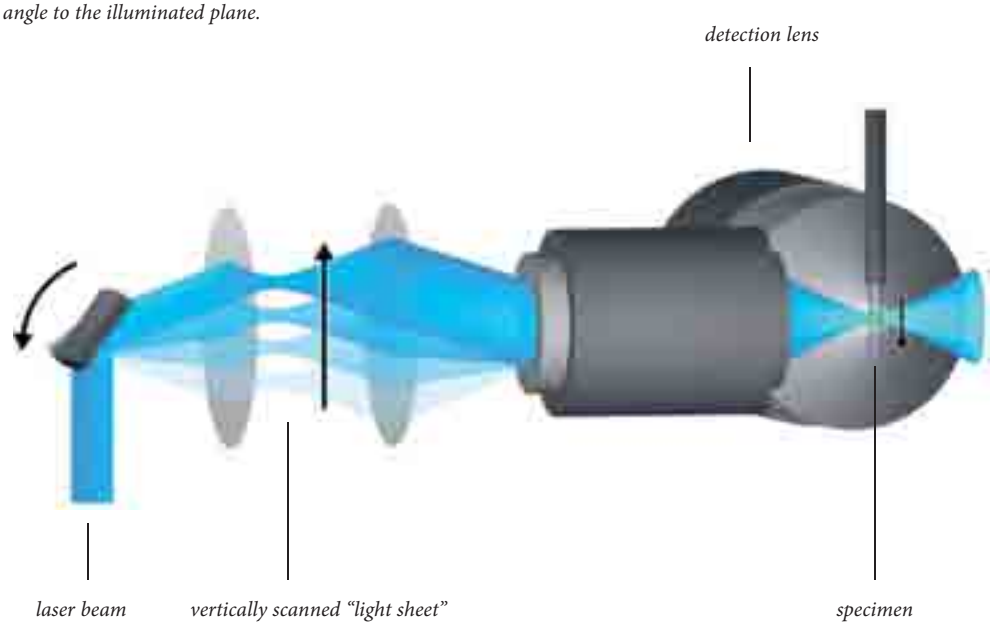
They monitored early development, a stage when a zebrafish embryo is basically a group of cells sitting on top of the yolk sack (the embryo's nutrient source). At first, cells in this group divide in a wave that moves out from the centre in all directions, like a ripple in a pool. By reconstructing the cell's movements, Philipp found that after a certain period, the pattern of cell division changes to a wave that moves out from the centre in one direction and splits up to continue along the periphery in two half-circles. The line between these two half-circles will later become the animal's body axis, defining the body's symmetry. So the scientists discovered that the zebrafish's body axis is defined before the embryo even starts expressing its own genome, at a much earlier stage in its development than was previously supposed.

They also studied gastrulation, the process by which cells from the embryo's single outer layer migrate inwards to form the different germ layers, which will eventually give rise to different types of tissue. "It takes textbooks two or three pages to describe this process, but when you see the movies of the digital embryo, you just get it," Annette says. What she and her colleagues discovered was that the textbooks are actually wrong. There has been an ongoing, sometimes fervent, debate in the field since the 1980s over how cells migrate to form these layers. The prevailing, textbook view was that cells involute – that is, they migrate to an opening in the embryo and roll over the margin to form a layer underneath, like the edge of a swimming cap rolling up around your head, on the inside. But some scientists argued that cells on the outer layer simply dive in, or ingress, to form a layer beneath. "With quantitative analysis," says Jochen, "we really settled the issue nicely: it turns out they were all right!" It's a question of where you look. On one side of the embryo, cells involute, while on the other side, they ingress. The controversy had arisen because different scientists were looking at different areas of the embryo without being able to determine exactly which area they were looking at, because they were unable to watch the whole process unfold. In overcoming these constraints, the EMBL scientists were able to uncover the truth.

The scientists have used the digital embryo as a developmental blueprint, by starting at an advanced developmental stage, marking the cells they know are involved in forming one of the zebrafish's eyes, and then tracking them back in time to find out where they originate. The EMBL scientists intend to do the same for other organs and tissues in the future.

With help from EMBL-EBI, they have already made their digital embryos publicly available on the Internet, along with tools for other scientists to analyse their own microscopy data. What they've achieved so far has sparked the interest of the scientific community, and even brought the researchers notoriety. Jochen says he felt like a rock star at the latest

Schematic illustration of a Digital Scanned Laser Light Sheet Fluorescence Microscope (DSLM): The laser beam illuminates the specimen from the side and rapidly scans a two-dimensional plane. Images are generated by fluorescence detection with a camera at a right angle to the illuminated plane.



zebrafish conference; Philipp has had invitations from scientific institutes around the world to present the digital embryo and discuss possible collaborations, and has also become an expert at talking to the press. According to Ernst, the secret to this success is open-mindedness. "The main thing," he vows, "is that you have to be willing to design completely new experiments to take advantage of the new technology, and it's hard to find people who can do that."

Ernst and his colleagues are certainly capable of thinking outside the box. In fact, ask them 'what next?' and you can feel the air sizzling with ideas – not only of how this technology could be applied and adapted to allow further studies of zebrafish and other species, but also of what should be done with the results.

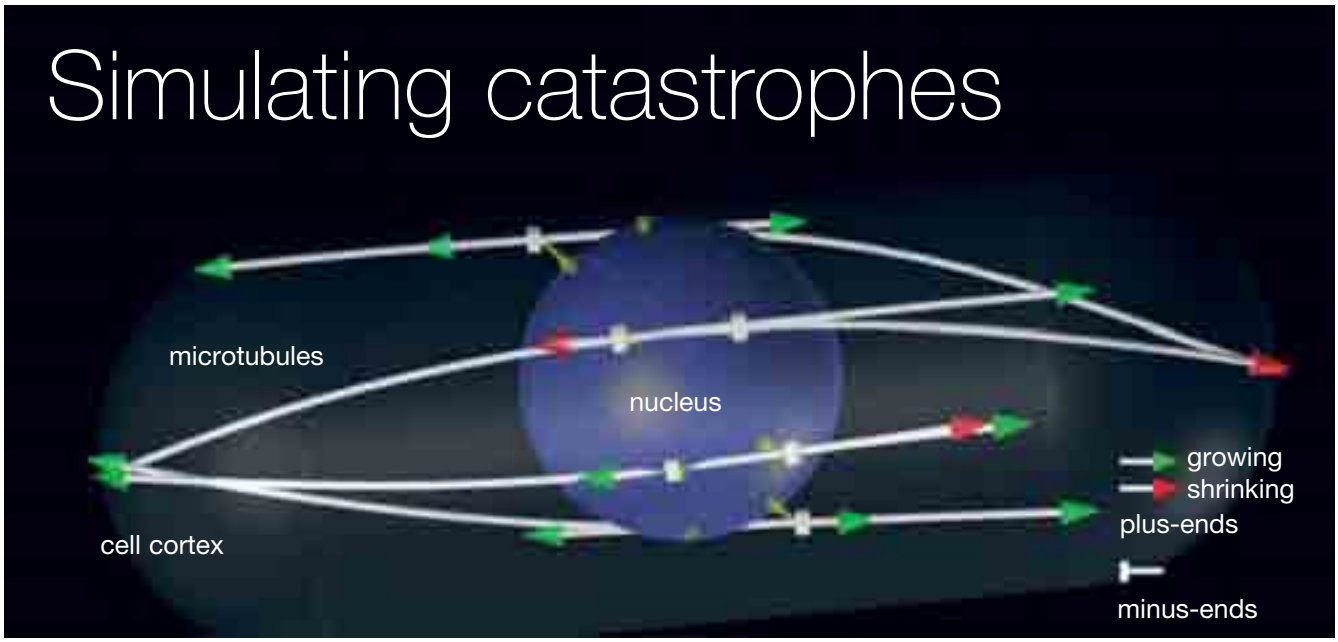
In the original Google Earth, as well as seeing the whole planet and zooming in on different places, users can add their own notes and markers and view those added by other people. Similarly, Jochen, Philipp, Ernst and Annette envision their digital embryos becoming what they call 'virtual embryos': resources in which other scientists could view developmental processes, zoom in for more information, and add their own annotations and results.

Setting up the digital embryo as a database in this fashion is an ambitious proposal, with many technical hurdles to overcome, but the scientists have already started discussing possible approaches with bioinformaticians at EMBL-EBI. And contribution from the community has also already started: scientists at the National Institute for Basic Biology in Japan are developing a way to create more lifelike simulations of cells to replace the dots representing nuclei in the current model.

In the long term, the EMBL scientists would like to expand the digital embryo's scope to other species, as this would enable scientists to quantitatively compare how different embryos develop, which would provide valuable insights into evolution. To that end, Philipp has recently been collaborating with colleagues from Scotland, Japan and the USA to explore the possibility of creating digital embryos for four other species – fruit fly, mouse, chicken and frog – and would like to continue to explore the possibilities in years to come.

So the digital embryo has lots of room for growth, and with growing numbers of people willing to help it develop, it appears to have a promising life ahead.

Keller PJ, Schmidt AD, Wittbrodt J, Stelzer EH (2008) Reconstruction of zebrafish early embryonic development by scanned light sheet microscopy. *Science* **322**: 1065-9



A computer simulation showing microtubules growing and shrinking.

Apparently, in cell biology, catastrophes aren't always bad. A microtubule catastrophe occurs when one of these straw-like protein filaments stops growing and starts shrinking. In fission yeast cells, far from being a disaster, this process is essential to ensure correct cell growth. Normally, these rod-shaped cells grow only at the tips – or poles – where microtubule catastrophes occur. "If these catastrophes occur elsewhere in the cell, it will grow abnormally and end up bent or branched," says François Nédélec, whose group at EMBL Heidelberg teamed up with Damian Brunner's, also in Heidelberg, to create a computer simulation of microtubule dynamics. This included modelling not only how microtubules grow and shrink, but also how they interact with the cell's inner lining – the cortex – and with the nucleus.

The prevailing view was that microtubule catastrophes must be regulated by a number of different molecules interacting at the cell poles, but the EMBL scientists' simulation enabled them to test another hypothesis: the possibility that microtubule catastrophes occurred at the cell poles due to the forces generated by microtubule growth itself. This alternative explanation had been previously proposed, but had not been tested due to a lack of experimental tools.

Nevertheless, fission yeast has been widely studied, so François, Damian and their groups had a wealth of experimental data at their disposal. This gave the scientists a good quantitative picture of what normal cells should do. They developed a simulation in which the forces generated during microtubule growth regulated microtubule behaviour, ran it thousands of times, changing the input values a little each time, and then looked to see if any of the results matched reality. Using this procedure, the scientists were able to generate cells that showed eight of the ten traits they had defined for normal cells based on the experimental data. "This indicated that microtubule growth and catastrophes were influenced by force," François says, "but those two mismatched traits made us suspect another influencing factor."

He and his colleagues speculated that long microtubules might be more unstable, and consequently more prone to catastrophes, than short microtubules. When the scientists adjusted their computer simulation to incorporate this effect of microtubule length, all the traits were matched. To make sure that these results were not due to the effect of length alone, the scientists ran a third simulation, in which they considered only the influence of microtubule length. The result: not all traits were matched. So by using computer simulations, François, Damian and their colleagues had discovered the determining factors behind microtubule growth and shrinking: force and length. "Our simulations proved that restricting catastrophes to the cell poles doesn't require any specific molecules to interact in those regions; microtubule dynamics are self-organised," Damian concludes.

By discovering how the machinery responsible for cell growth is positioned, the EMBL scientists have taken the first step towards understanding what determines a cell's shape. For their next step along that path, they would like to develop a model to distinguish the effects of force on different microtubule-associated proteins, as this would enable them to generate computer simulations of mutant cells with abnormal shapes.

Foethke D, Makushok T, Brunner D, Nédélec F (2009) Force- and length-dependent catastrophe activities explain interphase microtubule organization in fission yeast. *Mol Syst Biol* **5**: 241

When the exception becomes the rule

Life is full of surprises. For a long time scientists thought that genes, which carry the information needed to make proteins, were the only useful things to be found in DNA. Finding out that much more than the 2% of DNA that encodes proteins is transcribed into RNA created a big puzzle. How is all of this RNA made and what does it do?

Intrigued by these questions, Lars Steinmetz at EMBL Heidelberg and Wolfgang Huber at the EMBL-EBI took a closer look at transcription in yeast. The fairly simple model organism with a small genome allowed Zhenyu Xu and Wu Wei, two computational biologists in Lars' group, to simultaneously monitor all transcription events happening at different places in the genome. This global view of the process revealed that most promoters give rise not to one but to several transcripts. "But instead of firing in the same direction, we found that many promoters support transcription in two directions," notes Lars. "This was really surprising because transcription is conventionally considered a one-way process – comparable to reading a text."

In order to translate a sentence from a book, a translator needs to know not only where to start reading, but also in which direction the language is supposed to be read. Similarly, the cellular machinery involved in transcribing a particular sequence of DNA into RNA starts at defined promoter regions, and was thought to move only in one direction. But, as it turns out, the transcription machinery is one step ahead of the translator. It can read DNA code and produce RNA in two directions: upstream and downstream of a promoter. Scientists had occasionally encountered transcripts that appeared to have been generated in this way, but such 'antisense' transcripts were regarded as exceptions, and dismissed as noise. The EMBL scientists have now proved that such transcripts are very common and that bidirectional promoters are the rule, not the exception.

Having solved the mystery of how all the RNA found in cells is made, Lars and Wolfgang are now dealing with the second, more difficult question of what all these transcripts do. So far, all they have for answers are speculations. The transcripts could help regulate gene expression, or provide 'raw material' for evolution and acquire new roles in the future. "Or maybe we should ask not whether the transcripts have function but whether the act of transcription itself does," Lars says. Transcribing one stretch of DNA might help or hinder the transcription of a nearby gene, for example. "Most likely, the real answer is a combination of all of the above. Or it may come as yet another surprise," Lars concludes, as his team sets out to study the effects of each of the individual RNA molecules.

Xu Z, Wei W, Gagneur J, Perocchi F, Clauder-Münster S, Camblong J, Guffanti E, Stutz F, Huber W, Steinmetz LM (2009) Bidirectional promoters generate pervasive transcription in yeast. *Nature* **457**: 974-5

Location, location, location

Question: Imagine you're in IKEA, shopping for furniture to completely refit your new home. Do you: a) pull apart the shop displays and attempt to lug whole beds, cupboards and other large items intact out to your car, or b) get the items – still flat-packed in their wrappings – delivered to your home, where you assemble them in the right places as and when needed?

Hopefully, you picked 'b'; picking 'a' would just be silly. And it seems as though the cells of our bodies follow this commonsense rule. Biologists are realising that many of the proteins in our cells are not made in a centralised factory as once supposed, but built on-site at specific locations. Now, Anne Ephrussi and her team at EMBL Heidelberg have shed new light on this crucial process by discovering a new player involved in controlling where and when proteins are made.

The instructions for making proteins are encoded in DNA, which is housed in the cell's brain centre, its nucleus. To make a protein from these instructions, the cell first makes a copy of them using a chemical relative of DNA, called messenger RNA. The cell reads the instructions in the messenger RNA to stitch together the building blocks, or amino acids, in the right order to make the protein, in a process called translation.

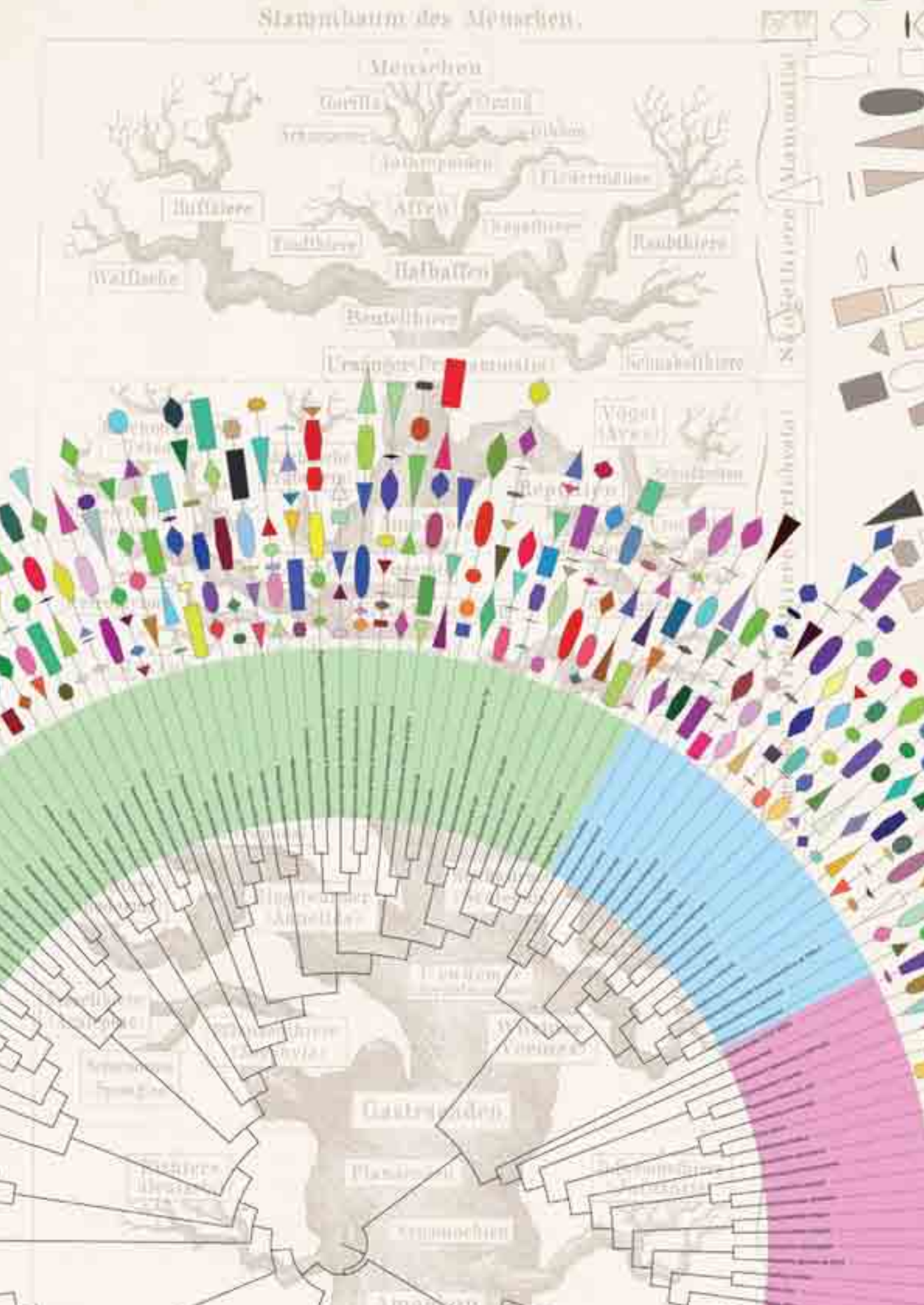
It's now becoming clear that many – if not most – of the cell's messenger RNAs are carried to where they are needed within the cell before they are translated into proteins. "It's about building machines where you want them," explains Anne. There are a number of reasons why cells do this. Some proteins, for example, can be toxic to a cell if they are produced in the wrong place. Others can act as a kind of grid reference system, localising themselves like signposts in particular parts of the cell, indicating, for example, which end of a cell is its top or bottom. If the cell in question is a fertilised egg, localised RNAs and proteins help an embryo develop normally by, for example, marking out its head end from its tail.

Anne and her team are working on one such egg protein, called *oskar*, found in the tail end of fruit fly eggs. If *oskar* is not restricted to the tail end but is also present at the opposite end, the result is a headless embryo. "If you express it in the wrong place you do disastrous things," says Anne.

Like all localised RNAs, *oskar* messenger RNAs are parcelled up with proteins that help target them to the right place – a bit like the packaging and customer address labels on IKEA furniture. These packages, called ribonucleoproteins (RNPs), can also contain proteins that prevent the translation of the RNAs until they reach their destination. RNPs are complex and so large that they can be seen with a light microscope.

To find out more about how *oskar* translation is controlled, Anne and her team designed an experiment to look for proteins that participate in that process. They identified one, called PTB, and found that it binds to *oskar* messenger RNA and blocks its translation. Intriguingly, PTB is also involved in packing multiple *oskar* RNA molecules together into large RNPs. This is very exciting, says Anne, because it shows there is a link between the way RNAs are packaged and how their translation is controlled. Now the team plans to study how the molecular motors that move RNPs around in the cell are attached, and how they move the RNPs to the right destination. Indeed, you don't want to be left standing in front of IKEA with your bed in a box!

Besse F, López de Quinto S, Marchand V, Trucco A, Ephrussi A (2009) *Drosophila* PTB promotes formation of high-order RNP particles and represses oskar translation. *Genes Dev* **23**: 195-207


Stammbaum des Menschen.
Menschen
Affen
Halbaffen
Beutelthiere
Raubthiere
Wallfische
Vögel
Reptilien

Biology in time

"Why cannot we move in Time as we move about in the other dimensions of Space?" Ever since H.G. Wells posed this question in his novella *The Time Machine*, science-fiction writers have experimented with the idea of time travel. What if we could travel to and fro along the fourth dimension, just as easily as we navigate the other three?

In a way, we don't really need to build machines to travel in time. Our bodies (and indeed, all organisms) are time machines of a sort, although admittedly, they only offer a one-way ticket. Just as it is vital to understand how biological processes operate in three-dimensional space, so it is crucial to learn how they are organised in time.

Timing is everything in many aspects of life. Molecular interactions within cells have to be organised in the right order and pace to ensure that they work properly. Such interactions can take place in tiny fractions of a second, and are extremely hard to track. But biology also operates on far longer timescales. Evolutionary change happens over millions of years. Life itself originated billions of years ago.

At EMBL, researchers have been studying biological process on all these time scales, from millisecond to millennium. Some, for example, are looking at how cells order the destruction of RNA to prevent errors in protein production. Others are working on proteins, such as p53, which defend us against the damage that comes with the passage of time. They can capture these moments more easily thanks to the work of EMBL colleagues who are developing technologies such as time-lapse microscopy movies and methods that let biologists watch how a molecule's structure changes in real time.

Yet more teams are investigating how some of these tiny, fleeting events end up controlling biological change over the long term. Certain genes and proteins that regulate how an organism's body shape unfolds, or how its cells develop over hours or days, can themselves change and drive the course of evolution over thousands and millions of years.

Science itself is also at the mercy of time, and a number of EMBL scientists are finding ways of speeding up the pace of research, such as developing new, more powerful computational tools. All this work will deepen our knowledge of biology, but may also give us the chance to take a metaphorical trip back into the deep history of life, to understand more about how it evolved. Perhaps we might also predict, or even alter, its course in the future. Given all of that, who needs a time machine?

Gáspár Jékely and Detlev Arendt

A real eye opener

"To suppose that the eye with all its inimitable contrivances [...] could have been formed by natural selection seems, I freely confess, absurd in the highest possible degree." What sounds like words taken straight out of a creationist's mouth is actually a quote from the founding father of the theory of evolution, Charles Darwin, himself. As Darwin confesses in this passage from *On the Origin of Species*, the eye has long been considered the Achilles' heel of his theory of evolution. Over the years it has served as the prime showcase for advocates of creationism and intelligent design theories. How could an organ as complex and perfect have arisen by random mutations and natural selection? And how can the large variety of eyes that we find across the animal kingdom be reconciled with the idea of a common origin?

Developmental biologist Detlev Arendt and his team at EMBL Heidelberg prove that answering these questions in the light of evolution is entirely possible. For more than 10 years they have been studying the evolution of the central nervous system in a marine rag-worm, called *Platynereis dumerilii*, for good reason: "*Platynereis* is a living fossil," says Detlev. "It still lives in the same environment as its ancestors around 600 million years ago and has preserved many ancestral features." Among these ancestral traits, as Detlev's previous research revealed, are those genes and cells that today make up the eyes of humans, vertebrates, fish and insects. In 2002, Detlev's group discovered that the gene Pax6, which controls the development of eyes and other sensory organs in humans, is also active in the eyes of the rag-worm, confirming early, evolutionary traces of more sophisticated eye systems. A few years later, in 2004, his group identified two types of cells in *Platynereis* as photoreceptors – cells that, thanks to a pigment molecule also found in vertebrates, are sensitive to light. "This means the last common ancestor that *Platynereis* shares with vertebrates, fish and insects must have had at least two types of photoreceptor cells representing key components of more recent visual systems," explains Detlev.

Studying *Platynereis* is thus probably the closest scientists can get to figuring out what eyes in bilaterian animals looked like when they first evolved. Because the eyes of their larvae are even more rudimentary than those of the adult worm, the round, only 0.2 millimetres long *Platynereis* larvae have become Detlev's main research subject. They live as zooplankton in the shallow waters of all moderate and tropical oceans and have the smallest and simplest eyes that exist – so basic that they are called 'eyespots' rather than eyes. The eyespots consist of only two cells: a light sensitive photoreceptor cell and a pigment cell. While this minimalistic arrangement is not enough to 'see' in the strictest sense – that is, to create images of objects – it allows the larvae to distinguish between light and dark and to determine the location of a light source. The latter ability is conferred by the pigment cell. It absorbs light and casts a shadow over the photoreceptor cell. The shape of the shadow varies according to where the light comes from and allows the larvae to work out its direction.

The directional sensitivity to light is the basis for a remarkable behaviour of zooplankton: phototaxis, or swimming guided by light. During dusk and dawn, the light guides zooplankton to the water surface where

The circular movement of this toy propeller is similar to that of P. dumerilii larvae swimming towards the light.

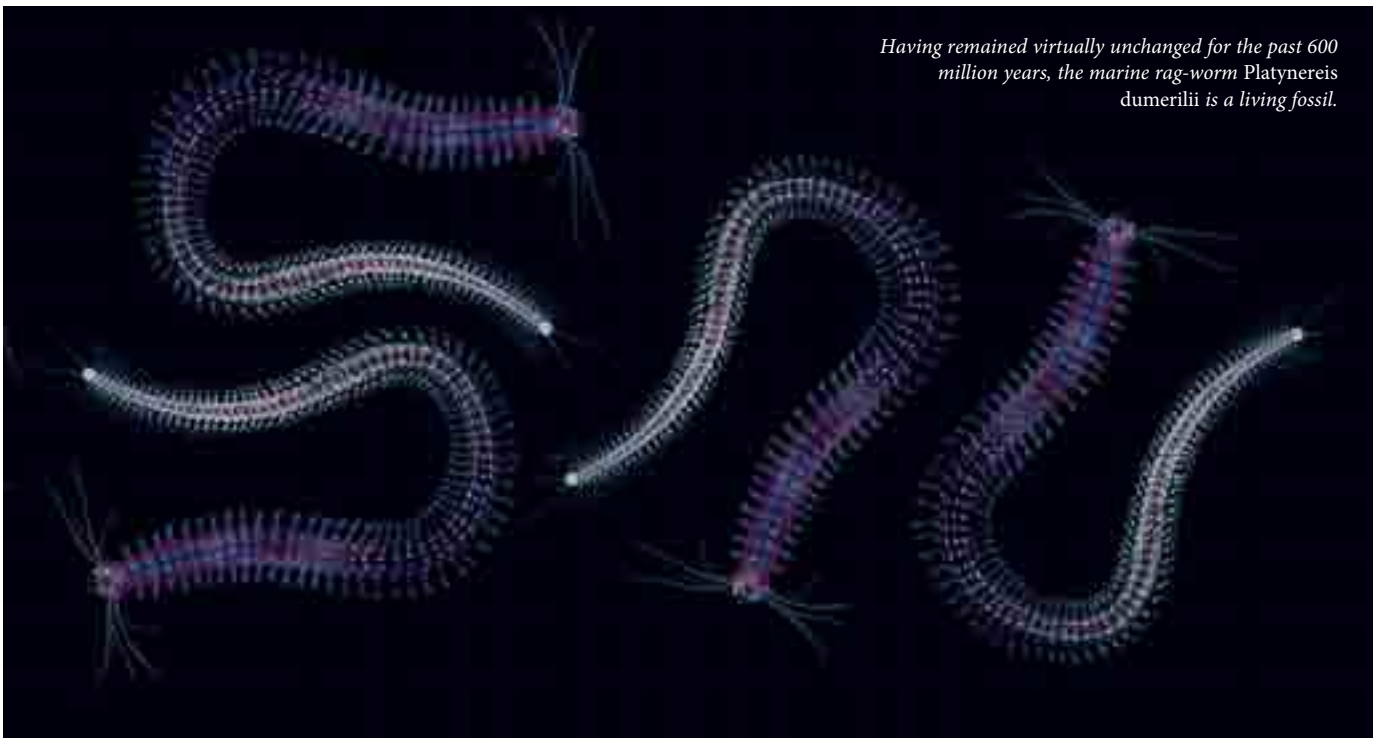
waves disperse them. An even distribution enhances their chance of survival, because it reduces competition for food and helps them to avoid predators. But during the day the sun is so strong that small organisms can become burned at the water's surface. Negative phototaxis, away from the light source, makes sure this does not happen. The daily vertical migration of zooplankton in the sea is the biggest biomass transport on Earth and has massive ecological implications.

Detlev and postdoctoral fellow Gáspár Jékely were curious to find out if something as simple as the eyes of the zooplankton larvae could be the driving force behind such a complex ecological phenomenon. They performed laser nanosurgery – a method developed by EMBL group leader Ernst Stelzer – to remove both of a larva's eyespots. "The result was as clear as it could be: no eyespots, no phototaxis," says Gáspár, who now heads a lab at the Max Planck Institute for Developmental Biology in Tübingen, Germany. "The logical next step for us was to find the mechanism that couples light sensing to motion."

Platynereis larvae move with the help of thin, hair-like projections called cilia that are located in a band around the equator of the spherical organism. The beating cilia displace water and propel the larva forward in a helical fashion. But how can this motion be directed towards light? The key is a direct connection between the light-sensing photoreceptor and the ciliated cells. By injecting a dye into a larva's photoreceptor, Gáspár found that its neural projection, its axon, forms a synapse with the neighbouring ciliated cells. "Essentially there is no nervous system in between," he explains. When light falls onto the photoreceptor, an electrical signal is generated that encodes the direction of the light source and the command to alter the beating frequency of the cilia. The signal travels down the receptor's axon until it reaches the synapse where it is relayed to the ciliated cells with the help of acetylcholine, a chemical messenger that is involved in the control of movement and muscle activation in other animals and also humans.

Shining light selectively on one eyespot makes the adjacent cilia slow down. The scientists measured the changes in beating frequency, and fed the data into a computer simulation that was developed by François Nédeléc – a group leader in EMBL Heidelberg's Cell Biology and Biophysics Unit – to study the larva's swimming behaviour. "It turns out that minimal changes in the beating of the cilia are enough to direct the swimming trajectory of the organism towards light," Gáspár summarises. "The predictions of the model exactly match what we see in reality." The computer model also showed that the larvae's helical mode of swimming is a prerequisite for successful phototaxis and increases the precision of navigation.

The combination of experimental and computational methods provided the first mechanistic account of phototaxis in a zooplankton larva, a mechanism that is likely shared by other marine invertebrate larvae, including those of other worms, jellyfish and sponges. A piece of basic research with big eco-



Having remained virtually unchanged for the past 600 million years, the marine rag-worm Platynereis dumerilii *is a living fossil.*

logical implications, as Detlev points out. "Climate change is a big concern at the moment. To know how it affects our oceans and the life in them, we first have to understand how plants and animals live, move and behave in the sea."

But scientists' understanding of eye evolution will also benefit from the new insights. "Phototaxis is an evolutionarily very old behaviour and one of the most fundamental tasks eyes have to fulfill in the sea. That means understanding phototaxis unravels the first steps of eye evolution. In fact, we assume that the first eyes in the animal kingdom evolved for exactly this purpose," says Detlev.

So, the findings also shed light on what Darwin referred to as 'proto-eyes', the evolutionary precursors of today's eyes and visual systems. The close coupling of light sensor to motor (photoreceptor to cilium) might well have been one of their key features and an important, early landmark in the evolution of animal eyes. Other scientists go even further in their interpretation of the results. John Spudich, a photobiologist at the University of Texas in Houston, thinks that Detlev's 'elegant study' identifies a photosensory mechanism that may be the "key evolutionary intermediate suggested by Charles Darwin's reflections on the evolution of human vision". What's for sure, however, is that Detlev's research has served to make the common origin of the eye and its evolution through natural selection plausible and tangible. With this, he has claimed back an important piece of territory for evolution from the hands of creationism.

Platynereis dumerilii's *eyes are among the most primitive in the animal kingdom, and can provide insights on the evolution of more complex eyes like our own.*

Jekely G, Colombelli J, Hausen H, Guy K, Stelzer E, Nédélec F & Arendt D (2008) Mechanism of phototaxis in marine zooplankton. *Nature* **456:** 395-9


Niels Gehring and Matthias Hentze

Reduce, re-use, recycle

Long before the green movement came up with the slogan 'reduce, re-use, recycle', cells had already implemented the so-called 3Rs into their inner workings. Many key molecules are only present in limited numbers. This provides cells with better control of when and where processes take place, but it also means that there have to be efficient ways to recycle and re-use molecules.

To better understand one such way is the goal of Matthias Hentze's group at EMBL Heidelberg. In collaboration with Andreas Kulozik's team at the Molecular Medicine Partnership Unit (MMPU), a joint unit between EMBL and the University Clinic Heidelberg, they study the translation of information from gene to protein, an extremely important process for cells.

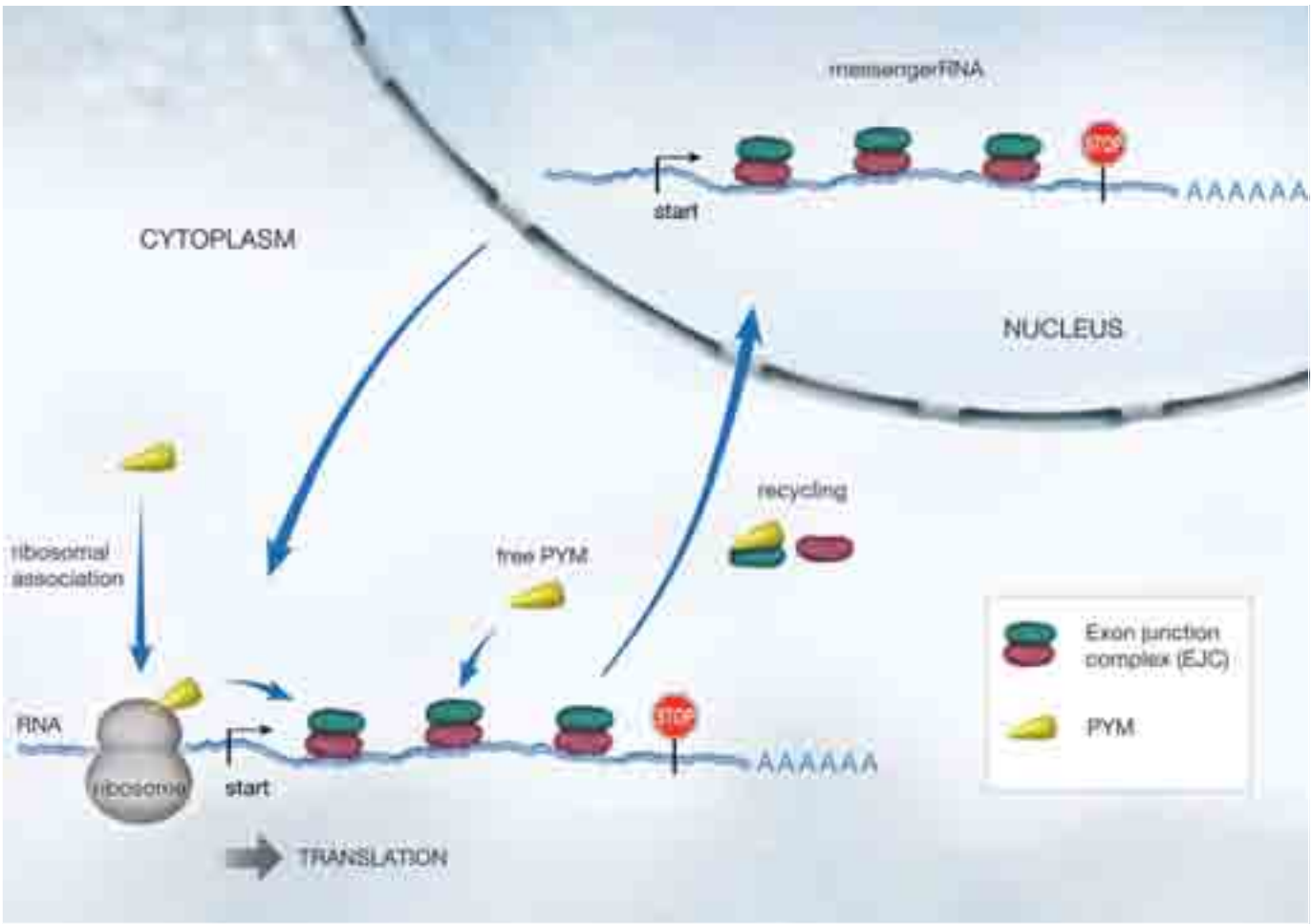
Unfortunately, translation is error-prone and as errors can lead to diseases, several proofreading mechanisms check for mistakes along the way. One of these mechanisms, called nonsense-mediated decay (NMD), is based on a molecular tag that is attached to messenger RNAs, an intermediate step in the translation from DNA to protein. The tag, called exon junction complex (EJC), tells the NMD machinery if an RNA is faulty or potentially dangerous and should be degraded.

Overall, a cell would need to mark around 400,000 sites with EJCs, but it only has 10,000 copies of one of the marker's components. This means EJCs must be broken down as soon as possible, so that their components can be re-used.

"For a long time everybody had assumed that ribosomes, the large structures that carry out protein assembly, simply iron out the EJCs as they pass," says Niels Gehring, a staff scientist in Matthias' lab. This logical explanation turned out not to be quite right. Niels discovered that cells lacking a protein called PYM do not disassemble EJCs properly so that the marker accumulates on messenger RNAs. In the opposite scenario, PYM is present in excess. EJCs no longer bind to the RNA and NMD is inhibited. Both results suggest that PYM is responsible for EJC disassembly and carries out the first step of their recycling.

> "This research is a showcase of how basic and applied research often go hand in hand," says Matthias.

PYM can be found on its own in the cell, but most of the time it tends to associate with ribosomes. This explains why – and how – EJCs are removed when the ribosome goes by, and could also ensure that they are not removed too early. If that happened, NMD would be compromised, as the proofreading machinery would have no markers to guide it. This in turn could have wider consequences, as NMD influences how diseases such as thalassaemia, Duchenne's muscular dystrophy and cystic fibrosis manifest themselves.



With the help of the protein PYM, cells recycle the limited number of exon junction complexes.

"This research is a showcase of how basic and applied research often go hand in hand," says Matthias. "Our findings fill an important gap in the understanding of a vital cellular process and at the same time have medical implications."

The research was conducted in the MMPU, a partnership designed especially to promote the cross-fertilisation between biologists and clinicians. The concept works extremely well, according to Matthias. "There are many examples of how basic research findings have prompted clinical studies in the unit in the right direction. In this case, however, it was the other way round. The interest in NMD originated in the clinic and guided our experiments." The common goal of both biologists and clinicians now is to find ways to modulate NMD pharmacologically to influence the development and course of genetic diseases.

Gehring NH, Lamprinaki S, Kulozik AE, Hentze MW (2009) Disassembly of exon junction complexes by PYM. *Cell*, **137**, 536–548


Andrew McCarthy

Beam me up

Biologists around the world are set to benefit from new state-of-the-art technological developments at EMBL's Hamburg and Grenoble outstations. The work will broaden access to a structural biology technique known as small angle X-ray scattering, or SAXS, which is playing a key role in helping researchers understand the structure and physiology of proteins that are otherwise hard to study. As well as increasing the availability of the technique, the developments are making SAXS experiments more efficient and easier to perform, and are improving the quality of the data they produce.

The work is being done by Andrew McCarthy's group at EMBL Grenoble, together with Dmitri Svergun's group at EMBL Hamburg, and forms two main projects. The first involved converting one of the beamlines at Grenoble from its previous use as an X-ray crystallography beamline into one devoted to SAXS for use in biology. Thanks largely to the work of Dmitri's team, SAXS is becoming increasingly popular as a technique in structural biology and demand for its use has increased. "One of the main motivations for this work was the oversubscription of the SAXS beamline in Hamburg," explains Adam Round, a staff scientist in Andrew's group. Although there is a SAXS beamline in Grenoble, it is mainly used by physics and materials scientists.

Andrew, Adam and their colleagues at the neighbouring European Synchrotron Radiation Facility (ESRF) began by modifying one of the beamlines, ID14-3, for SAXS. The conversion is now complete and the beamline has been accepting users (and has been used by 59 external users) since November 2008. "The beamline is open to anybody who has an interest," says Andrew. "We aim to offer people beamtime for their experiments within six to eight weeks of their application."

His team is also working in collaboration with the Institut Laue Langevin (ILL) to give users combined access to the ILL's small-angle neutron scattering facility. This technique offers data that nicely complements that obtained by SAXS, and combining the two onto one platform means users can get the all the data they need from a single visit to the Grenoble site.

The second project involves the development of a robot that can automatically change the test solutions on a SAXS beamline. The problem with changing samples manually is that users normally only have a limited amount of time to work on a beamline, and changing samples by hand is laborious, time-consuming and, when performed by scientists exhausted after many continuous hours of data collection, prone to human error.

So Adam and his colleagues developed a prototype of an automated sample changer, together with colleagues at the Fraunhofer Institute for Manufacturing Engineering and Automation in Stuttgart, Germany. The machine is faster, more efficient and more stable than human users, meaning scientists can collect more data, of higher quality, than before. The prototype was tested on the Hamburg SAXS beamline X33. "It was a great success," says Adam.

Florent Cipriani and his team at EMBL Grenoble have now teamed up with Adam to refine this design. The second-generation prototype is smaller, faster, more efficient and will require less sample volume per measurement. Adam hopes it will undergo further evaluation at Grenoble by May 2009, before being installed there, with a duplicate system planned for the Hamburg SAXS beamline. "We're working hard on it," he says.

Charles Girardot, Julien Gagneur, Luis Gutiérrez, Katarzyna Oktaba, Jürg Müller and Eileen Furlong

Thanks for the memory

Henry Molaison, better known as H.M., was probably the world's most famous amnesiac. Following a brain operation intended to cure him of epilepsy, he lost the ability to lay down new memories. People used to wonder what that meant for him: how could a man with no memory have a sense of his own continuity over time – in other words, a sense of self?

The idea that memory is essential to identity also applies to cells. The entire genome of an individual is present in all its cells, but thanks to the existence of mechanisms for regulating gene expression, different cells express different sets of genes, and so assume different identities. Hence a muscle cell is not the same as a neuron, which is different again from an immune cell.

So far so good, but cells divide constantly throughout an animal's life. If the identity of a cell is not written in its genes, the substance of inheritance, how does a daughter cell remember and assume its parent's identity? In particular, how does it do so when the instruction that drove the cell to adopt a particular fate in the first place is no longer present in later stages of development? The fact that cells do not revert to a blank state after each division suggests that some form of cellular memory is at work.

At EMBL Heidelberg's Gene Expression Unit, Jürg Müller studies the nature of that cellular memory. In recent years, his group has discovered that cellular memory is encoded in chromatin, the combination of DNA and protein that makes up chromosomes. Jürg's group studies Polycomb group (PcG) proteins, a family of molecules that form part of the cellular memory system in both animals and plants. PcG proteins place chemical marks on chromatin components, and Jürg thinks these marks contribute to the memory of cellular identity that survives cell division and is propagated. They also instruct the cell's transcription machinery either to transcribe a gene or to bypass it, leaving it silent.

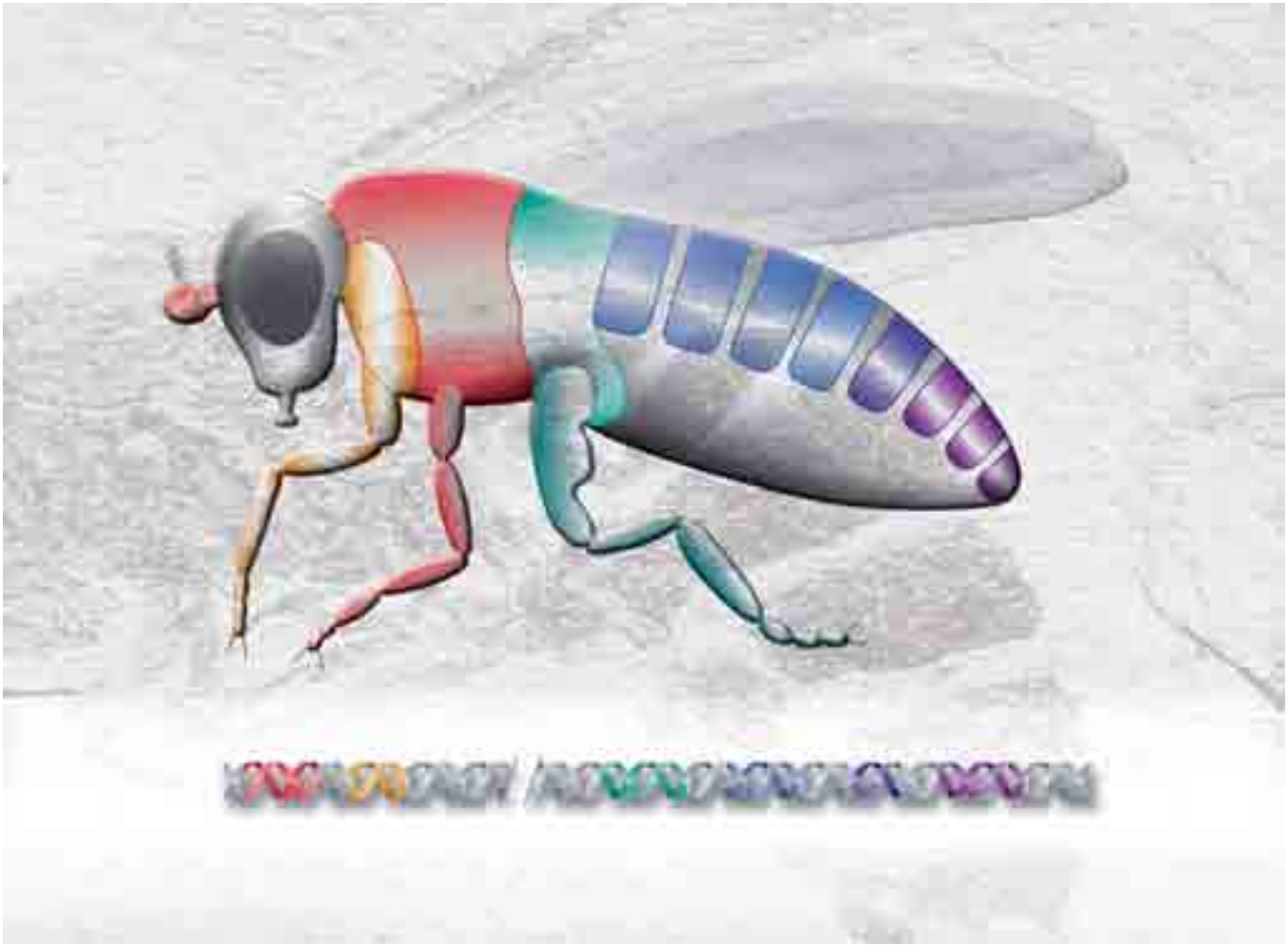
Among the best-known targets of the PcG proteins are the *HOX* genes, which are vital for specifying the body plan of an animal from early in development. In order for *HOX* genes to impose different identities on cells in different body regions, and hence to drive the formation of different anatomical structures along the main body axis, different *HOX* genes must be activated in different cells. "However, it is equally important that *HOX* genes are kept inactive in cells where their products are not wanted," Jürg explains. "The function of the PcG proteins is to freeze this transcriptional 'off' state as it was set in the early embryo, and to maintain that state, in the case of the *HOX* genes, throughout the animal's life."

The members of Jürg's group have identified several of the genes that encode PcG proteins in the fruit fly *Drosophila*. They have also shown that these proteins exist in large complexes that bind to target genes – that is, genes to be silenced – at specific DNA sequences called Polycomb Response Elements (PREs). Exactly how they get to those genes and what they do once they are there was a puzzle for a long time, since only one PcG protein, Pho, binds directly to DNA. However, studies by Jürg's and other groups have now shed light on that problem. "We think that the complex that contains Pho is a platform that allows the other PcG protein complexes to dock onto their target genes," he says. These other complexes then place the memory marks on chromatin that make the 'off' state of target genes heritable.

In 2006, Jürg's group made the unexpected discovery that PcG proteins bind to *HOX* genes whether or not those genes are transcribed, but that the pattern of memory marks on the genes is different depending on whether they are active or inactive. To try to find out more about how PcG proteins work, Kasia Oktaba, a PhD student in Jürg's lab, used a method called ChIP-on-chip [page 35] to look at the target genes of the Pho protein complex across the entire fly genome.

With support from bioinformaticians Julien Gagneur and Charles Girardot in the group of Eileen Furlong, who is Joint Head of the Gene Expression Unit at EMBL Heidelberg, Kasia identified more than 100 target genes of the Pho complex. Her analyses suggest that, as with the *HOX* genes, the Pho complex serves as an assembly platform for other PcG protein complexes at these genes.

What were those target genes? Unexpectedly, the researchers found that they included genes that control the cell cycle, which may explain why, in PcG mutants, that cycle goes awry,



Specific genes must be turned on (and others off) in different parts of a fruit fly embryo, to give rise to an adult with differentiated tissues.

cell proliferation runs out of control and tumours form. More generally, they showed that PcG proteins are more versatile than had been thought, since cell cycle genes are expressed in all cells, cyclically. "So the system is obviously used to control expression of certain target genes in a more dynamic way, and not just by freezing a gene in the 'off' state," says Jürg.

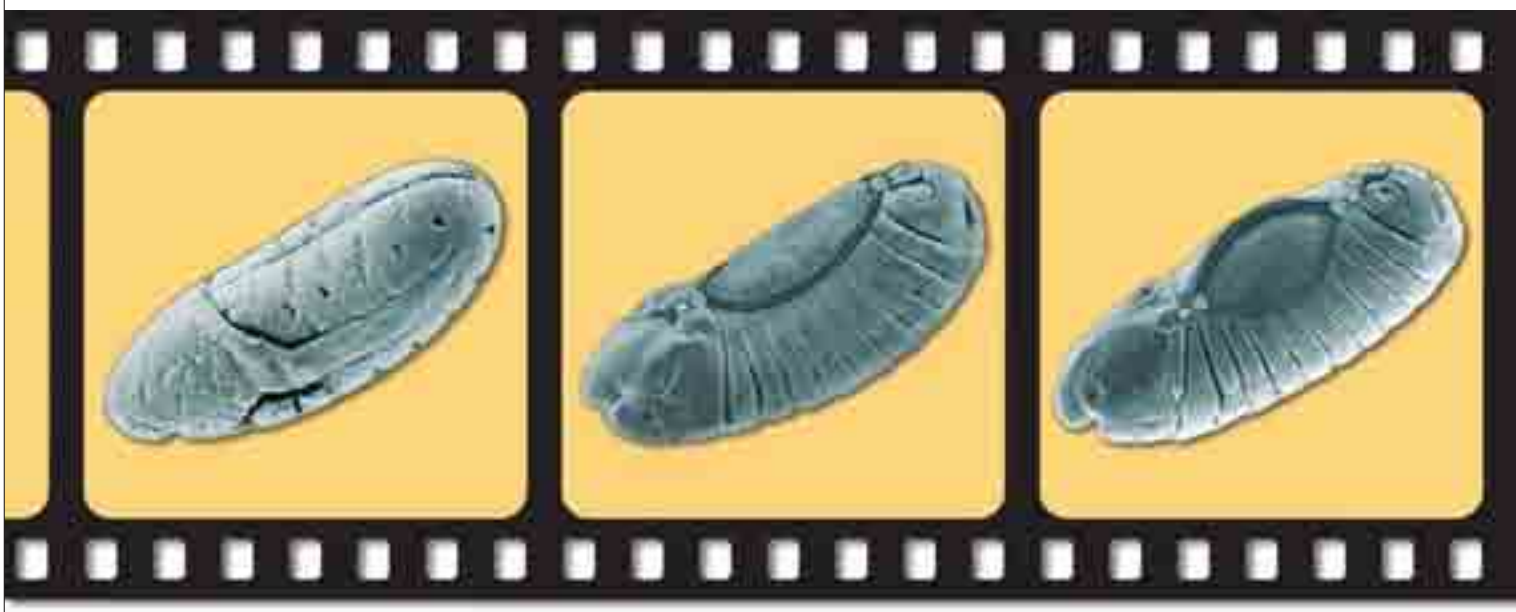
The group also found that genes responsible for the three-dimensional patterning of limbs are under PcG control. "If PcG proteins are removed from cells in developing limbs, these genes are expressed in the wrong place and the cells forget what they were supposed to become," says Jürg. For example, the front part of the wing develops as a mirror image of the back part, and the bottom part mirrors the upper part, whereas these wing sections normally develop according to distinct patterns.

Other researchers have used ChIP-on-chip to show that PcG protein complexes bind to the equivalent developmental regulators in stem cells in mammals. It has been known for a long time that similar genes control the body plans of mammals and flies, Jürg says, but the finding that PcG protein complexes regulate the same sets of target genes in the two lines came as quite a surprise, since many other aspects of the regulation of these genes have changed during evolution. Mammalian PcG proteins are also implicated in cell cycle regulation, he says, though in the context of the cell cycle, PcG proteins have different functions in mammals and flies.

Could these two roles of the PcG system – the freezing of a transcriptional state, and the regulation of the cell cycle – ever be combined, to make some cancers heritable? After all, as H.M. never knew, memories can be good as well as bad. For now, it's an open question. "PcG proteins have indeed been implicated in cancer progression in humans and in mouse models," says Jürg, adding that the evidence suggests that their expression is upregulated as cancer cells progress from a benign to a malignant state. However, he adds, "Whether the PcG system is used to make the cancer state of cells heritable is not known."

Oktaba K, Gutierrez L, Gagneur, J, Girardot C, Sengupta AK, Furlong EE, Müller J (2008) Dynamic regulation by polycomb group protein complexes controls pattern formation and the cell cycle in *Drosophila*. *Dev Cell* **15**: 877-89

Development in slow motion



In time-lapse imaging, film frames are captured at a certain interval, say two minutes, and then played back as if they followed on seamlessly from one another. The effect is to concertina, or speed up, the event being filmed. Think of time-lapse photographers' favourite subjects: cloudscapes, or flowers opening. Time-lapse imaging is also popular at the microscopic level, and biologists frequently use it to show more clearly how a process evolves. But distorting reality comes at a cost, and time-lapse imaging can lose or degrade features of a process that would show up more clearly in real time.

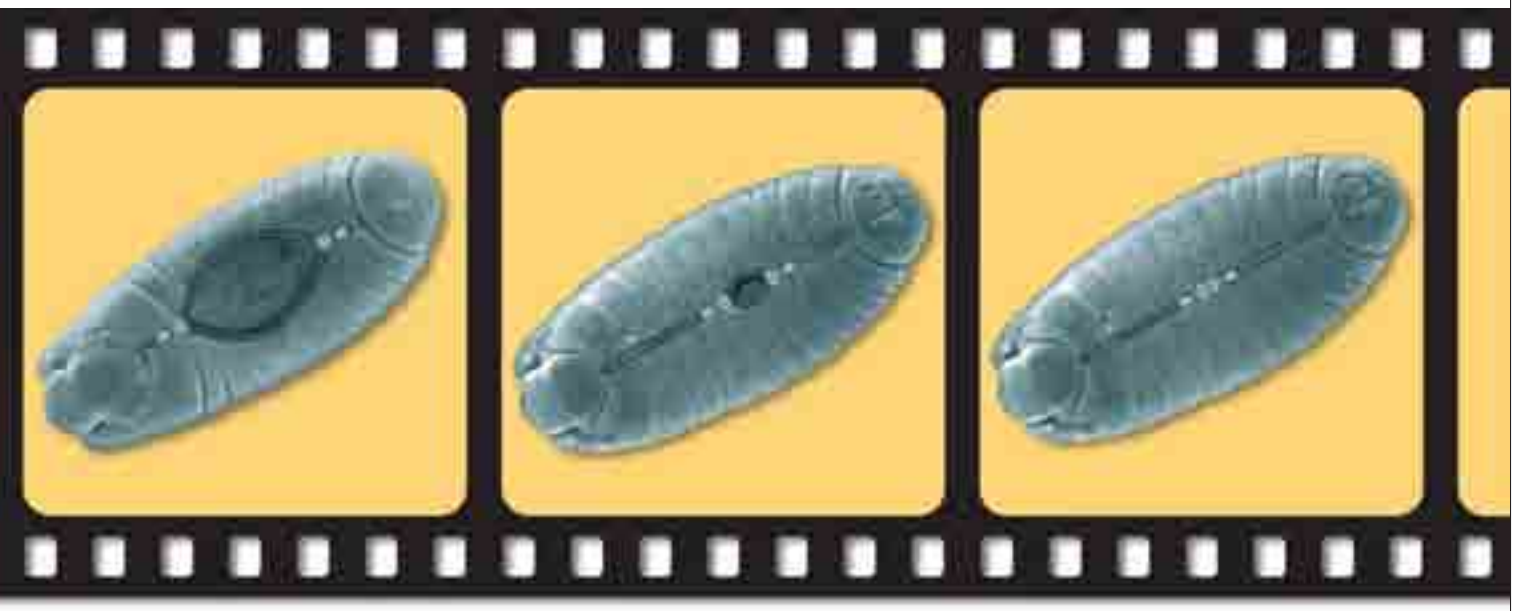
This is what happened recently in Damian Brunner's lab at EMBL Heidelberg. Damian is interested in dorsal closure in the fruit fly *Drosophila*, a process that takes place in the final stages of embryonic development. A large opening remains in the epithelium or skin covering the embryo's back, until the epithelium stretches and the hole closes from both ends, like a zipper.

Dorsal closure is considered a model for wound healing, and Damian is interested in how it comes about. Time-lapse movies of the three-hour process suggest that it starts with the contraction of cells that fill the opening, called amnioserosa cells. At about the same time, a band or cable of the protein actin, which runs around the opening, causes the epithelial cells at its edge to stretch across it, until eventually cells on either side of that opening meet each other in the middle.

Based on those accelerated movies, researchers thought that the actin cable was behaving like a drawstring, tightening around the slowly contracting amnioserosa cells – and that the contraction of both was triggered by the same, as yet unknown, signal. Then Damian noticed something strange about the movies: the amnioserosa cells seemed to be twitching nervously.

To investigate that twitching, PhD student Aynur Kaya in his lab made new movies in which contiguous frames were only about five seconds apart, as opposed to two minutes – in effect slowing them down to something approaching real time. They made an exciting discovery: "All of a sudden we saw that this twitching was actually a dramatic pulsing," says Damian. As it turns out, amnioserosa cells pulse their whole lives: "They are like a very primitive muscle that contracts and relaxes," he explains.

To try to understand how the forces created by the pulsing might aid dorsal closure, physicist Jerome Solon created a computer simulation of the pro-



Slowing down the film allowed scientists to uncover how dorsal closure really happens in fruit fly embryos.

cess. This was a new departure for the group, but Damian says the technology has been immensely helpful. Experimental data is fed into a simulation until it models the real process fairly accurately, at which point it can be used to make predictions which can then be tested experimentally. So the researchers work in a back-and-forth way between experiment and simulation – an approach that is becoming increasingly important in modern systems biology – until they arrive at a satisfactory explanation of what they see under the microscope.

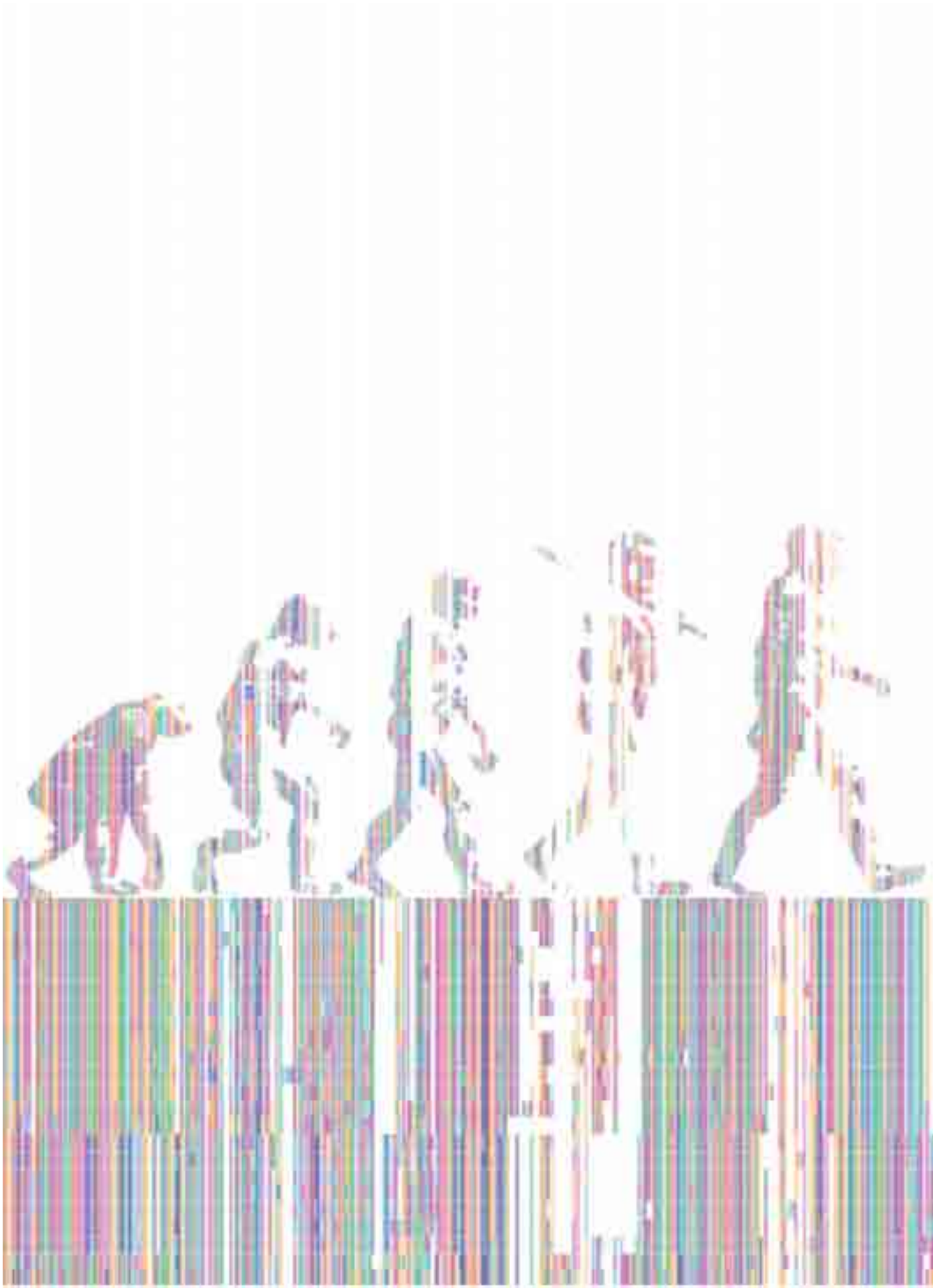
The explanation that Jerome and Aynur have arrived at, as a result of their collaboration, is that amnioserosa cells pulse constantly and evenly, pulling and pushing on the cells of the surrounding epithelium as they do so. At a certain signal, the actin cable forms, allowing the epithelial cells to continue to contract, but preventing them from relaxing. In other words, it behaves like a ratchet, which only allows movement in one direction. The result is the closure of the dorsal opening.

The simulations even allow the researchers to ask, what is the advantage of this strategy over, say, a drawstring strategy for the embryo? One possible answer is that it allows the system to cope with a certain amount of noise. If the amnioserosa cells were contracting gradually over time, some would be bound to exert more force than others, leading to uneven closure. "But if they are only allowed to contract in short pulses, they all get many chances to pull," says Damian, "so any differences are evened out."

If that is true, it could apply not only to dorsal closure and wound healing, but also to many developing tissues, since moving tissue around is central to development. In each case, different cell populations may provide the contracting force and the ratchet, but the basic mechanism remains the same: "You have a field of cells that creates the force," says Damian, "and then you add ratchets that lock certain cells into the state where they should move." At least, that's what the simulations seem to suggest; now it's back to the experimental drawing board.

Solon J, Kaya A, Colombelli J, Brunner D (2009) Pulsed forces timed by a ratchet-like mechanism drive directed tissue movement during *Drosophila melanogaster* morphogenesis. *Cell*, in press

A new way of looking at differences between genetic sequences gives a more accurate picture of evolution.



Mind the gaps

It's a scene that preys on the mind of anyone planning to perform DIY work on a home. You embark on a straightforward painting job, but as soon as you peel back the wallpaper, an ugly, more fundamental problem rears its head, such as a massive crack in the brickwork. Fixing it takes a lot of time and effort, but doing so improves the structural integrity of the whole house.

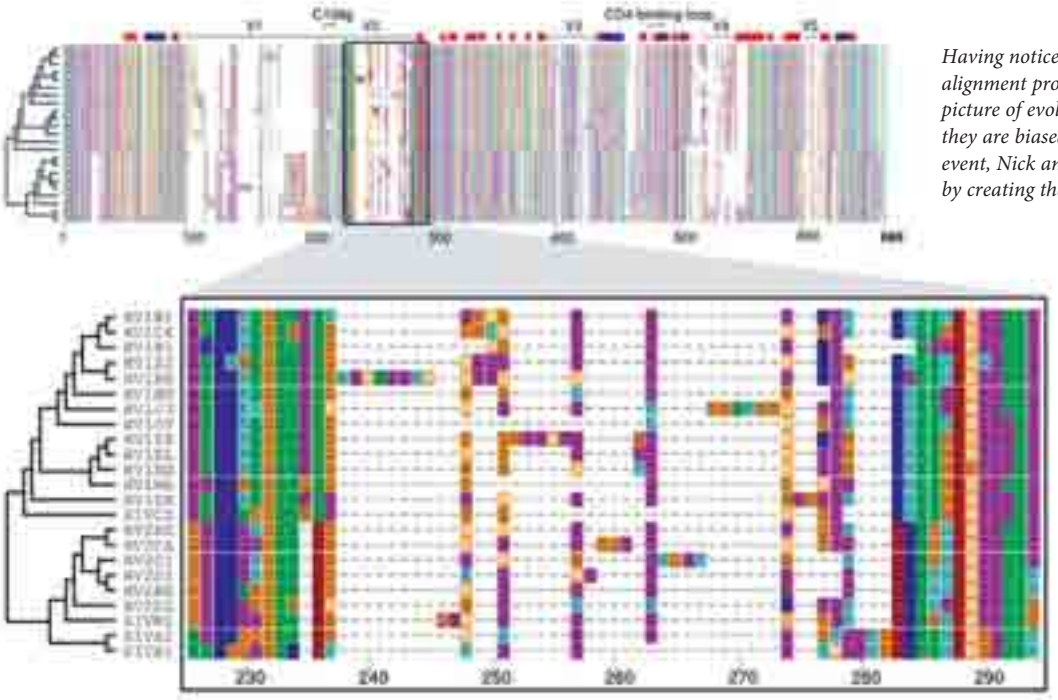
Something similar happened to Ari Löytynoja and Nick Goldman at EMBL-EBI when they set out to develop a new computer program to compare proteins between different species. Instead of simply improving existing comparison methods, they found that the very basics of these methods are incorrect and that the same error has been repeated in all of them. They proposed a correction for this error and developed a new method that promises to give biologists a more accurate and lifelike picture of how genes and proteins evolve. As well as improving scientists' understanding of basic biology, the work could also ultimately transform vaccine and drug design.

It's a far cry from Ari and Nick's original project, studying amino acids – the building blocks of proteins. These are strung together in long chains, which fold into different shapes and eventually form the final structure of a protein. To understand more about how proteins evolve, biologists compare the sequences of these proteins, to see which amino acids have changed over evolutionary time. To do this, they need to line the sequences up against each other, and work out which amino acids in one protein correspond with those of another. This process is known as alignment, and is also used by geneticists to compare DNA sequences.

To do this, bioinformaticians create programs that test lots of possible alignments between two protein or DNA sequences, and then assign a score to each alignment, based on how likely it is to be correct. Ari and Nick wanted to refine these programs by placing more emphasis on the structure of the protein. But Ari realised that there was an even bigger issue he had to tackle first: there was a fundamental problem with the way bioinformaticians were calculating the scores in the first place. The programs were biased against a particular kind of evolutionary change, and the alignments that the programs were suggesting were often very wrong.

Evolutionary changes in DNA sequences are what make matching genes across different species so tricky. The more distantly related the species, the longer evolution has had to act, and the trickier it is to get a reliable match. What's more, changes to a DNA sequence can take several different forms, some easier to match than others. Most problematic are changes that cause letters to be added (insertions) or removed (deletions) from a sequence. Take the human DNA sequence CAGA, for example, and compare it with a chimp sequence that has an extra letter: CA**T**GA. As well as having to match the sequences correctly, the computer has to decide how to account for the extra T in the chimp sequence. Is it an insertion in the chimp gene, or did the original common ancestor have a T at that location which has since been deleted in humans?

Computer programs have tackled this by comparing sequences from several different species, pair by pair, and working back to the roots of the evolutionary tree. The approach is based on a clever idea of representing the result



Having noticed that existing sequence alignment programs give an inaccurate picture of evolutionary relationships because they are biased against a particular kind of event, Nick and his group solved the problem by creating their own, unbiased, programme.

of each comparison of two sequences as a new sequence, which stands for the ancestor of the two. In a similar way, such an ancestral sequence can then be compared with another sequence, progressively going further and further back in time. So, for example, a program might compare human with chimp, then the human-chimp ancestor with gorilla, and finally the human-chimp-gorilla ancestor with orang-utan. If the program comes across an extra letter in one sequence, it will insert a gap in the other one to try to line up all the letters. So AT in human compared with AGT in chimp becomes A-T (human) aligned with AGT (chimp). The computer then compares the human-chimp ancestor (A?T), where '?' represents the uncertainty of that character, with gorilla (AT). It has to insert another gap, this time in the gorilla sequence (A-T), to match up the letters. Finally, it compares the human-chimp-gorilla ancestor (A?T) with orang-utan (AT), and, again, must introduce a gap in the orang-utan sequence to line up the two sequences.

There are a number of ways these sequences could have come about. The common ancestor of humans, chimps, gorillas and orang-utans could have had AGT as its sequence. The G may have been lost independently in each of the three lineages leading to orang-utans, gorillas and humans. Alternatively, the common ancestor may have had AT in that place, with the G being inserted in the chimp sequence.

Bioinformatics programs use statistics to choose the most likely scenario from all the possibilities. For example, losing a G three times in the same place over the course of great-ape evolution is far less likely than having an extra G crop up once in the chimpanzee lineage. To help the computer program calculate the most likely alignment, bioinformaticians give each kind of DNA change a 'penalty' – a kind of mathematical black mark. The more penalties a given alignment clocks up, the less likely it is to be the right one. So the computer does the maths and picks the alignment with the fewest penalties. But Nick and Ari noticed that the way programs dole out penalties is unfairly biased against insertions. Going back to the earlier example, if a program creates a gap to align human A-T with chimp AGT, it assigns that gap a penalty. Then, when it creates a gap in the gorilla sequence, it adds a second penalty, and then a third when aligning the orang-utan sequence. So the program penalises a single insertion event (in the chimp gene) three times. On the other hand, if chimp had AT as its sequence and all others AGT, the program would correctly penalise this deletion only once.

Things get much worse when scientists try to align sequences that have several insertions and deletions, as is common when comparing genes from more distantly related species, such as mouse and human, or in organisms that evolve rapidly, such as viruses. Insertions and deletions that take place close to each other are especially problematic. "Once you've got nearby events, there are different ways of reconciling them, which is when it goes wrong," says Nick. Comparison programs that penalise insertions multiple times but deletions only once inherently favour solutions that contain lots of deletions to align the sequences. "This has led to puzzling results as we know that insertions should happen but we rarely see them in our sequence alignments," says Ari.

A number of researchers have tried to address the issue by tweaking the scoring methods. "But they did it in a crude way that applied to both insertions and deletions," says Nick. These led to programs aligning non-matching insertions and positioning gaps in the sequences that create the impression that multiple deletions have taken place. "So you get all the gaps scrunched up into little tight regions," says Nick. Effectively, the attempts to paper over the cracks in the brickwork had failed. Nick and Ari decided on an approach that paid more attention to the process of evolution. When their program encounters an insertion or deletion, it creates a gap in the sequences and adds a penalty as usual. Crucially, however, it records a little 'flag' associated with the gap, and then uses information from evolutionarily related sequences to work out whether the gap represents an insertion or a deletion. If it is an insertion, the flag remains with the gap to stop the computer adding more penalties when it compares that section with other, more distantly related sequences. If, however, the gap turns out to be a deletion, the flag is removed.

The pair tested their program, called PRANK, against several others and found that it was better at aligning sequences from closely related organisms, and far superior at aligning distantly related sequences. "We were respecting the evolutionary events over time," says Nick. "That element was being ignored by the other methods."

The work has far-reaching consequences in a number of fields. For example, previous attempts to align an HIV protein called gp120 with its counterpart in SIV, a related virus which infects chimps, suggested that one particular part of the protein was evolving rapidly by switching one kind of amino acid for another, as well as becoming shorter. PRANK, however, revealed a different story: that region of the protein is evolving as a result of lots of insertions and deletions. Improved understanding of this gp120 region is vital for the development of vaccines and drugs, as changes in it are associated with HIV disease severity and the efficiency of a patient's immune response.

Now that PRANK is working (and available on the EMBL-EBI website for anyone to use), Nick and Ari can finally get back to their original project, safe in the knowledge that they are building it on solid foundations.

Löytynoja A, Goldman N (2008) Phylogeny-aware gap placement prevents errors in sequence alignment and evolutionary analysis. *Science* **320**: 1632-1635

An inspiring environment to do science – the EMBL-EBI campus in Hinxton.

Platypus genome fits the bill

It's an animal so bizarre that many zoologists at first insisted it was a hoax. Now, more than 200 years after Europeans first discovered it, the duck-billed platypus has had its genetic blueprint, or genome, analysed by an international consortium of scientists, including researchers at EMBL-EBI. As well as helping biologists understand more about this strange and secretive creature, the work is revealing new insights into how mammals evolved and why their genomes, including our own, behave the way they do.

It's easy to see why 18th-century scientists were sceptical when explorers brought the first dried specimens back from the animal's home in South Australia and Tasmania. With its beak, webbed feet, fur and eccentric egg-laying habit, the platypus looks like some practical joker has glued it together using bits of reptiles, birds and mammals. But it soon became clear that the platypus was no fake. What's more, biologists eventually realised it was a crucial missing link in the evolutionary history of mammals such as ourselves.

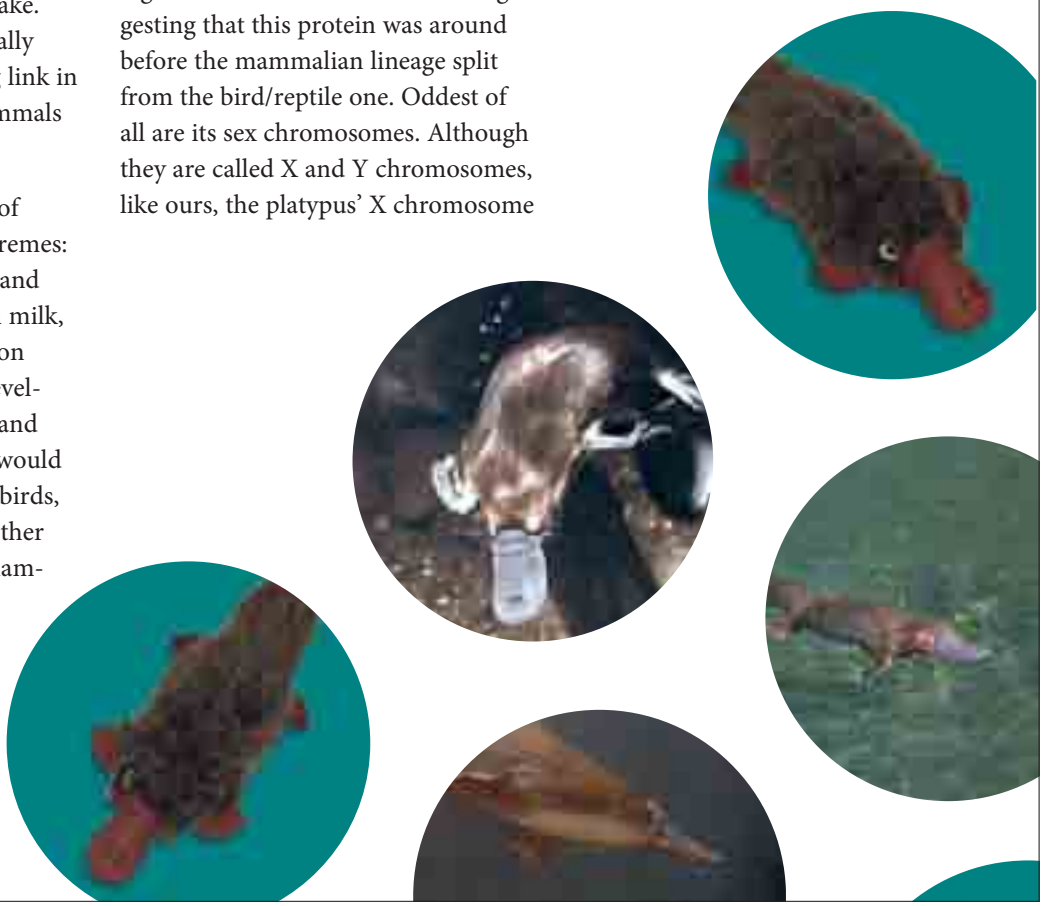
The platypus is one of a group of mammals known as the monotremes: animals that lay eggs like birds and reptiles, yet rear their young on milk, like mammals. About 315 million years ago, creatures that had developed the ability to lay eggs on land split into two groups: one that would eventually go on to give rise to birds, dinosaurs and reptiles, and another that would give rise to small, mammal-like reptiles. The latter group of animals branched into two further groups: one that gave rise to the monotremes, and the other that resulted in placental mammals, such as ourselves, and the marsupials, such as the kangaroo.

Ewan Birney and his team at EMBL-EBI helped to analyse the genome sequence of a female platypus called Glennie. Their studies revealed a number of intriguing insights into mammalian evolution. The genes for a group of milk proteins called caseins, for example, have a similar structure to those of humans. This means the ability to produce milk is likely an ancient one that predates the branching of the mammals 166 million years ago.

Perhaps the biggest surprise is that the platypus genome is as much of an outlandish mixture of mammalian, bird and reptile-like features as is its body. It has, for example, four genes involved in making the jelly-like coating of unfertilised eggs in common with other mammals. But it also has a gene for an egg protein called vitellogenin, found in birds and fish, suggesting that this protein was around before the mammalian lineage split from the bird/reptile one. Oddest of all are its sex chromosomes. Although they are called X and Y chromosomes, like ours, the platypus' X chromosome is much more like that of its bird equivalent than that of a mammal. And unlike us, they have not two but ten sex chromosomes. "The sex chromosomes of the platypus show it is as weird molecularly as it is physically," says Ewan.

Sadly, the platypus is one of just five monotreme species alive today, and its survival is under threat from climate change and habitat destruction by humans. The genome sequence should help ecologists understand more about this shy, mysterious animal and its little-known habits. Hopefully, such knowledge will ensure that the platypus stays around to let future generations wonder at its weirdness.

Warren W et al. (2008) Genome analysis of the platypus reveals unique signatures of evolution. *Nature* **453:** 175-184




Dmitri Svergun

Shaping up your guardian angel

They call it the guardian of the genome: a protein that watches over your DNA, on the lookout for damage that could turn a cell cancerous. For many years, the complete structure of this protein, called p53, remained mysterious. Now, researchers at EMBL Hamburg in collaboration with other leading European laboratories have developed new methods to elucidate the structure of p53 and other proteins like it. As well as giving cell biologists new insights into how p53 carries out its vital functions, the work promises to unlock the structures of hundreds of other proteins that have so far resisted analysis. "This field is really exploding," says Dmitri Svergun, a group leader who headed the work at the Hamburg outstation.

p53 is one of a recently discovered group of proteins containing major parts of what are known as intrinsically disordered regions, or IDRs. Unlike 'classical' proteins, in IDRs the chains of protein building blocks, or amino acids, do not fold up into a fixed three-dimensional structure. Instead, they stay largely unfolded, or disordered. As well as presenting a puzzle – how can a protein have a function if it lacks a fixed structure? – IDRs are a problem for structural biologists. Because they don't form folded structures, they cannot be studied with techniques such as macromolecular X-ray crystallography (MX).

And they need to be studied. A number of disease-related proteins, such as tau, which is involved in Alzheimer's disease, are almost completely unfolded. Many more proteins – up to 80 per cent of cancer-associated proteins, for example – contain both folded and unfolded sections. While some methods provided the structures of the folded parts of p53 and gave limited information about the disordered regions, scientists need a much more detailed picture.

Together with Sir Alan Fersht at the MRC Centre for Protein Engineering in Cambridge and Mikel Valle from the Centro Nacional de Biotecnología in Madrid, Dmitri solved this puzzle by bringing together a number of different methods.

Key to the work was a technique called small angle X-ray scattering, or SAXS. SAXS allows scientists to study the structure of molecules of any size in solution providing a picture of the overall shape of the molecule and how it flexes. The team combined SAXS data with information from MX, nuclear magnetic resonance spectroscopy and electron microscopy. They showed that the free p53 is a rather open cross-like assembly made of four copies of the protein, which collapses in the presence of DNA to tightly embrace the latter. This is how the flexibility of IDRs helps the protein to fulfil its function of constantly watching over the DNA in the cell.

Using a combination of NMR and SAXS, the researchers were able to work out which parts of a disordered section that is essential for p53's control were flexible, and which were rigid. They then used SAXS to look at how this section bound to a partner protein that controls p53's activity. By combining all this information, they finally produced a detailed picture of how the full p53 is assembled and how it functions.

Tidow H, et al (2008) Quaternary structures of tumor suppressor p53 and a specific p53–DNA complex. *PNAS* **105:** 5762–67

Wells M, et al (2008) Structure of tumor suppressor p53 and its intrinsically disordered N-terminal transactivation domain. *PNAS* **104:** 12324–29

Endless forms most beautiful

"...From so simple a beginning, endless forms most beautiful and most wonderful have been, and are being, evolved." This sentence, the last in *On the Origin of Species*, encapsulates the burning question that Charles Darwin set out to explain with his theory of evolution: where did the astonishing variety of living things come from? Now, 200 years after Darwin's birth, biologists are building a remarkably detailed picture of the biological mechanisms that create this amazing diversity and how we might be able to tap into them to understand more about our origins and illnesses.

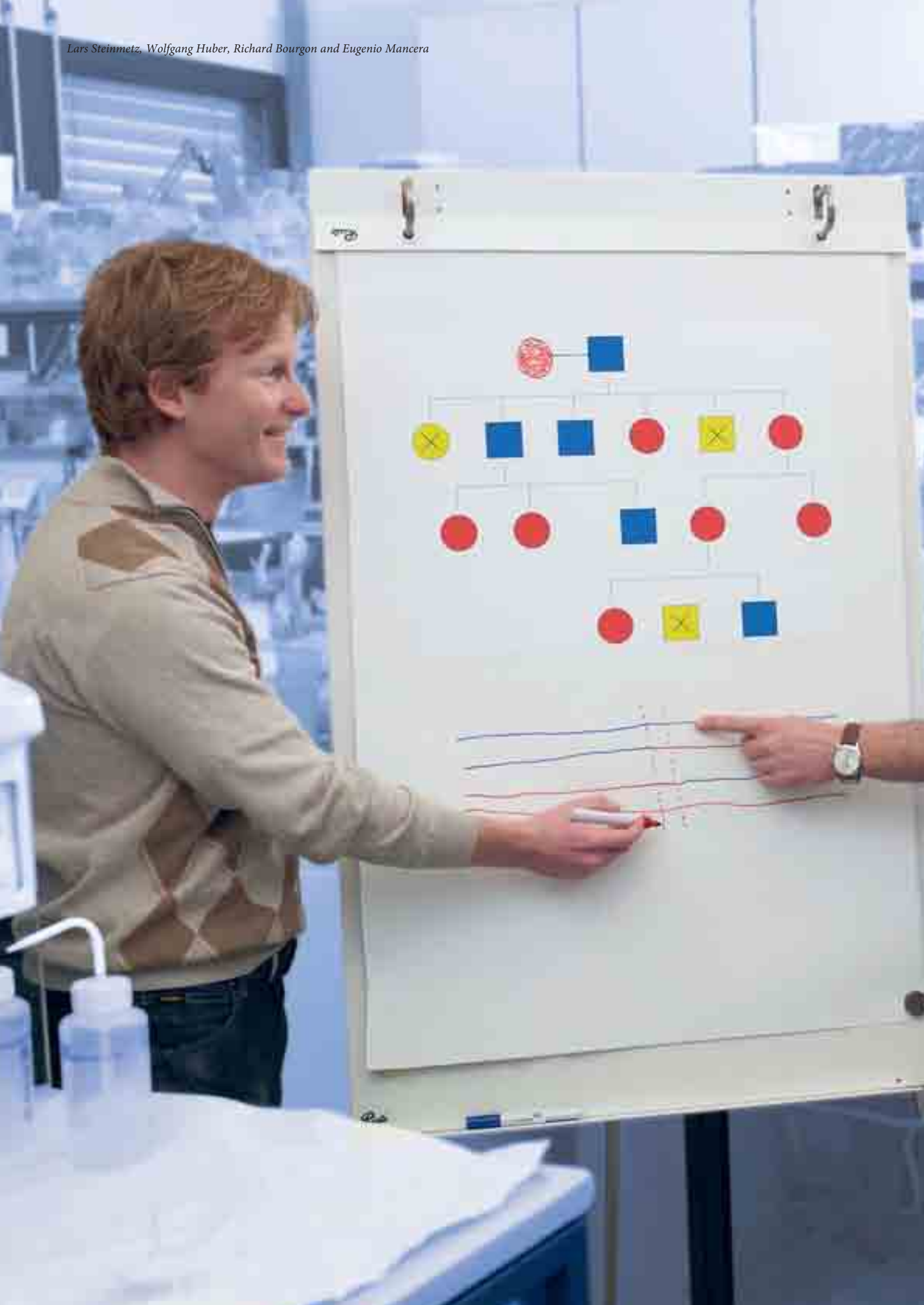
One of the most impressive things about Darwin's theory is that he published it before Gregor Mendel's work on the laws of inheritance had been widely circulated, before the concept of the gene existed, and before the discovery of DNA as the molecule of heredity. These days, biologists have the luxury of interpreting evolution in the context of understanding how sex shuffles chromosomes like decks of cards to generate genetic diversity, how DNA spells out instructions for making the next generation, and how this can be linked to an organism's shape and physiology, its phenotype.

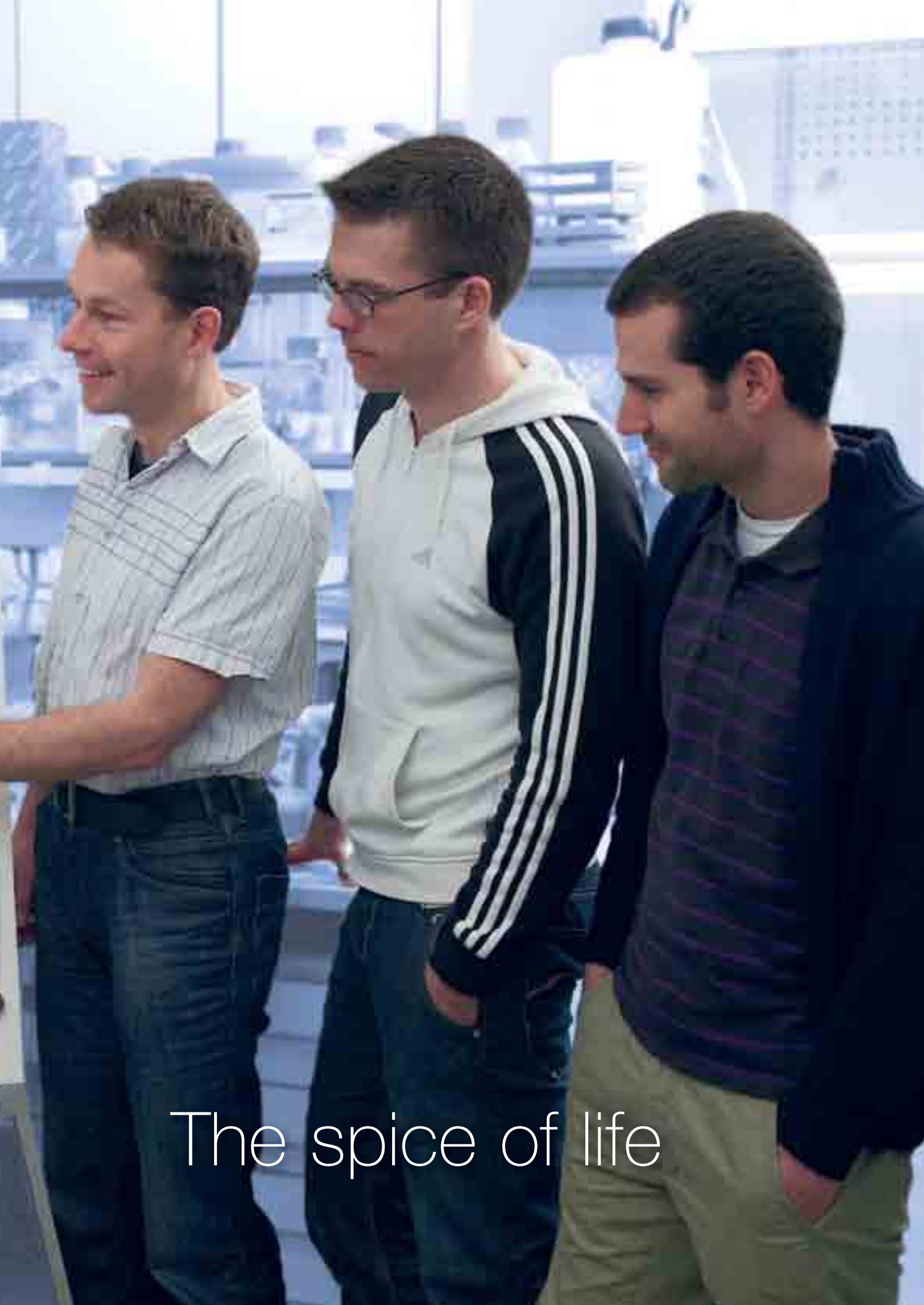
But such discoveries have raised as many questions as they have answered. EMBL researchers, for example, are currently working on ways to map the process of DNA shuffling to trace the variations in genes that can contribute to common diseases such as obesity and diabetes. Their colleagues, meanwhile, are studying how these variations actually raise disease risk. Others are looking into how the chromosomes that control an organism's sex are themselves controlled.

Of course, evolution does more than explain variety in the present. By its very nature, it is a window to the past. New tools developed at EMBL will allow researchers to uncover deep evolutionary relationships and study the evolution of genes and proteins, to better understand how they work normally and how they go wrong in disease. These tools will also allow researchers to mine text and data for hitherto undiscovered connections that will help them see how cells and organisms work as systems.

Scientists are now at a point where they know enough about biology to start engineering artificial varieties of organisms from scratch. This field, synthetic biology, brings a whole new set of ethical and scientific problems, which were raised at a science and society conference at EMBL this year. Perhaps 200 years from now, people will not only wonder at the beautiful and endless forms evolved by natural selection, but at the ones humans themselves have had a hand in creating.

Lars Steinmetz, Wolfgang Huber, Richard Bourgon and Eugenio Mancera




The spice of life

The reason that the vast majority of siblings aren't identical is because they inherit different combinations of their parents' genes. During the formation of every germ cell – an egg or a sperm – each parent's own genes are reshuffled. Each of their children therefore receives a unique combination of the genes of his or her four grandparents, rearranged and given an extra novel twist by any mutations that happened to be introduced during the production of the germ cells.

This reshuffling, called genetic recombination, creates the diversity within a species that is ultimately the secret to its strength. By exploiting that diversity, a species can adapt to a changing environment. However, this reshuffling also influences the inheritance of disease genes. Lars Steinmetz, Joint Head of the Gene Expression Unit at EMBL Heidelberg, and his group, together with the group of Wolfgang Huber at EMBL-EBI in Hinxton, UK, have now produced the most detailed map of genetic recombination to date.

In sexually reproducing species, cells undergo a process called meiosis, the duplication of their chromosomes followed by two nuclear divisions that gives rise to a haploid germ cell – that is, a cell with half the chromosome complement of its parent. In humans, for example, a sperm or an egg contains 23 unpaired chromosomes, compared with the 46 chromosomes found in pairs in other human cells. During fertilisation, egg and sperm fuse nuclei to produce a full set of chromosomes.

Recombination happens during meiosis, just before the first nuclear division, and it can take two forms: crossovers or non-crossovers. In crossovers, chromosomes swap large segments or even whole arms. Non-crossovers are less dramatic, and involve copying small sections of DNA from one chromosome to another, with no reciprocal exchange. Both mechanisms help to shape the genome across subsequent generations, however, and both contribute to the breaking up of blocks of genes that would otherwise be inherited together.

Lars' and Wolfgang's groups created their recombination map for budding yeast, *Saccharomyces cerevisiae*. In Heidelberg, PhD student Eugenio Mancera mated two yeast strains and induced meiosis in them to produce around 200 haploid spores, or segregants. Lars chose the strains – one standard lab strain and one clinical strain, isolated from a patient – because their genomes, both of which had previously been sequenced, diverged sufficiently to provide enough points of comparison.

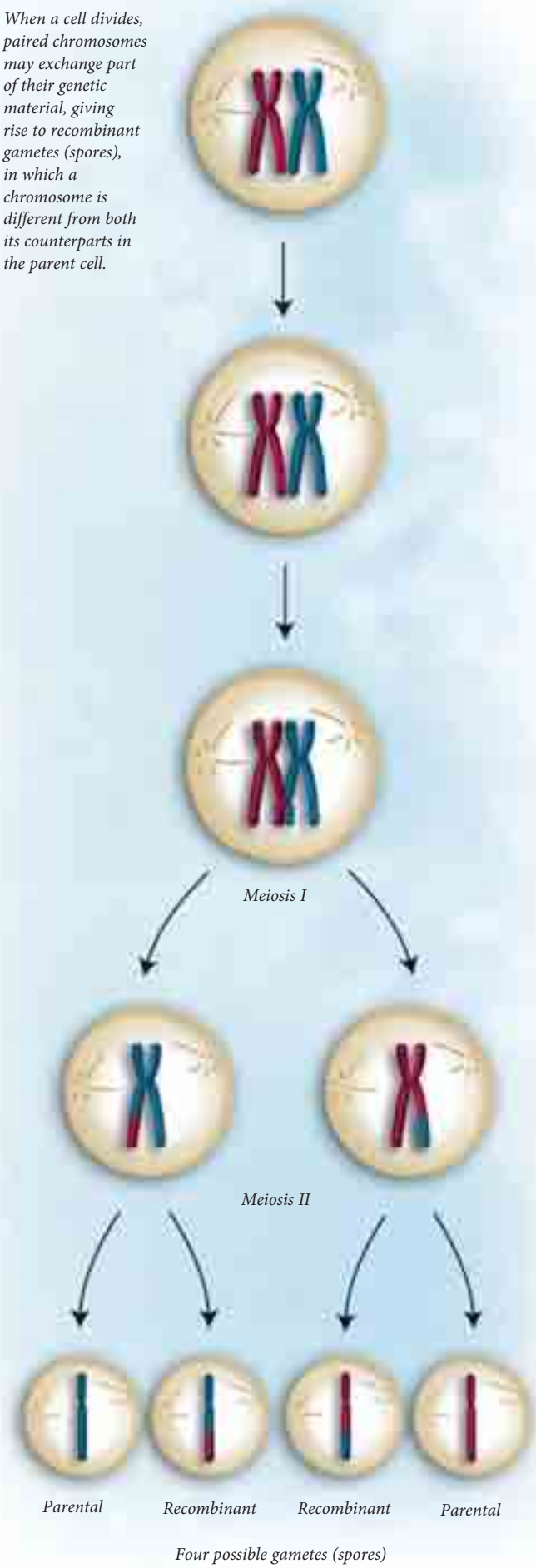
In all, there were 52,000 markers, or positions in the DNA sequence where the two genomes varied. These included insertions, deletions and, predominantly, single-nucleotide polymorphisms (SNPs) – places in the genome where different alleles or gene variants occur. Using arrays composed of DNA probes taken from the two parental strains, the researchers then 'interrogated' those markers in the spore genomes, to look for differences in behaviour. The arrays they chose were so-called tiling arrays, in which the probes either overlap or are very close together on the genome, providing a more unbiased analysis, and higher resolution, than traditional arrays in which the probes are further apart.

It was at this point that Wolfgang's group came in. A computer algorithm they had developed especially for the purpose, called ssGenotyping, used the data generated by the arrays to identify the genetic make-up, or genotype, of the spores at each locus the probes interrogated – that is, to find out which of the parents' genetic alternatives it had inherited at that place. To confirm the accuracy of their approach, they sequenced a handful of those loci, and compared the results with the genotyping calls returned by the arrays. "Once we had high-confidence genotype calls in hand, we could use these to infer the recombination events that each segregant had participated in," says Richard Bourgon from the Huber group.

Thanks to the combination of the Huber group's algorithm and the high-density coverage of the genome provided by the tiling arrays, the researchers produced a map with a resolution 20 times higher than that of any existing yeast map, and more than 360 times higher than that of a recent map of human recombination. "Traditionally, marker density has been low, so there have often been multiple recombination events that happened between two consecutive markers," Wolfgang explains. "With our technology we have multiple markers per recombination event. This means that we can detect these events unambiguously, look at their fine structure, and determine where in the genome non-crossovers have taken place."

The map reveals that about one per cent of the yeast genome undergoes sequence conversion during a single meiosis, or that more than 150 recombination events take place during a typical meiosis. For the first time it shows up non-crossovers across the whole genome – those events that have traditionally been so hard to see. The map also reveals that the recombination rate depends on the location in the genome, and that genomic 'hotspots' exist which favour either crossovers or non-crossovers. Previously, researchers had shown that crossovers can interfere with one another, making it unlikely that two would happen in close proximity. Now that the map has rendered non-crossovers visible, it has become clear for the first time that crossovers and non-crossovers can also interfere with each other.

"This knowledge is important to understand the mechanisms of how interference comes about," says Lars, adding that those mechanisms aren't yet understood, even for crossover-crossover interference. It is probably also important for understanding why there are differences in distribution of crossovers and non-crossovers, because interference may be the cause of those differences. Both types of recombination event are triggered in response to double-strand breaks (DSB) in DNA, which they repair. "If the



When a cell divides, paired chromosomes may exchange part of their genetic material, giving rise to recombinant gametes (spores), in which a chromosome is different from both its counterparts in the parent cell.

Four possible gametes (spores)

decision to follow one recombination pathway at one location of the genome affects the pathway decision at another location, then interference must happen before the pathways branch," he says. "That could be as early as DSB formation."

The existence of non-crossover hotspots also has implications for genetic mapping. Genetic linkage mapping is a technique used to determine the relative positions of genes on a chromosome and is used, for example, to link genes with an inherited trait. It relies on the principle that the closer together two genes are, the more likely they are to be inherited together. Or, to put it another way, the further apart they are, the more likely they are to be separated during meiosis by a recombination event.

To date, linkage maps have been built using observed crossovers, which are easier to spot than non-crossovers since the exchange of DNA is reciprocal and hence, the effects larger. But non-crossovers can also break up gene blocks, through one-way transfer of genetic material. As Lars puts it, "Non-crossover hotspots create holes in linkage maps." That means that conventional linkage maps may miss the essential detail when it comes to studying associations between genes and inherited traits. From now on, Lars and Wolfgang say, it is clear that if researchers want to capture all the genetic variation in a set of offspring, they will need to sequence the complete genomes of those offspring, in order to be able to identify the holes.

In the past, Lars' group has studied correlations between meiotic recombination and phenotypic variation – that is, variation in the observable characteristics of an organism. Within that context, the two yeast strains he chose for the current study could provide an interesting comparison, since the clinical strain has some unusual features, including the fact that it grows at high temperatures and is capable of killing an immunocompromised mouse. "None of these traits exist in the standard lab strain," says Lars.

He is confident that many of the same kinds of recombination behaviour that his and Wolfgang's groups have uncovered in yeast will eventually be identified in the human genome, and that such information will prove useful in understanding how disease genes are passed from generation to generation. To date, around 2000 disease genes have been identified in humans which are inherited in simple, Mendelian fashion, and a few hundred that contribute in more complex ways to common diseases such as type II diabetes, hypertension and schizophrenia. Before scientists can accurately predict how common diseases are inherited, says Lars, two things must happen: "First we need to identify all disease genes and the intricate ways in which they interact, and second, we must have high-resolution maps of recombination in humans."

Mancera E, Bourgon R, Brozzi A, Huber W, Steinmetz LM (2008) High-resolution mapping of meiotic crossovers and non-crossovers in yeast. *Nature* **454**: 479-85

Infinite variety

Scientists at EMBL-EBI are playing a key role in an ambitious project that aims to create the most detailed catalogue of human genetic variation yet undertaken. The initiative, called the 1000 Genomes project, will sequence the genomes of 1200 people to discover what genetic variation they share. As well as giving biologists new insight into what makes each of us individual, the project will help researchers link genetic variation with the risk of developing common diseases such as diabetes and heart disease.

The 1000 Genomes Project follows in the footsteps of a similar, earlier undertaking called the HapMap Project. This looked for single letter differences that are shared within a population – called single nucleotide polymorphisms, or SNPs – in the genomes of 270 individuals. The HapMap Project tested around half a million SNPs scattered through the genome, but because the SNPs were previously known, most of them were present in 5% or more of the population. It has already proved useful in flagging genes that could be involved in common diseases.

The new project involves nine sequencing centres and many research institutes around the globe and will look at SNPs in more detail, identifying those present in as little as 1% of the population. What's more, it will also scrutinise the genomes for other differences, such as extra or missing letters, or larger sections of DNA that have been moved, removed, copied or switched around, a phenomenon known as structural variation. "We will be able to see things at much higher resolution," says Paul Flicek, the team leader who heads EMBL-EBI's contribution to the work.

The project will scan the genomes of people from a range of different ethnic groups, such as Tuscans from Italy and Yoruba from Nigeria, and will help scientists refine the original 'reference' sequence obtained by the Human Genome Project in 2001. But dealing with all the data will be no easy task. Modern sequencing centres can now sequence an entire human genome in a day, a feat that took the original Human Genome Project ten years to complete. Researchers will need to manage and interpret huge streams of raw data.

This is where EMBL-EBI comes in. In collaboration with colleagues at the US National Institute of Biotechnology Information in Bethesda, Maryland, Paul's team, together with Jan Korbel's structural variation group at EMBL Heidelberg, is methodically collecting and verifying the data as they are produced, and putting them online as soon as possible. "We are making the data available to the scientific community in advance of publication," says Paul. As well as having to build the infrastructure to collect and store vast volumes of data, Paul and his colleagues need to monitor the information as it travels from the bench to the databases, to ensure that it doesn't get mixed up or corrupted. This might not sound so glamorous, but it is vital to the success of the project. "It mostly comes down to a very large job of keeping track of lots and lots of little pieces of information," explains Paul. "The project requires all the mundane stuff to be done at the beginning – otherwise the data would be wrong." But it's a challenge Paul and his team relish. "It's a lot of fun being involved in such a big project," he says.

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www.1000genomes.org

Top left to right: Asifa Akhtar, Jop Kind, Nick Luscombe and Paul Bertone, second row

Keeping the transcriptional train on track

Humans and fruit flies have a surprising number of things in common. Take their sex chromosomes, for example. While the females of both species have two copies of the X chromosome, males have an X and a Y. It's a simple and elegant arrangement for determining gender, but the inherent asymmetry creates a potential problem: males risk producing a smaller amount of the proteins encoded by genes on the X chromosome than females. Both flies and humans have evolved a mechanism to compensate males for this shortfall, but there the similarities end, because the mechanism differs radically between the two species.

In humans, dosage compensation, as the mechanism is called, occurs through the silencing or inactivation of one female X chromosome, whereas in the fly it is achieved through a twofold increase in expression of genes on the single male X. How this happens is still largely unknown in both cases. But in the Gene Expression Unit at EMBL Heidelberg, Asifa Akhtar is trying to get to the bottom of it.

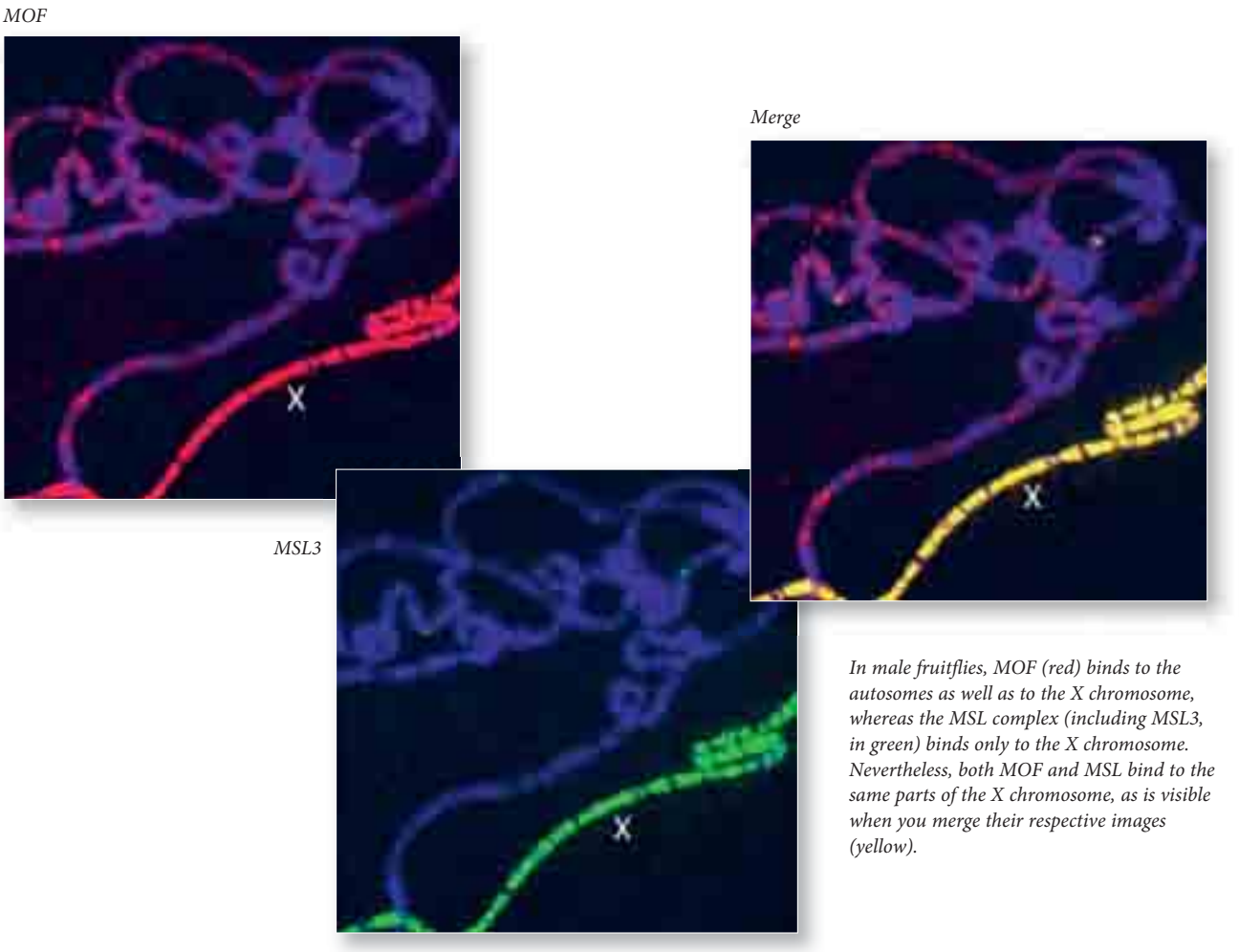
Asifa studies dosage compensation in the fly because, while it has the same arrangement of sex chromosomes as mammals, it is easier to manipulate genetically. In the last few years, she has focused her studies on a certain protein complex – called male-specific lethal (MSL) because males cannot survive without it, whereas females can – and more recently, on a particularly intriguing component of that complex called MOF.

MOF stands for *m*ales-absent-*o*n-the-*f*irst, because the gene that encodes it is on the first chromosome (the X chromosome in flies), and males that lack it are 'absent', or not viable. MOF is an enzyme that is thought to promote gene transcription by modifying the histone proteins around which DNA is wrapped in chromatin, the material of chromosomes. In essence, it relaxes the structure of the chromatin, allowing the cell's transcription machinery to gain access to the DNA.

Using antibodies to the MSL complex, researchers have shown that it binds to many sites along the X chromosome. The question that Asifa wants to answer is, what is the molecular mechanism that guides MSL to the male X chromosome, and what determines where on that chromosome it binds?

Because MOF targets a particular amino-acid component of a particular histone, a modification that is very common on the upregulated male X chromosome, researchers have suspected that it might be involved in attracting the MSL complex to that chromosome, initiating dosage compensation. To test that idea, Asifa's group collaborated with the groups of Nick Luscombe and Paul Bertone at the EMBL-EBI in Hinxton, UK, and used a technique called ChIP-on-chip [see page 35] to scan the fly genome for genes to which MOF and other MSL proteins bind.

Because it was genome-wide, the ChIP-on-chip survey generated a huge amount of data that Asifa analysed with Juan Vaquerizas, a postdoc in Nick's lab. It was a true collaboration, she says, because Juan analysed the ChIP-on-chip data while Jop Kind, a former PhD student in her lab, performed biological experiments in parallel.



In male fruitflies, MOF (red) binds to the autosomes as well as to the X chromosome, whereas the MSL complex (including MSL3, in green) binds only to the X chromosome. Nevertheless, both MOF and MSL bind to the same parts of the X chromosome, as is visible when you merge their respective images (yellow).

The results of their analysis revealed a major surprise: although the MSL complex binds only to the male X chromosome, MOF binds to all chromosomes. Intriguingly, however, its binding pattern in the male X chromosome is different from that in autosomes (chromosomes that are not involved in sex determination) and in the active female X chromosome. "Most transcription factors don't show different binding patterns in X chromosome genes and autosomal genes," Asifa says, "but MOF does."

Transcription factors are proteins that determine what genes are active when. When the researchers looked more closely, they saw that in autosomes and the active female X chromosome, MOF binds to the promoter region – the beginning of the gene, where transcription starts. In the male X chromosome, however, MOF appears to bind all along the gene, with binding 'peaks' at the beginning and the end. In experiments in which the researchers selectively knocked out the other MSL proteins from cells, they found that MOF remained bound to the beginning of the gene. "So it looks like the default status of MOF is to sit at the promoter," Asifa says.

She suspects that MOF may contribute to two independent transcription events. Its more general function may be to bind to the gene promoter, where it relaxes chromatin to allow transcription to start. In combination with the MSL complex, however, MOF may bind all along the gene. This latter function, Asifa speculates, may contribute to the mechanism underlying dosage compensation.

According to this hypothetical scenario, if you think of the transcription machinery moving along a gene like a train moving along a track, then tightly packaged units of chromatin represent rocks on the line that risk derailing the train, and MOF is a lubricating mechanism for clearing those rocks. In the autosomes, MOF is only present at the beginning of the track, so the train may occasionally be derailed. In other words, transcription may not be perfect and some DNA may go untranscribed, but it may not matter too much because

there is a second copy of each gene on the paired chromosome which compensates. In the male X chromosome, where there is no backup copy, MOF lubricates the entire length of the track, and the metaphorical train is therefore more likely to complete its journey. MOF may thus enhance the efficiency of transcription when transcription can't afford to fail.

That would explain why MOF binds to the whole length of genes on the male X chromosome, but not why there should be a second peak in MOF binding at the 3' end of those genes – that is, at the non-promoter end – when MOF is in the presence of other MSL proteins. Asifa says she can't explain that second peak for the moment, but she has a hypothesis which she would like to test: "One of the ways you could potentially make transcription more efficient is by looping DNA, that is, by joining the two ends of the gene together."

In such a scenario, the transcription train, having run through to the end of the gene, need merely continue on its tracks to begin transcribing it all over again. Maybe, Asifa suggests, MOF is responsible for closing that DNA loop in male X chromosome genes, by sitting at both ends and keeping them lubricated. Suggesting ways in which transcriptional efficiency might be enhanced is one thing, however. "Explaining how you can make a twofold difference is another, and that problem will keep us busy for a long time," she says.

According to Jop, who is now working as a postdoc at the Netherlands Cancer Institute in Amsterdam, MOF has its counterparts in mice and humans. "The finding that MOF binds to promoters in the entire genome therefore likely reflects an evolutionarily conserved regulatory process that is independent of dosage compensation," he says. Moreover, he adds, the binding of MOF to the male X chromosome probably reflects "a fly-specific adaptation in which MOF, a potent transcriptional activator, is 'lent' to the single male X chromosome to double its activity".

That makes sense, Asifa says, because the mammalian equivalent of MOF is not male-specific lethal – it is essential to survival in both sexes. "You would expect that if it was doing something on all chromosomes," she says. "So it looks like what the MSL complex has done is to hijack an essential protein."

The other fly MSL proteins also have their counterparts in mammals, which suggests that although different dosage compensation strategies have evolved in the two species, some of the key molecular players remain the same. That's exciting, Asifa says, because it turns out that dosage compensation might not be so different in flies and mammals after all.

"Over the last few years it has become apparent that the active X chromosome in female mammals is upregulated twofold as well," she says. In other words, though female mammals do indeed silence one of their X chromosomes, the expression of genes on the remaining X is upregulated, just as it is in the fly's male X chromosome. This is so the dose of X-linked genes that both sexes receive is brought in line with the dose of autosomal genes – also in pairs – that they receive. "So it is possible that there are more similarities between humans and flies than we had anticipated," Asifa says.

Kind J, Vaquerizas JM, Gebhardt P, Gentzel M, Luscombe NM, Bertone P, Akhtar A (2008) Genome-wide analysis reveals MOF as a key regulator of dosage compensation and gene expression in *Drosophila*. *Cell* **133**: 813–828

Interview with the new Heads of the Gene Expression Unit Lars Steinmetz and Eileen Furlong.

Striking new paths: from genomes to biological systems

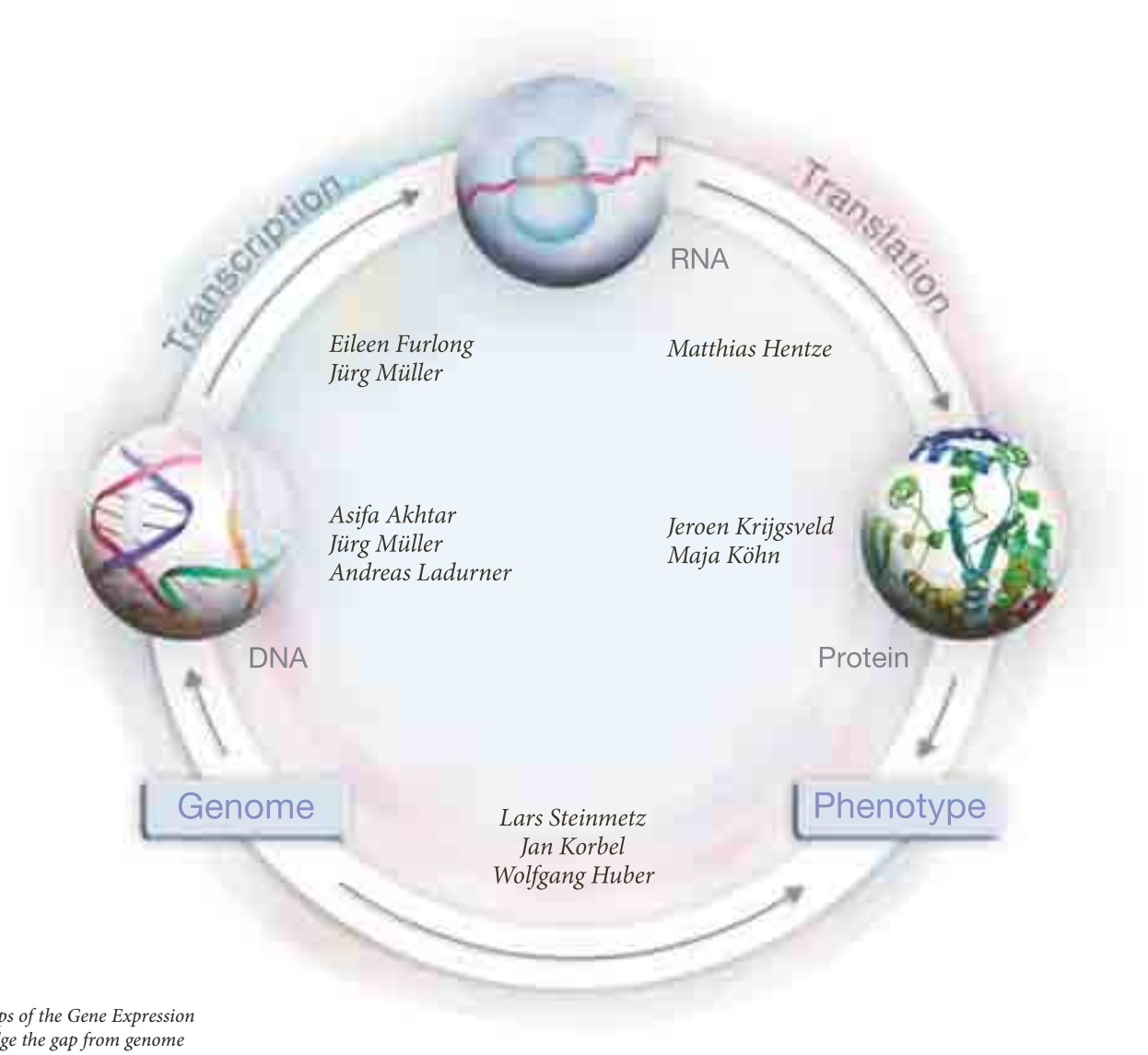
Since January 2009, EMBL's Gene Expression Unit has two new Joint Unit Heads: Eileen Furlong and Lars Steinmetz. Eileen and Lars have been group leaders at EMBL for several years – enough time to become familiar with the structure and direction of the Unit. But both also bring some new ideas of their own. To remain at the forefront of molecular biology, EMBL encourages its scientists to identify and pursue cutting-edge research topics early on and to adapt flexibly to changing scientific trends. Reflecting this spirit, and guided by the comments and recommendations of a very favourable scientific review of the Unit in May 2008, the newly appointed Unit Heads have used their first months in office to start revamping strategies, directions and even the name of the Gene Expression Unit.

How would you describe the research that is done in the Gene Expression Unit?

Eileen: The overall research theme of the Unit has always been to bridge the gap from genome to biological systems or from genotype to phenotype. How does the information contained in DNA determine how complex biological systems work? Which are the key steps, processes and molecules conveying genetic information into phenotype? And how are they controlled? We have excellent groups working on all levels of the gene expression process. Asifa Akhtar, Andreas Ladurner and Jürg Müller focus on the DNA and chromatin level: how does the structure of chromatin influence gene expression? The next level is transcription, the copying of DNA into RNA. How the temporal and spatial timing of transcription is regulated is addressed in Jürg's and my groups. RNA can be modified in various ways and bound by regulatory proteins which determine if and how much of the encoded protein will be produced. This is the area of expertise of Matthias Hentze. His group is loosely associated with the Gene Expression Unit. Once translated into protein, Jeroen Krijgsveld's group uses quantitative proteomic methods to investigate different aspects of the proteome. Maja Köhn's lab develops new types of inhibitors of protein function, and is applying them to phosphatases, which make post-translational modifications. As proteins are the agents that perform functions in a cell, and that make one cell different from another, they determine what we call a cell's phenotype. Lars Steinmetz' and Jan Korbel's groups bridge the gap from genome to phenotype by investigating how different changes or variation in the DNA affect the function of biological systems. Wolfgang Huber's group has the strong mathematical and computational background necessary to decipher and integrate the data masses resulting from large-scale experiments. He collaborates with many groups inside and outside the Unit and among many other things provides an important link between them all.

What are the new plans for the Unit?

Lars: The leitmotif 'from genome to biological systems' will stay the same, but our strategy will change slightly. There will be a shift more towards high-throughput and global approaches such as functional genomics, proteomics and systems biology. It will be like zooming out to have a look at the bigger picture of the same processes.



The groups of the Gene Expression Unit bridge the gap from genome to phenotype.

At the same time, we do not want to lose our focus on the details and thus will also continue to draw on the Unit's traditional strengths in detailed mechanistic studies using biochemistry and genetics. The ability to address questions at different scales and its interdisciplinary approaches are the Unit's biggest assets and will also guide future recruitment activities.

Eileen: Our plans for the Unit will not come as a surprise to anyone. What we have in mind is a natural progression of the path pursued already by previous Gene Expression Heads including Iain Mattaj, Elisa Izaurralde and, more recently, Jan Ellenberg. The latest group leader appointments have already brought valuable expertise in genomics, proteomics, mathematics, computer science and chemistry into the Unit – all the disciplines you need for successful systems biology. What we are doing now is expanding and integrating these efforts. We also intend to continue the recent trend of integrating more chemistry and chemical biology.'

Lars: What we are planning also fits well into the greater scheme of biomedical research and medicine beyond the Unit and EMBL. The next years will see a paradigm shift in the way medicine is carried out. The trend is moving away from therapies tailored towards entire populations, more towards personalised treatments. There will also be a shift from reactive medicine concerned with the curing of symptoms towards preventative approaches that detect and treat diseases at the molecular level before symptoms become apparent at the organismal level. Basic research and technology developments in the area of genome biology will be key to implementing such a change, because it requires a thorough under-

standing of biological systems and molecular mechanisms as well as new technologies.

It sounds like technology development will play an important role in the Unit's future activities.

Eileen: We are definitely planning to expand the Unit's technology development efforts. New technologies push the limits of science. The questions we address with our experiments are always restricted by what technology allows us to do. Having engineers and technology developers as part of the Unit facilitates direct exchange with the biologists. In this way, new instrumentation can be customised to researchers' needs, so that research questions can shape technology rather than the other way round.

What kind of technologies do you have in mind?

Lars: One priority will certainly be nanotechnology. For example, we would like to start doing the same global biological measurements that are now only possible across organisms or cultures on a single cell level. Gene expression profiling, for example. With nanotechnology and microfluidics techniques, one could envision developing custom 'lab on a chip' applications that combine all steps of a genomics experiment in one device. Such a chip would have many chambers, each harbouring one cell, and would also contain the chemical methods to extract their DNA or RNA. This kind of technology would greatly speed up large-scale genomics research, decrease experimental variability and make it more efficient. But so far nobody has been able to integrate and automate all the different steps into a single device.

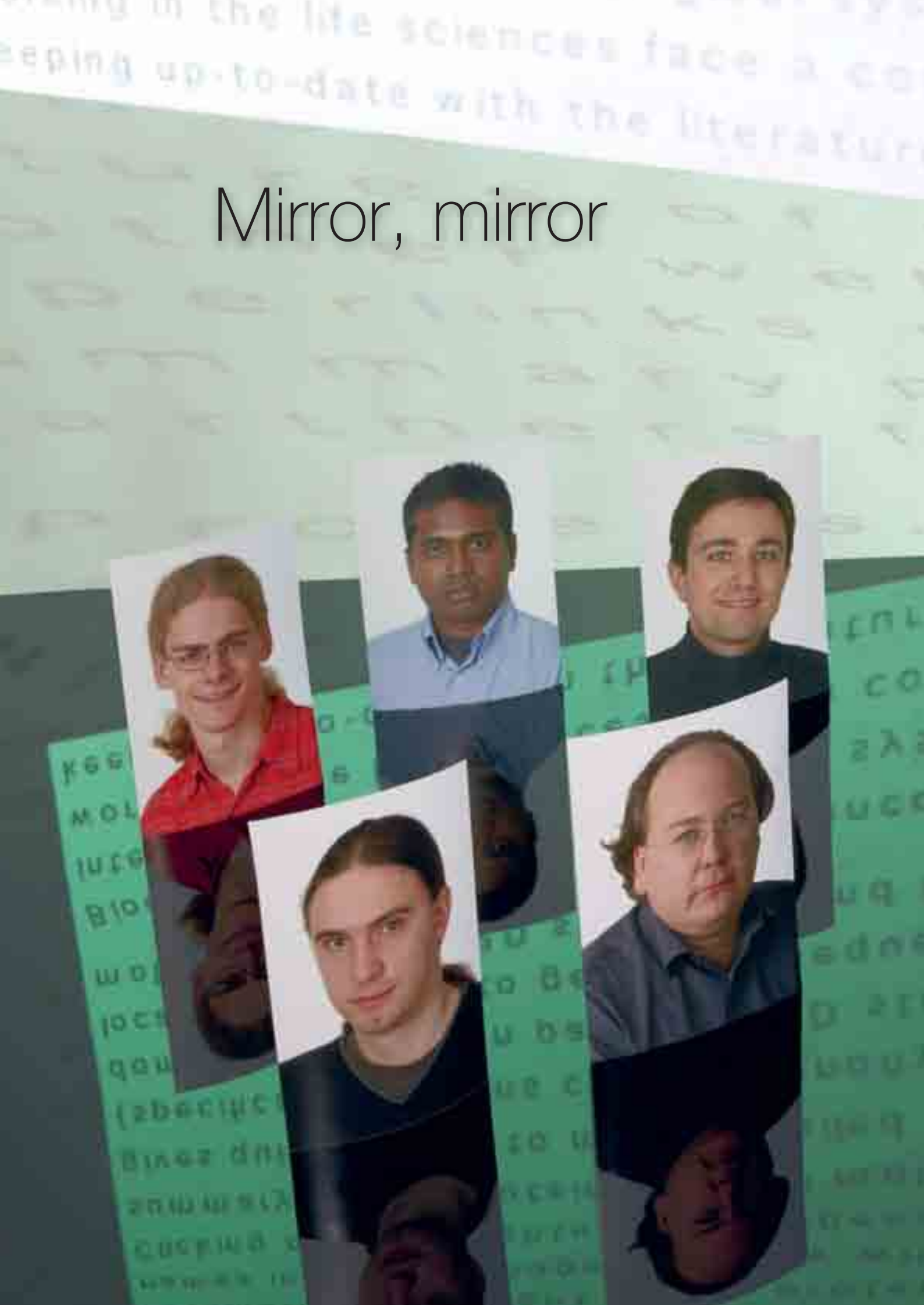
Eileen: It is also important to apply more engineering principles to biology – for example, to construct artificial circuits of molecular networks. These types of synthetic biology approaches can provide a very powerful mechanism to test hypotheses about the behaviour of network components and dynamics. With DNA synthesis technology it is also possible to readily generate proteins with new functionality and introduce them into a circuit to see how it changes the system's behaviour.

You have decided to rename the Gene Expression Unit. What will the new name be and why did you choose it?

Lars: We felt that 'gene expression' was too narrow a description to encompass all groups currently in the Unit, and even more so with respect to the kind of people we would like to recruit. In the future the Unit will be called the Genome Biology Unit, which embodies its new, broadened scope. The new name stresses the focus on genome-wide studies and will make it easier to recruit interdisciplinary staff. Biology is moving very rapidly and the new Genome Biology Unit hopes to take an active role in shaping these exciting new developments.

EMBL Heidelberg's social hub.

Mirror, mirror



Back row: Michael Kuhn, Venkata Satagopam, Lars Juhl Jensen, Heiko Horn, Nigel Brown, Theodoros Soldatos

Front row: Evangelos Pafilis, Reinhard Schneider, Georgios Pavlopoulos and Sean O'Donoghue

Astronomers call it the radio sphere: the boundary of the radio transmissions radiating from Earth into space, created by humans broadcasting strong radio signals such as TV carrier waves around the planet. We've been sending them out since the mid-1930s, long enough for them to have travelled tens of light years and encountered other planets and stars.

The question is: are there other intelligent civilisations out there, and if so, what would they make of our broadcasts? Perhaps they are trying to piece together an understanding of humanity, having only our radio and TV programmes to work on. If so, they are missing out on all our conversations, emails and phone calls, and thus only scratching the surface of the vastly complex set of interpersonal relationships and interactions that reveals what it is to be human.

In many ways, biological researchers trawling the worldwide web for information on genes or molecules are facing a similar problem. Type a term into Google, for example, and you can only search what is known as the 'surface web', the small proportion of the Internet that is visible to search engines. There is a much larger, uncharted region, the 'deep web', which is invisible to the programs that index content by crawling from page to page via links. Much of the data held in these invisible areas is included in databases, such as bioinformatics databases.

So a biologist looking for information on, say, a human muscle protein called myosin and the other molecules it interacts with, might look it up in one database, but be unaware of information in another database that could be relevant. A search engine might help find relevant information on the surface web, but miss the deep web and the information it holds about myosin's molecular relationships. And given that biologists in different fields often use different names and terms for genes and molecules that are in fact related, it's very easy for a researcher to miss out on making a link to new, potentially useful information.

To forge connections between data in the deep and surface webs, and to tackle the multiple-naming problem, Reinhard Schneider, a team leader in EMBL Heidelberg's Structural and Computational Biology Unit, and his colleagues have developed a new set of software tools for biologists. Their approach comes under the umbrella of a new frontier in Internet software development called the semantic web. As the name suggests, this involves giving semantics, or meaning, to the data on the web, so that computers can make more sense of it and deliver more useful information to users.

One way of adding semantics into the mix is by adding 'tags', or labels, to pieces of data so that a computer program has a way of cataloguing them. It's a bit like adding captions to the ever-growing collection of photographs you may have on your computer, so that you can search through them easily to find the ones you want. Better still, if all your friends agree to use the same dictionary of words to caption common objects in their pictures, you'll be able to search through each other's albums and find what you want quickly and easily.

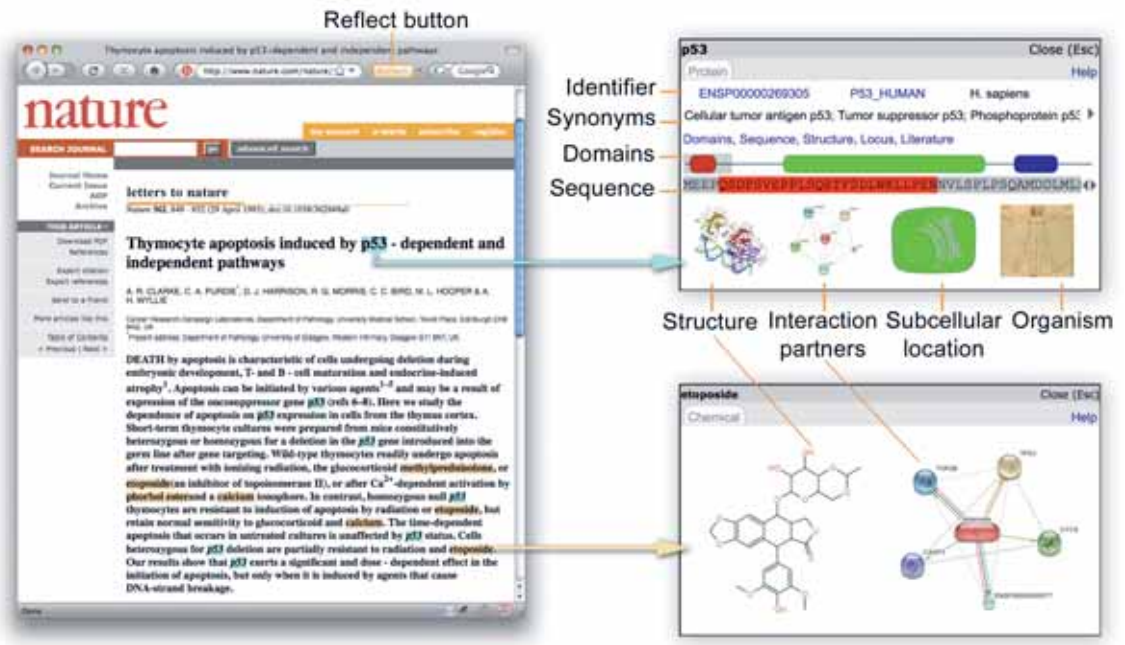
This all sounds great in principle, and in fact, a number of organisations – such as PubMed, an online literature resource, and a small number of scientific publishers – already do this to some extent. The trouble is, adding tags to documents and other bits of data is a tedious and time-consuming task that offers little incentive for people to undertake. "That's an additional annotation job, which does not pay off immediately, so no one wants to do it," says Reinhard. What's more, not everyone uses the same words to describe the same things, making it harder for software to find the right connections between pieces of data.

But Reinhard and his team have developed software that adds tags to biological data automatically, saving users the job of having to do it themselves. Although there are already programs available that do something similar, few are specifically tailored to the needs of life scientists, or they have limited functionality. One of these new tools is called Reflect. Reflect is an example of 'augmented web browsing': software that users can either download or access via a third-party site to add an extra dimension of information-gathering to their browsing. Such software automatically adds tags to words it recognises in the text of a webpage, by turning them either into links to relevant web pages or into 'entity popups': small popup windows that open when a user clicks on a highlighted word.

Reflect was designed with three aims in mind: first, to be easy to use; second, to deliver the result in a few seconds at most; and finally, to include entity popups that gave concise summaries of the key features of the item, as well as web links to the relevant data sources. Reinhard says: "We managed to keep it very simple, so really everybody can use it."

Users have the option of downloading a small software package, or plug-in, that works with their web browser, or they can visit Reflect's website at http://reflect.ws, and simply type in the URL of the webpage they want to see. The Reflect server at EMBL then automatically tags any words it recognises on the webpage. It can do this thanks to its vast dictionary, which currently contains the names and synonyms of more than 4 million small molecules and 1.5 million proteins from 373 organisms.

The tagging itself is rapid, taking only a few seconds. The popups include information such as molecular structure, sequence and names of the molecules with which the item is



With Reflect, information about proteins or genes mentioned in a webpage is just one click away.

known to interact. If biologists want to tag documents, such as scientific papers, they can upload these to an extension to the Reflect server called OnTheFly, which can tag PDF, PowerPoint or Word files. Users can also upload spreadsheets containing their data, so that Reflect can tag it for them before they submit it to a journal or a database. "To do this by hand is very tedious," says Reinhard. "With Reflect, it's just one click." Reflect will also allow users to correct any mistakes in its tagging, or to add any missing data. "If you have a lot of people doing this, you could add more semantic information to your data," says Reinhard.

Reflect is currently in its first version and has been up and running since the end of 2008. Reinhard hopes the tools will transform the way biologists do research, perhaps helping them make connections or ask questions they might not have done before.

The tools could also be useful for database annotators, as well as being a means for scientific publishers to enhance their content. The industry has already recognised the potential benefits of the technology: Reinhard's team is the winner of the first Elsevier Grand Challenge, a competition inviting researchers to develop tools to help life scientists deal with the increasing amount of online biological data.

Of course, the more connections you make within biological data, the more complicated it becomes. There comes a point when it is too complex to follow, even with the help of a diagram. This is where another of the team's new tools, Arena3D, proves useful. Developed by Georgios Pavlopoulos and his colleagues, Arena3D allows biologists to visualise connections between their data by grouping related data, such as sequences or diseases, into flat layers which are then staggered in a three-dimensional layout. These layers can then be spaced out to make it easier to see the connections that exist between one layer and the next. Researchers can manipulate the layers and explore both direct and indirect connections between datasets.

This means that, as well as making relationships easier to see, Arena3D can help biologists discover new ones. Reinhard's team has used these features, for example, to uncover hitherto hidden links between Huntingtin, the protein involved in Huntington's disease, and other proteins. Arena3D is freely available for download or use at http://arena3d.org.

Both Reflect and Arena3D seem set to become invaluable tools for 21st-century biology. Exactly what an extraterrestrial would make of them is, of course, another matter. Would they contact us to share their scientific knowledge? Maybe. Or perhaps they would simply ask us to remember the neighbours and try to keep the TV noise down.

Pavlopoulos G, O'Donoghue S, Satagopam V, Soldatos T, Pafilis E, Schneider R (2008). Arena3D: visualization of biological networks in 3D. *BMC Systems Biology* **2**, 104

Pafilis E, O'Donoghue S, Jensen L, Horn H, Kuhn M, Brown N, Schneider R. (2009). Reflect: Augmented Browsing for the Life Scientist. *Nature Biotech*, in press

http://arena3d.org

http://reflect.ws

Liliana Minichiello, Mumna Banchaabouchi and Viviana Triaca

A rose by any other name

Since the late 1950s, mammalian physiologists have been fascinated by a phenomenon known as the Bruce effect, in which a pregnant rodent exposed to the odour of an unfamiliar male, in the three days following conception, spontaneously aborts or reabsorbs her embryo. Almost nothing is known about the mechanisms underlying this effect, but in 2008, Liliana Minichiello's group at EMBL Monterotondo stumbled serendipitously on a brain system that could be involved.

Odour plays a key role in the sexual life of rodents. Odorous chemicals in male urine, in particular, can affect a female's hormonal status, ovulation, sexual receptivity and mate choice. Since these odours seem to favour fecundity, scientists suspect that they may be harmful to pregnancy. The Bruce effect is often regarded as a dramatic illustration of this, since the odour of a strange male triggers abortion in close to 100 per cent of the pregnant females that are exposed to it in that critical early window, with the result that they begin to ovulate again and become receptive to the new male's attempts at copulation.

Two sensory systems must be in good working order for these social odours to guide a mouse towards successful mating: the main olfactory bulb and the vomeronasal organ. Both are involved in the perception of odours, though the vomeronasal organ has long been thought to play a dominant role in mediating the reproductive functions of odours. The work of Liliana's group, however, suggests that the role of the main olfactory bulb has been underestimated.

The group's discovery was serendipitous because the researchers weren't interested in the Bruce effect. In fact, they were – and still are – interested in an entirely different question: the role of a certain brain molecule, a receptor for the enzyme tyrosine kinase, in the neuronal process thought to underpin learning and memory – long-term potentiation. In pursuing that line of enquiry, they had created a mutant mouse which lacked an important component of the receptor in question. Noticing that the main olfactory bulb in the female mutant mice contained more cells that expressed another enzyme, tyrosine hydroxylase (TH), than wild-type controls, Che Serguera, a postdoc in Liliana's lab, decided to investigate what role the modulation of TH plays in the female olfactory bulb.

The main olfactory bulb is interesting because it is one of the few regions of the mammalian brain where neurons continue to be born throughout life. Che was also armed with two pieces of information about TH: first, that in the olfactory bulb it is required for the synthesis of the neurotransmitter dopamine, which is involved in the discrimination of odours; and second, that it is modulated by the female hormone oestrogen. Given that oestrogen regulates the ovulatory cycle, he decided to study the expression of TH in the olfactory bulbs of wild-type female mice over that cycle, and to look for changes in their perception of odour over the same period, with the goal of explaining in physiological terms a possible link between ovulatory cycling and olfaction.

Che's first finding was that TH levels were equivalent in male and female main olfactory bulbs, and did not seem to change significantly over the ovulatory cycle in

females. One condition, however, did have an affect: pregnancy. Pregnant females from two mouse strains both showed about a 30 per cent increase in TH-positive cells in that brain structure over the first three or four days following conception. TH levels then remained high for at least eight days, before falling again around 12 days after conception.

The researchers were intrigued: could they be looking at a dopamine-mediated neuronal mechanism which prevents the mother from losing her embryo, but only once that embryo has implanted itself in the uterus lining – in other words, only once her investment in it is fairly certain? "The female goes through so many changes in pregnancy, that to lose the embryo would amount to a serious energy loss," Liliana says, "so it is quite likely that a protective mechanism is in place."

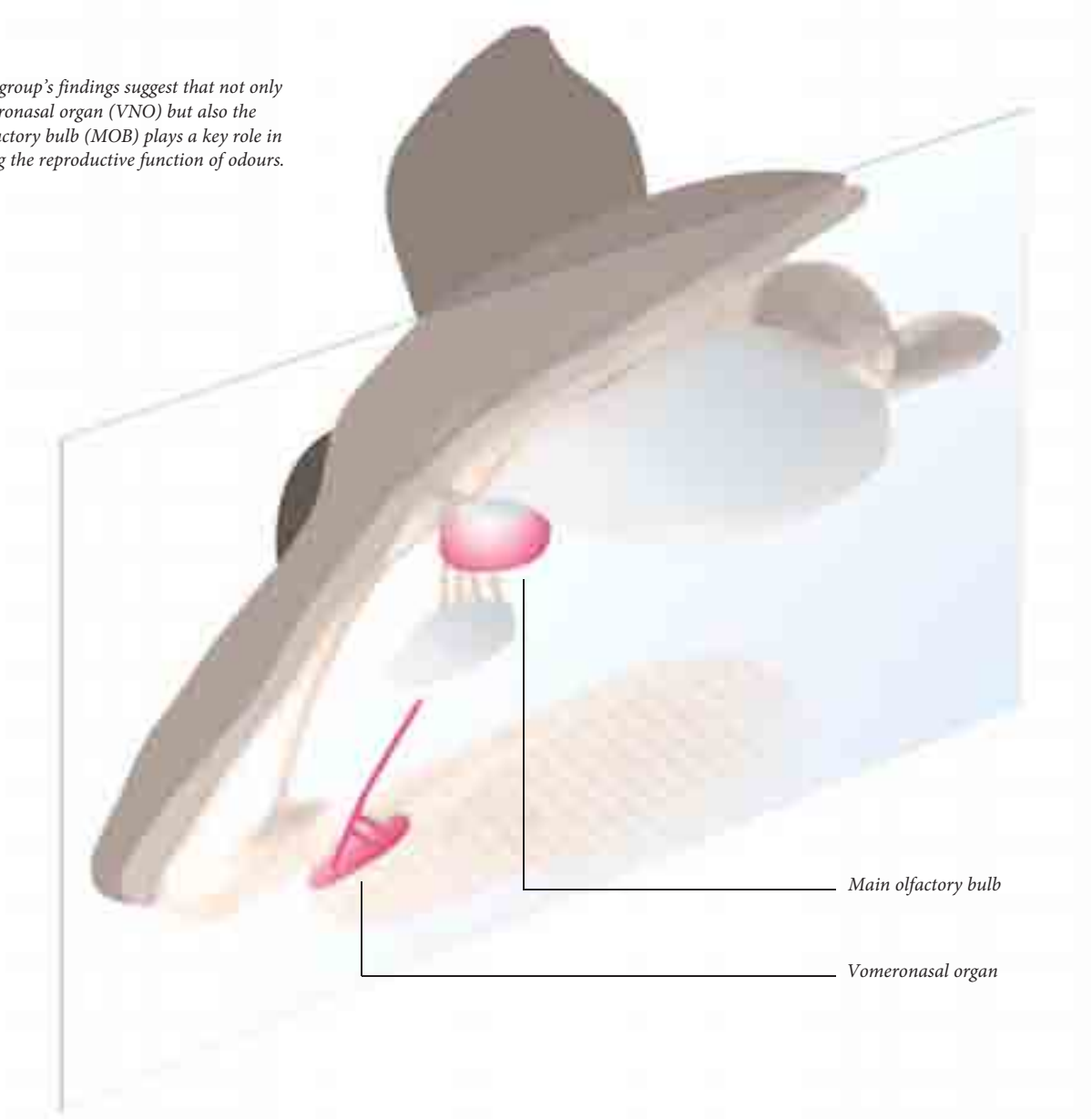
By contrast, the researchers speculated, the first three days following conception might not benefit from this protective mechanism – the rise in TH, and the concomitant rise in dopamine – which might explain why, in that early, pre-implantation phase, the mouse is vulnerable to the Bruce effect. Another possible explanation for their findings is that, once implanted, the embryo sends out protective signals to ensure its own survival. The researchers decided to investigate further.

Che treated pregnant mice with a dopamine antagonist, a chemical that binds to dopamine receptors thereby blocking the action of dopamine itself. When these mice were exposed to the urine-impregnated bedding of an unfamiliar male between six and eight days after conception – that is, in the middle of the implantation period, when they would normally have been protected from abortion – a significantly higher number aborted their pregnancies compared to mice that did not receive the dopamine antagonist. Those mice that received the antagonist but were exposed to the bedding of the male that had impregnated them were also unlikely to lose the embryo.

"We think we're looking at a dopaminergic sensory barrier," Liliana says, adding that the changes in TH levels they see during pregnancy – and hence the changes in dopamine levels in the main olfactory bulb, to which the synthesis of TH contributes – are indeed associated with changes in the mice's sensitivity to odours. Female mice typically prefer to smell male urine than female urine, and prefer low molecular weight fractions of that urine to higher ones. So to test their hypothesis, the researchers separated male and female urine into low and high molecular weight fractions, and studied females' ability to discriminate between those fractions at different stages of pregnancy.

"At the beginning of their pregnancy, until about three days, they would spend more time smelling the low molecular weight sample of males," says Liliana. "But between four and six days, they no longer discriminated much between the low molecular weight fractions of male and female urine." So as the number of dopaminergic neurons in the main olfactory bulb increased, the mice's ability to discriminate odours decreased.

When the researchers looked at expression levels of a marker of neuronal activation, the protein c-Fos, in the mouse brains, including the main olfactory bulb, they found that they were significantly reduced in pregnant females compared with ovulating females, or with pregnant females that had received the dopamine antagonist. In other words, those mice that had been deprived of their sensory barrier, and were once more



Liliana's group's findings suggest that not only the vomeronasal organ (VNO) but also the main olfactory bulb (MOB) plays a key role in mediating the reproductive function of odours.

vulnerable to the pregnancy-disrupting effects of strange male urine, showed much more activity in the part of the brain responsible for discriminating odours than those that had not.

Though the study led Che and Liliana away from the main subject of their research, to which they have now returned (Che has taken up a new post with the French national health and medical research institute, INSERM, in Paris), there is one follow-up question Che hopes one day to be able to answer: what is the molecule or combination of molecules in male mouse urine that triggers abortion in females, the perception of which is blocked by this dopaminergic sensory barrier? "I think it's really a complex array of molecules that carries information about the identity of an individual," he says.

The nature of that array continues to elude the researchers. For now, Liliana is happy to point out the wider significance of their discovery: "What is very important is that the main olfactory bulb definitely has a reproductive function," she says, "because that is something that has always been in doubt." For some very important aspects of reproduction, notably the maintenance of pregnancy, it seems that the vomeronasal organ can only perform its mediating role in tandem with the main olfactory bulb.

That finding may have relevance to the link between olfaction and pregnancy in humans, whose vomeronasal organ is defunct, though they have a fully active main olfactory bulb. Though nobody is suggesting that the Bruce effect is at work in people, pregnant women do report changes in their sensitivity to smell, and it's possible that some of the same brain mechanisms are at work in both cases – and even that those changes in sensitivity could be an evolutionary relic of the Bruce effect.

Serguera C, Triaca V, Kelly-Barrett J, Banchaabouchi MA, Minichiello L (2008) Increased dopamine after mating impairs olfaction and prevents odor interference with pregnancy. *Nat Neuroscience* **11**: 949-56

EMBL EMBO

Systems and Synthetic Biology

Scientific and Social Implications

9th EMBL/EMBO joint conference on Science and Society

EMBL HEIDELBERG, GERMANY

7-8 NOVEMBER 2008

Science and Society

Systems biology has been one of the laboratory's 'buzz phrases' for the last year or so. According to the EMBL Programme 2007-2011, it is 'the natural next step for EMBL to take'. The move towards a holistic approach rather than a reductionist one flies in the face of what life scientists have always been taught, but it's increasingly recognised that the study of complex interactions in biological systems can lead to a more realistic understanding of biological processes – in other words, studying a number of components simultaneously is a much more effective way to address the pluralism of cause and effect in networks. As one of the fathers of systems biology, Denis Noble, put it in a lecture at EMBL back in 2007, "Transmission of information isn't just one-way; there's downward causation from all levels. Neither is there a privileged level of causality."

EMBL has long boasted a multidisciplinary approach to research, and this presents the perfect, ready-made breeding ground for a systems-based *modus operandi*. "EMBL has gathered much of the expertise required to take a systems approach over the past ten years," Director General Iain Mattaj has said. "Now we want to combine and extend these efforts for a deeper understanding of complex biological phenomena." At EMBL, we have the data resources, the computer know-how and the research techniques, supplemented by cross-disciplinary approaches – in particular the EMBL Centres for functional genomics, imaging and computational biology – and we have the biologists, chemists, physicists, mathematicians, computer scientists and engineers needed to achieve everything under one roof, from analysing the collective behaviour of amino acids to understand a protein structure to creating working models of whole organs.

This year's Science and Society Conference – the ninth – tackled the subject and related issues with its theme, 'Systems and Synthetic Biology: Scientific and Social Implications'. A joint venture by EMBL's Science and Society representatives and those of its sister organisation EMBO, the annual conference calls upon leaders in its chosen field to present the relevant issues with thought-provoking presentations that initiate debate and discussion. As the University of Newcastle's Tom Kirkwood put it on the first day of 2008's event, "It's fantastically important that we look at these problems from a broader perspective, and that's exactly what meetings like EMBL's Science and Society Conference achieve."

While Tom Kirkwood addressed the importance of a systems biology approach to understand "one of the biggest issues we have to face in the human condition", ageing, another of the founding fathers of the field, Leroy Hood, talked about the paradigm shift required in the way we think about science in light of this emerging discipline. "Biology has always been about taking things apart," said the founder of the Institute for Systems Biology in Seattle, going on to describe his own efforts at achieving a systems approach to disease, in particular cancer and prion diseases such as Alzheimer's. Other speakers and participants were in agreement that a sea change is required at the teaching stage in order to encompass such a shift in perception.

Other lectures and discussions during the two-day event covered advances in synthetic biology – a field which is

Clockwise from top: An interested audience filled the Operon at EMBL Heidelberg to listen to talks by provocative speakers such as Leroy Hood, question discussion panels, and even carry debates over into the conference's coffee-breaks.

intrinsically linked with systems biology, as a result of its emphasis on the reconstruction of biological systems – and the use and possible abuse of new technologies and their societal implications, with such names as American physicist, philosopher and historian Evelyn Fox Keller and the University of California's David Deamer joining the debate. The non-academic angle was well represented by participants from AstraZeneca and the European Patent Office. A constant strength of the Science and Society Conference is the series of panel discussions organised as part of the programme, attended by scientists and non-scientists alike – and which can become so lengthy and heated that they often throw the entire schedule off-kilter.

The visitor numbers are testament to the success of the symposia. In 2008, 254 participants attended the event from 17 countries, with 63% coming from Germany and the farthest travellers coming from the United States. The packed auditorium year after year has been one of the arguments behind Heidelberg's need for a larger events facility, the Advanced Training Centre, which will be completed later in 2009.

THE IMMORTAL LIFE OF HENRIETTA LACKS

This year it was one of the visiting speakers who sparked the most interest and discussion by presenting a topic which challenged the veracity of something that most researchers had always thought of as fact, revealed the two sides of a coin where ethics and the consequences of scientists' actions were concerned and, as such, opened a Pandora's box of questions and considerations.

HeLa – the first human cell line ever grown in culture – is one of the most widely used and important laboratory tools in medicine and research. HeLa cells have helped researchers uncover the secrets of cancer and viruses, and have led to important advances like stem cell research, gene mapping, cloning and *in vitro* fertilisation. Sent to labs in their billions around the world – and beyond – HeLa cells have been used to develop the polio vaccine, blown up at nuclear test sites and even flown in space shuttle missions.

Even if you've used HeLa cells yourself in your research, you probably haven't given much thought to their origin – and even if you have, you can be forgiven for getting it wrong. As recently as 1994, the McGraw-Hill Dictionary of Scientific and Technical Terms was still describing HeLa cells as 'human cancer cells maintained in tissue culture... originally excised from the cervical carcinoma of a patient named Helen Lane'.

The truth is, there never was a Helen Lane. The 'He' and 'La' of the name actually stands for Henrietta Lacks, a housewife from Baltimore and mother of five children, who died from that same cancer in 1951 at the age of just 31. But the story here isn't just about a pseudonym or a cover-up.

Rebecca Skloot, a freelance science writer based in Memphis, Tennessee,

Henrietta Lacks

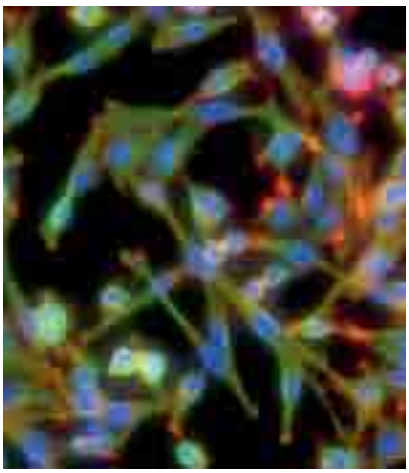
visited EMBL Heidelberg on 24 November to give a Science and Society Forum lecture, 'The Immortal Life of Henrietta Lacks', which is also the title of her forthcoming book. "Although Henrietta's cells did wonders for science, they had troubling consequences for her family," explains Rebecca, who, since her early days as a biomedical student planning to become a veterinary scientist, has been fascinated by the story. "Her husband, an impoverished tobacco farmer with a third-grade education who struggled to afford housing and healthcare, didn't learn about the cells until 25 years after Henrietta's death."

That itself was only due to a twist of fate. The cells had been taken without Henrietta's or her husband's knowledge or consent – not unusual in those days – but it wasn't until 1974 that suspicion grew that they might be infiltrating the world's stock of other cell cultures. Researchers approached the Lacks family for blood and tissue samples – anything to find genetic markers to help identify the cells.

For a family with a third-grade education, this sudden news that their mother's cells were alive in a lab – that 'she', 25 years dead, was being tested, manipulated, blown up and sent into space – was bewildering. Even with the understanding that the researchers were testing for hereditary material, it was confusing. "Deborah Lacks, Henrietta's youngest daughter, thought she'd get a phone call telling her whether she was going to live or die. She never heard back from the researchers and soon had the first of what would become several breakdowns," says Rebecca.

The story raises some serious questions about informed consent and patient confidentiality. "The case is often held up as an example of scientists acting unethically – they didn't ask her, and then they sold the cells and got rich on them. But it's not that simple. What the scientists did wasn't maliciously intended at all, but it wasn't just a couple of cells; to somebody, that was their mother."

Rebecca hopes that the sale of her book will eventually enable a scholarship fund to be set up in Henrietta's name. "Members of the Lacks family feel they've been passed over in the story. They know their mother's cells started a medical revolution and are now bought and sold around the world, but they're pretty sure that someone, somewhere, has profited from their mother's death – and it's not them."



A Year in the Life of EMBL

May

How personal is my genome?

This and other related questions were addressed during the second EMBL-EBI Science and Society symposium in Cambridge on 23 May. The half-day meeting brought together geneticists, social scientists and anthropologists at Fitzwilliam College at the University of Cambridge, UK, to discuss the implications of recent advances in sequencing technologies and the questions raised by the advent of personal genomics – such as of its impact on discrimination in society. The attendees particularly appreciated the balanced picture presented during the symposium, which covered a wide range of the concerns and arguments about personal genome services.

Lab Day 2008

On 10 June, scientists from all outstations gathered in Heidelberg for another EMBL Lab Day. As in previous years, it was an excellent opportunity to network and catch up with old friends in an informal atmosphere. Apart from traditional activities such as the poster competition and the graduation ceremony, this year also saw the presentation of the first John Kendrew Award by former EMBO Executive Director John Tooze to Antonio Giraldez and Giovanni Frazzetto, and the launch of the Alumni Wiki, a new source for networking and career development for alumni. Other non-scientific aspects of Lab Day included a theatre production and a football game.

June

July

2008

EMBL goes Athens

EMBL's new EIPOD programme was the topic of many interesting questions at the 33rd annual Federation of European Biochemical Societies (FEBS) congress, held in Athens from 28 June to 3 July. The majority of the visitors to the EMBL stand were PhD students or postdocs who had already heard about EMBL but wanted to find out more about career opportunities and working conditions.

Staff Association Summer Party

It was one of the hottest days of the year, but that didn't deter the crowds from joining the Staff Association Summer Party 2008 at EMBL Heidelberg, where the canteen staff had already prepared chilled cocktails and a wide range of delicious international specialties. Further highlights were an attractive entertainment programme with live bands and DJ music. The sale of the tombola tickets yielded the record sum of €5,000 for the cancer charity Waldpiraten camp.

A Visit from Israel

On 17 July more than 20 visitors from Heidelberg's twin town of Rehovot in Israel visited EMBL. After a comprehensive tour of the Core Facilities, the mayor and other dignitaries, among them the president of the Weizmann Institute, left for a bigger tour of the city.

July

Not blinded with science

As the biggest gathering of scientists, politicians, media and outreach people in Europe, the third European Science Open Forum (ESOF) attracted more than 4,000 participants from 18-22 July in Barcelona for scientific sessions, exhibitions and other activities which even included drama and dance productions. As a partner organisation of EIROforum, EMBL was also present with a stand and EMBL staff members contributed to the conference by hosting sessions on communication and education.

Time to travel

An EMBL-wide reunion took place over one week in August at the International Union of Crystallography Congress in Osaka, Japan, which celebrated its 60th anniversary with more than 2,600 participants from 66 countries. The EMBL booth was manned by scientists from Hamburg, Grenoble, Heidelberg and EMBL-EBI who were also involved in the congress by presenting talks, software demonstrations and posters.

August

Passing on the torch

It was the end of an era when the European Life Sciences Organisation (ELSO) held its last annual congress on 30 August to 2 September in Nice. EMBL attendees were involved in the programme with talks, minisymposia and subgroup meetings. After eight years of organising this high-profile international forum for molecular life science research, ELSO will hand over the organisational reins to EMBO, which will launch a new series of meetings as a platform for outstanding lectures, workshops and networking opportunities, starting in Amsterdam on 29 August to 1 September 2009.

First European Science & Society Summer School

'Deconstructing and ordering living organisms' and 'Re-making life: new bioentities and their meanings' were the two major themes at the first European Science & Society Summer School (E4S) held in Heidelberg on 25-30 August. The attendees particularly praised the culture of reflection and the way the talks brought together the proverbial 'Two Cultures' represented by the 20 PhD students and post-docs from the life sciences, humanities and social sciences.

2008

Faculty retreat

Faculty members old and new came together at the Golfhotel Stromberg near Frankfurt for two days of discussion and talks away from the bench. For the 20 new group leaders hired since the last retreat, it was an excellent opportunity to network and look for potential new collaborations.

Welcome to the predoc network

The Portuguese capital was the venue of this year's predoc retreat on 5-7 September. The three days provided ample opportunity to discuss science and issues of interest such as the introduction of the new full-time Dean of Graduate Studies which was unanimously regarded as hugely beneficial.

September

2008

Happy anniversary!

It may be hard to believe now, but the Kinderhaus had only eight children when it opened its gates in 1988. Since then, it has steadily grown and now offers 98 places. Reason enough to celebrate its 20th anniversary on 26 September with a special summer party, which included many activities for the children and national specialties prepared by the parents. The head of the Kinderhaus particularly wanted to thank her staff and EMBL general and social services for the amount of support they had received over the years.

A top ceremony for the Advanced Training Centre

Thursday 25 September saw the traditional 'topping out' ceremony for the Advanced Training Centre which celebrates the last beam being placed at the top of a building. With the basic structure now finished, most of the work will be focused on the interior before the EICAT groups and management, administration, and the Office of Information and Public Affairs move into the new building in late 2009.

September

October

Nature Award for Peer Bork

On 24 October, Peer Bork was one of three scientists to be awarded a *Nature* Award for Mentoring in Science. He was nominated for his outstanding leadership skills and method of mentoring. The ceremony was held at the Bode Museum in Berlin, and speakers included the German Federal Minister of Education and Research.

Postdoc retreat in France

Aix-les-Bains at the heart of the Savoyen region of France provided this year's location for the postdoc retreat on 10-12 October, where 51 postdocs took the opportunity to talk science and discuss other topics related to their position, such as taxes on fellowships, the pension scheme and the mentorship programme for new postdocs at EMBL. The focus of the retreat was on increasing 'connectivity' between the attendees, such as by generating more publicity for the Postdoc Association.

October

Nature at the crossroads

Starting in Heidelberg on 23 October, the 10th PhD Student Symposium, 'Decision making in Biology – Nature at the crossroads', offered valuable insights into the immeasurable number of decisions made every second by biological systems. More than 200 attendees came together to share outstanding research including a presentation on 'Getting in and out of mitosis' by Nobel Laureate Tim Hunt. Other highlights were the 'meet the speakers' dinner and the panel discussion on how to justify basic research to the public.

Joining forces

January saw the launch of two retreats. Structural biologists came together on 23-24 January for their structural biology retreat in Bad Dürkheim near Mannheim for what is planned to be the beginning of a regular series of biennial get-togethers. One item on the agenda was to discuss a common strategy for EMBL's involvement in the pan-European initiative INSTRUCT (the Integrated Structural Biology Infrastructure), which is aimed at building a network of infrastructures and platforms for integrated structural biology.

It was also the debut of the new Chemical Biology retreat, which took place in Heidelberg. With more chemistry groups and projects now at EMBL one of the chief aims was to identify where overlap of expertise and applications will foster new collaborations. The next meeting is planned for 2010.

January

2009

The fountain of youth?

Will stem cell research mean the end of ageing? When Head of EMBL Monterotondo, Nadia Rosenthal, gave an Insight Lecture for the Set-Routes programme about 'Stem Cells and Regeneration', she had more than 160 students from four local schools hanging on her every word. The lecture was followed by a panel discussion about careers in science.

EMBL abroad

The MIT Career Fair, organised by the European club at the Massachusetts Institute of Technology in the USA, on 24-26 January offered another opportunity for EMBL to advertise jobs and its wide range of training programmes to students aiming to return to Europe. The fair was very well attended and provided an excellent platform to pursue EMBL's strategy to increase its visibility. EMBL also attended other careers fairs in Berlin (Federal Foreign Office) and in Lausanne (Federal Department of Foreign Affairs) in February.

'Bashed neeps' and 'Champit tatties'

In honour of the most famous Scottish poet Robert Burns, 150 guests came together on 14 February for Burns Night Supper at EMBL. As in recent years, the gastronomic affairs were accompanied by literary extras such as the "address to the Haggis" and a rendition of "Holy Wullie's Prayer" followed by Ceilidh, the traditional Scottish dance.

February

Our planet and its life

On 12-16 February EMBL took the opportunity to advertise itself at the annual meeting of the American Association for the Advancement of Science (AAAS) in Chicago. As the world's largest general science organisation, AAAS brings together scientists, policy-makers, teachers, journalists and members of the public – an ideal setting for EMBL to strengthen Europe's presence at a major American conference and to network with other European organisations. This year's theme, 'Our Planet and Its Life: Origins and Futures', called to mind the 200th anniversary of Charles Darwin's birth and the 150th anniversary of the publication of his book *On the Origin of Species*.

March

Official opening of NMR

The rector of Heidelberg University was one of the guests of honour at the official opening of the upgraded high-field nuclear magnetic resonance centre (NMR) on 13 March in Heidelberg. Topics on the agenda of the one-day symposium included the recent advances of NMR in terms of technology and applications, and its increasingly important role in structural biology.

Open Day at EMBL-EBI

EMBL-EBI's Outreach and Training team organised its 7th Open Day – formerly the Masters Open Day – on 12 March at EMBL-EBI's premises in Hinxton, UK. Thirty-three students from 11 institutions spent the day learning about life at the institute, its research, services and data resources, and the EMBL International PhD Programme.

VIP visit from Down Under

On 30 March, Professor Richard Larkins AO, Vice-Chancellor and President of Monash University in Melbourne, Australia, visited EMBL Heidelberg to meet Director General Iain Mattaj and Head of EMBL Monterotondo Nadia Rosenthal in preparation for taking up office as the first chair of EMBL Australia's council in July this year.

March

European get-togethers

In March and April, EMBL hosted a series of visits from all over Europe, among them a group of 41 Finnish biochemistry and biotechnology students from the Institute of Medical Technology in Tampere. They were welcomed to EMBL Heidelberg on 12 March in a visit initiated by a former Finnish trainee at EMBL. They were followed by a group of Greek students on 1-2 April from the Costeas Geitonas High School in Athens, whose visit was organised by a SET-Routes school ambassador. The students were introduced to EMBL and its PhD programme and heard talks by group leaders and PhD students. The days were rounded off with a lab visit.

It's Girls' Day at EMBL

As it does every year, EMBL again participated in Girls' Day on 23 April. The idea of this day is to encourage young women to try out some technical and scientific jobs, to test their skills and to find the strength to choose a non-traditional profession. The 22 girls and 4 boys were hosted at EMBL on an individual basis to offer an insight into 'typically male' jobs. Activities included an introduction to the basics of photography in the Photo Lab and the extraction of the participants' own DNA.

2009

April

Music, maestro!

EMBL Heidelberg hosted 25-year-old Russian piano virtuoso Nikolai Tokarew in his sell-out Heidelberger Frühling recital on 16 April. The prize-winning young artist treated an audience of 320 people to pieces by Rameau, Tchaikovsky, Mussorgsky, Ravel, Rosenblatt, Liszt and Debussy.

Index of group & team leaders

Annual Report 2008-2009

Planning, co-ordination, editing

Lena Raditsch, Anna-Lynn Wegener

DG Report

Iain Mattaj

Science writers

Claire Ainsworth, Sonia Furtado, Laura Spinney, Anna-Lynn Wegener

Graphics

Petra Riedinger

Photography

Hugo Neves, Christine Panagiotidis, Udo Ringeisen, Marietta Schupp

Cover and inside cover design

Petra Riedinger based on a picture by the Gilmour group (cover) and Schultz group (inside cover)

Layout and composition

Nicola Graf

Contributors

Christian Boulin, Caroline Hadley, Vienna Leigh, Angela Michel, Silke Schumacher
Special thanks to the EMBL group leaders and Heads of Units

Additional photography and graphics

Stephen Curry, Imperial College (3), Stefan Welti (11), Typoform and the Royal Swedish Academy of Sciences (RSAS) (18, 19 bottom), ESRF (28), Rob Ruigrok (29, top left), Steven Todd (74, bottom right), Dr. G Dallas & Margaret Hanna © California Academy of Sciences (74, bottom centre), Healesville Sanctuary (74, second from top), Kurt Stüber, MPI Köln (76), Florian Fuchs (103), febs.org (105), esof2008.org (106), Uta Mackensen (106, bottom), Ernst Stelzer (107), Evangelia Petsalaki (107, bottom), Gregor Reither (109), Maria Hondele (109, bottom), Klaus Grewer (110, right), colellaphoto.com (111, left), Angela Michel (113, top)

Print

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EMBL Heidelberg
Meyerhofstraße 1
69117 Heidelberg
Germany

Tel. +49 (0)6221 387 0
Fax +49 (0)6221 387 8306

EMBL Grenoble
6, rue Jules Horowitz, BP 181
38042 Grenoble, Cedex 9
France

Tel. +33 (0)4 76 20 72 69
Fax +33 (0)4 76 20 71 99

EMBL Hamburg
c/o DESY
Notkestraße 85
22603 Hamburg
Germany

Tel. +49 (0)40 89 902 0
Fax +49 (0)40 89 902 104

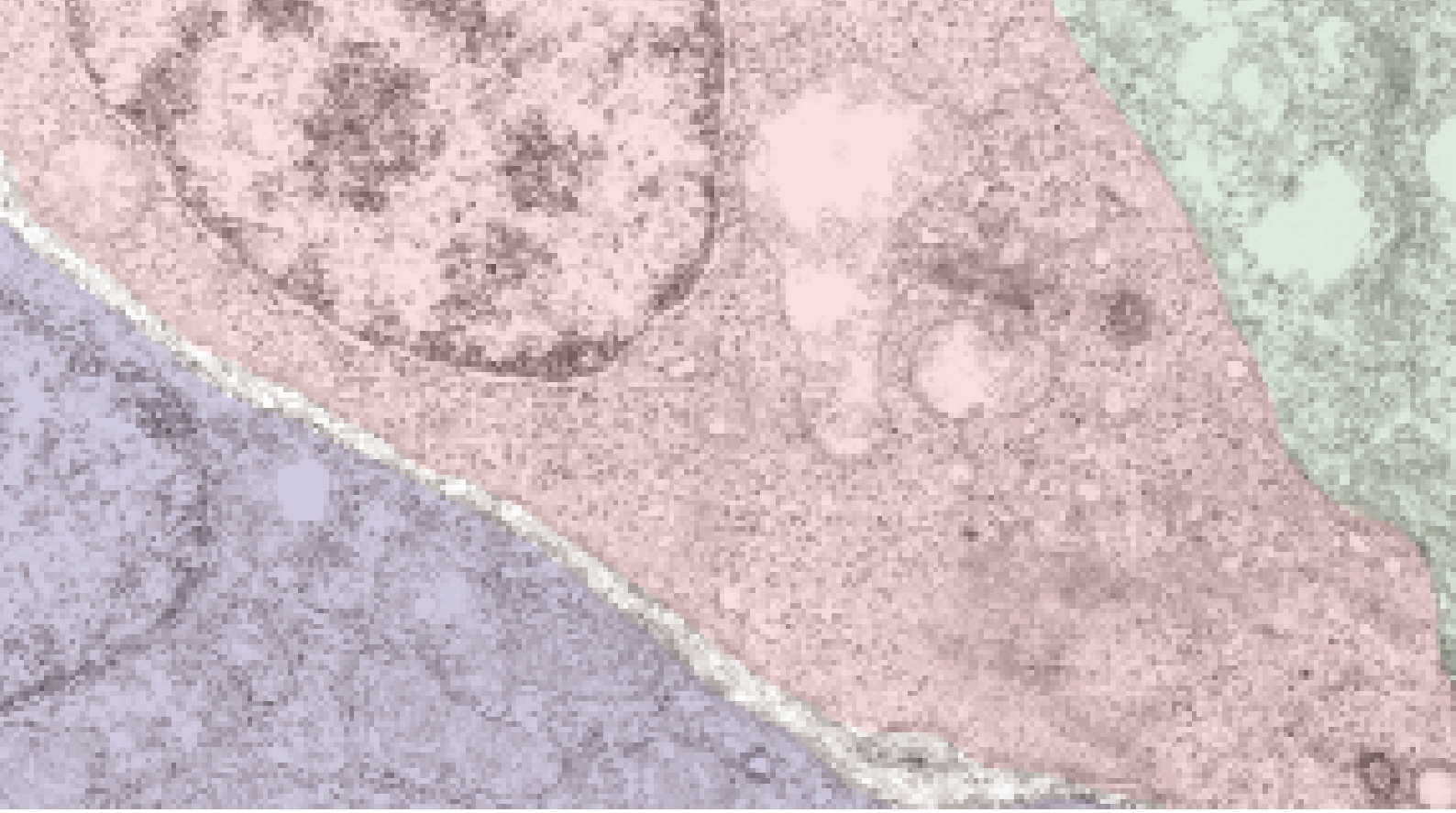
EMBL-EBI
Wellcome Trust Genome Campus, Hinxton
Cambridge CB10 1SD
United Kingdom

Tel. +44 (0)1223 494 444
Fax +44 (0)1223 494 468

EMBL Monterotondo
Adriano Buzzati-Traverso Campus
Via Ramarini, 32
00016 Monterotondo (Rome)
Italy

Tel. +39 06 900 912 85
Fax +39 06 900 912 72

www.embl.org



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