

Annual Report 2011-2012

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European Molecular Biology Laboratory

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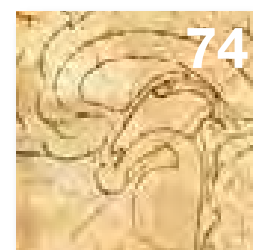
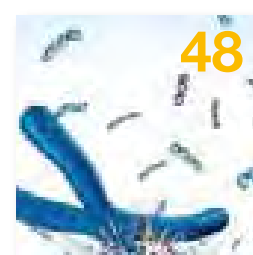
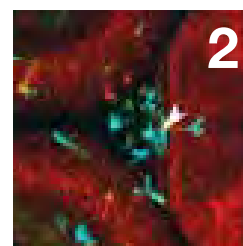
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Thank You!

This year my message is very simple but extremely important: thank you! Firstly to our member states, which for almost 40 years have valued and supported the work EMBL is doing for life science research in Europe. At the end of last year they approved an Indicative Scheme that will allow us to implement many aspects of our ambitious 2012-2016 Programme. At a time when many countries are in financial difficulties this is a sign of strong appreciation for EMBL and we are both proud and extremely grateful. In addition, the UK government pledged generous support to create the hub of the emerging infrastructure for biological information ELIXIR at EMBL-EBI. This impetus, together with the support of the other 10 ELIXIR member countries, who committed not only to several national nodes but also to an initial central budget for ELIXIR, represents the first step towards making the new pan-European infrastructure a reality.

Thank you also to those who have been instrumental in shaping, guiding and leading EMBL in the recent or more distant past. Some have just left EMBL, in particular Nadia Rosenthal, Head of EMBL Monterotondo, and Graham Cameron, Associate Director and Head of Services at EMBL-EBI. Both contributed enormously to the development of these successful outstations. EMBL's second Director General, Lennart Philipson, who played a key role in shaping the whole Laboratory, sadly passed away in 2011. To all of you we are heavily indebted and thankful.

We also thank those who have either joined EMBL or taken on new roles in the past year, especially Rolf Apweiler, Ewan Birney, Phil Avner, and Malcolm Jolliffe, who have the task of filling the large holes left by Graham and Nadia and the imminent departure of the Head of Finance, Keith Williamson. We need your ideas and energy to propel EMBL into the future.

And finally, I would like to thank all EMBL staff and fellows, those producing excellent science and those working in the background to keep the Laboratory running and improving. It is your work that makes EMBL the success story that it is – and some highlights of this work are featured in this report.

Iain Mattaj

State of the Laboratory

The end of 2011 brought very positive news that will allow the Laboratory to plan optimistically for the future. At its winter meeting at the end of November, EMBL Council approved the EMBL Programme 2012-2016 and the accompanying Indicative Scheme. It foresees an annual budget increase of 2% (without inflation adjustments) that amounts to an overall sum of roughly €500 million over five years. EMBL greatly appreciates this expression of support by its member states, which for many countries comes at a time of serious financial difficulty. The delegates also agreed to review the Indicative Scheme at the end of 2013 after evaluating how their financial



New EMBL-EBI Associate Directors Rolf Apweiler and Ewan Birney, with retiring Associate Director Graham Cameron.

situations have developed. The financial commitment will allow EMBL to implement many aspects of its ambitious 2012-2016 Programme. Within the framework of Information Biology, the Programme's main theme, thematic research areas will be: bridging the scales of biological organisation, biology in four dimensions, predictive networks and models, generating quantitative data, analysing, integrating and exploiting quantitative data, inter-species variation, intra-species variation and disease models and mechanisms. There are also wide-ranging future plans for EMBL activities in the areas of service, training, technology transfer and international relations and integration.

It so happens that the start of the new EMBL Programme coincides with the departure of several people who have substantially shaped the main

Laboratory and its outstations. After 30 years at EMBL, Graham Cameron, EMBL-EBI's Associate Director, retired at the beginning of March 2012. Graham was instrumental in the foundation of EMBL-EBI in 1993, and without his infinite commitment, enthusiasm and persistence the EBI would simply not be what it is today – Europe's leading bioinformatics research institute and service provider. In his role as Associate Director, Graham oversaw all EBI service activities and was dedicated to continuously improving them to always offer the best possible support to scientists around the world.

Even though the gap Graham has left will be difficult to fill, two highly qualified successors have already been identified. Following an international search, EMBL-EBI team leaders Rolf Apweiler and Ewan Birney were appointed joint Associate Directors of the outstation as of April 2012. Both EMBL senior scientists have had long and successful careers at EMBL-EBI and are familiar with the challenges and complexity of providing world class bioinformatics services. Since 2007, Rolf and Ewan have been jointly running the EBI's large PANDA team that develops public-domain protein and nucleotide databases and tools. Graham has been working closely with Ewan and Rolf over the past months to ensure the smooth transition of stewardship of the world's most comprehensive range of freely available molecular databases and we consider ourselves lucky to have found such an excellent solution.

EMBL Monterotondo is also undergoing a change in leadership this year. After more than 10 years as Head of EMBL's Mouse Biology Unit in Italy, Nadia Rosenthal left EMBL in May 2012 to take up the position of Scientific Head of EMBL Australia, a network formed by the eight major Australian research universities and CSIRO to implement EMBL's first associate membership. She also holds the post of Director of the Australian Regenerative Medicine Institute at Monash University in Melbourne. Nadia has done an outstanding job in directing the Mouse Biology Unit. She recruited excellent scientists, inspired a productive and creative scientific atmosphere and established EMBL Monterotondo as a central hub in the international network of mouse biology.

In November 2011 EMBL Council appointed Philip Avner as Nadia's successor. Philip has been the Head of the Developmental Biology Department at the Institut Pasteur in Paris. His research interests are in the area of mouse genetics and genomics and the relationship between genetics and epigenetics. In his work at the Pasteur Institute, Philip has proven that he possesses great leadership and strategic skills and EMBL is delighted to welcome him among its faculty. Philip is already spending time in Monterotondo and will be there full-time from September 2012.

Sadly, EMBL also had to say a final goodbye this year. Lennart Philipson, EMBL's second Director General, passed away on 26 June 2011. Lennart headed EMBL for over a decade between 1982-93, a crucial time for molecular biology when different scientific disciplines in the life sciences were becoming increasingly interlinked. He reorganised the Laboratory into new scientific and instrumentation Units, with a profound impact on both scientific success and the development of innovative technologies in areas such as microscopy. Most influentially, he foresaw the power of bioinformatics approaches and ensured that EMBL became a stronghold of research and service activities in this area. Throughout his career, Lennart was renowned for bringing together the right combinations of talent to achieve goals. He held a number of important positions on both sides of the Atlantic and was deeply passionate about the importance of basic research in the life sciences as an international activity. Lennart will be fondly remembered as a great scientist, colleague and friend.

Research

EMBL scientists frequently receive top marks in scientific reviews, they publish papers in high-impact journals and are heavily cited. Still, it is always reassuring when the quality of their work also receives recognition by prestigious funding agencies, such as the European Research Council. In the past year, EMBL Associate Director Matthias Hentze and senior scientist Detlev Arendt have both been awarded an ERC Advanced Investigators Grant. Therefore, together with the grant awarded in 2010 to joint Head of the Structural and Computational Biology Unit, Peer Bork, three of the highly competitive ERC grants are held by EMBL's senior faculty. EMBL's junior faculty has been equally successful. In 2011 team leader Christiane Schaffitzel in Grenoble and staff scientist Rocio Sotillo in Monterotondo both received ERC Starting



Nadia Rosenthal,
former Head of
EMBL Monterotondo.

Independent Research Grants following closely in the footsteps of EMBL Heidelberg group leaders Marcus Heisler, Takashi Hiiragi and Francesca Peri, and EMBL Grenoble group leader Ramesh Pillai, who were awarded ERC grants in 2010 (p. 64).

Inter-Unit Collaborations

There are many examples of teamwork at EMBL that has led to scientific breakthroughs, but not so many involve interactions between scientists from three EMBL sites. Rarer still does the work result in two *Nature* papers. But such was the case with research by scientists at EMBL Grenoble, EMBL Monterotondo and EMBL-EBI, who generated new insight into how cells protect themselves against so-called transposons – sequences of DNA that move from place to place within the genome. Ramesh Pillai at EMBL Grenoble, Dónal O'Carroll at EMBL Monterotondo, and Anton Enright at EMBL-EBI took a divide-and-conquer approach to study all three Piwi proteins that mouse cells employ to protect themselves against transposons. Dónal and Anton's groups showed that, before birth, one Piwi protein makes it easier for another to enter the nucleus and inactivate transposons (p. 26). And Ramesh's group found that the third Piwi protein works after birth, mopping up any leaks in this repression (p. 26).

Through a collaboration enabled by their shared EMBL Interdisciplinary Postdoc (EIPOD), Eileen Furlong in Heidelberg and Ewan Birney at EMBL-EBI unveiled a new model for how genetic switches called enhancers recruit the molecules that activate them. In the process, they also discovered that en-

hancers and their activators can be used as clues to a cell's developmental history (p. 32).

Another shared EIPOD project between Hinxton and Heidelberg took a close look at a protein known to promote cancer metastasis. In this collaboration, the groups of Maja Köhn in Heidelberg and Janet Thornton at EMBL-EBI found that excessive amounts of this protein probably disrupt the cell's scaffolding, allowing cancerous cells to change shape and move around the body (p. 52).

Mouse Biology

Apart from changes of leadership, Monterotondo also enjoyed an exciting year in terms of research. Among the highlights of 2011 was the discovery that the brain's wiring is shaped during development by cells called microglia pruning the connections between neurons. The findings by Cornelius Gross and his group indicate that changes in how microglia work might be a major factor in neurodevelopmental disorders like autism, in which brain wiring is altered (p. 74).

Developmental Biology

The Developmental Biology Unit in Heidelberg underwent its four-yearly review in 2011. The panel rated the research programme as outstanding and stated that for its size, the Unit ranks amongst the best in the world. The Head of Unit, Anne Ephrussi, was congratulated on her leadership, mentoring and her scientific performance. The Scientific Report features her work on how *oskar* RNA moves around fruit fly egg cells with the help of a mechanism that determines its destination and guarantees it will travel fast enough (p. 24).

Computational Biology and Bioinformatics

An international consortium led by EMBL-EBI developed a new standard for describing the effect of a compound on a biological entity – the Minimum Information about a Bioactive Entity (MIABE), which makes the interchange of public data on drug discovery more fruitful (p. 63).

In Heidelberg, in a continuation of an extensive analysis of *Mycoplasma pneumoniae* that was kick-started with three back-to-back papers in *Science* in 2009, the groups of Anne-Claude Gavin and Peer Bork have gained further insights into how this bacterium makes the most of its relatively few genes. Working in collaboration with EMBL alumni Luis Serrano and Rob Russell, they have shed light on how *M. pneumoniae* controls its protein levels, and how it uses chemical tags to enable those proteins to multi-task (p. 78). At the beginning of 2012, in addition to his research activity and his role as joint Head of the Structural and Computational Biology Unit, Peer Bork took on the responsibility for coordinating all bioinformatics activities at the Heidelberg site. His aims are to better utilise the potential of this growing discipline, adapt to the changing research landscape, minimise redundant activities, and promote an interactive and supportive bioinformatics community in Heidelberg.

Structural Biology

José Marquez and his team in Grenoble have developed a way of predicting which proteins are most likely to crystallise depending on the temperature at which they usually function (p. 13). Stephen Cusack's group have used this method to determine the structure of RIG-1, which has revealed how this protein sounds the alarm when it detects viruses invading the cell (p. 66).

EMBL Hamburg was reviewed in September 2011. The panel particularly praised the outstation's development of software and synchrotron instrumentation as well as its research activities that address biological questions with structural biology approaches. As an example of the latter, Matthias Wilmanns' group combined X-ray crystallography, small angle X-ray scattering – in collaboration with Dmitri Svergun – electron microscopy and atomic force microscopy to obtain an unprecedentedly detailed view of the elastic part of myomesin. This protein links muscle filaments, and the study reveals how it is able stretch to two and a half times its original length (p. 60).

EMBL SCIENTIFIC PUBLICATIONS AND COLLABORATIONS

Total number of peer-reviewed publications: 384

Internal collaborations: Publications co-authored by more than one EMBL group leader: 36

External collaborations: 914 in total of which 77 resulted in publications

In November last year structural biology groups from Grenoble, Hamburg, Heidelberg and the EBI came together for a two-day structural biology retreat to exchange ideas about structural biology methods and challenges and to foster new collaborations.

Genome Biology and Cell Biology and Biophysics

Working in collaboration with the German Cancer Research Centre (DKFZ) and the University Clinic Heidelberg, Jan Korbel's group in the Genome Biology Unit discovered that an inherited mutation is likely to be the link between exploding chromosomes and medulloblastoma, which is a type of paediatric brain cancer and the second most common cause of childhood mortality in developed countries (p. 48). Jan Ellenberg's group in Cell Biology and Biophysics shed light on other congenital diseases in 2011 (p. 70). They discovered that as an egg cell forms, the cellular machinery that separates chromosomes is extremely imprecise at fishing them out of the cell's interior. This phenomenon could be behind errors in the number of chromosomes in an egg cell, which can lead to conditions like Down's syndrome and is the leading cause of miscarriage and female infertility.

Services

Bioinformatics Services

EMBL-EBI hosts Europe's most comprehensive biomedical data resources and makes them freely available to the scientific community. They are used heavily by scientists working in academia or industry around the world and in 2011, there were on average 5.3 million requests on the services per day. There has also been a steady growth in the number of user addresses accessing the services: this number increased by 6.1% between 2010 and 2011.

As in previous years, all core data resources have grown substantially in 2011. For example, the European Nucleotide Archive (ENA) – the DNA sequence database – received more than 10^{14} bases. This represents a four-fold increase since last year, and means that 75% of the data have been in the archive for one year or less. To keep up with these growing data volumes, scientists at EMBL-EBI spend a lot of their time developing effective mechanisms for data compression. In the past year, the ENA team developed a new format, called the CRAM format, which compresses DNA sequences by storing only differences between aligned and



Servers in the Hinxton data centre.

reference sequences. Expectations are that, at least for the mid-term, the use of the CRAM format will stabilise storage costs despite the increasing data flow rate.

In the context of ever-increasing data volumes and the cost and effort their storage and utilisation means for the EBI, the announcement from the UK Government of a £75 million commitment from the Department for Business, Innovation and Skills' Large Facilities Capital Fund for Europe's emerging infrastructure for biological information (ELIXIR) was fantastic news at the end of 2011. The new funding will allow the construction of ELIXIR's central hub at EMBL-EBI on the Wellcome Trust Genome Campus in Hinxton. In 2010, the Biotechnology and Biological Sciences Research Council (BBSRC) supported the EBI with a £10 million investment to strengthen its data handling capacity. Thanks to this support the EBI leased two new state-of-the-art data centres in London and the migration of databases to these new data centres has since been a major ongoing effort. 18 databases were transferred to the London Data Centre throughout 2011.

Other bioinformatics service highlights of the past year include:

- The Ensembl Genomes project, a resource featuring genomes of plants, fungi, bacteria, protists and non-vertebrate metazoans, was launched in 2009 and remarkably now contains 335 species from across all five kingdoms of life. At the end of 2011, the Ensembl team and external collaborators launched PhytoPath, a bioinformatics resource for plant pathogens, and PomBase, a resource for fission yeast.
- Throughout 2011 the InterPro, ENA and UniProt teams developed an integrated portal for

metagenomics researchers who study all the genomes present in a given environment.

- The EBI launched a new and integrated enzyme portal early in 2012, which mines and displays data about proteins with enzymatic activity from public repositories and includes biochemical reactions, biological pathways, small molecule chemistry, disease information, 3D protein structures and relevant scientific literature.
- In July 2011 EMBL-EBI was awarded a contract for the continuation of UK PubMed Central (UKPMC), the free online literature resource for life science researchers. According to this contract, the EBI will lead the project. Now five years old, UKPMC has grown from a simple mirror of the National Center for Biotechnology Information (NCBI) PubMed Central site to a stand-alone site providing access to a repository of over two million full-text biomedical research

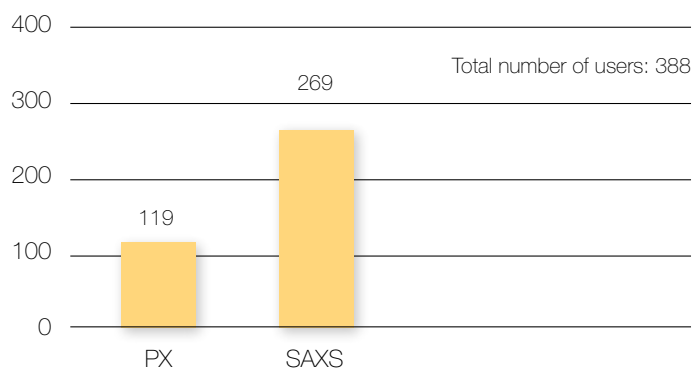
articles, more than 25 million citations from PubMed and Agricola, patents from the European Patent Office, UK treatment guidelines and biomedical PhD theses.

Structural Biology Services

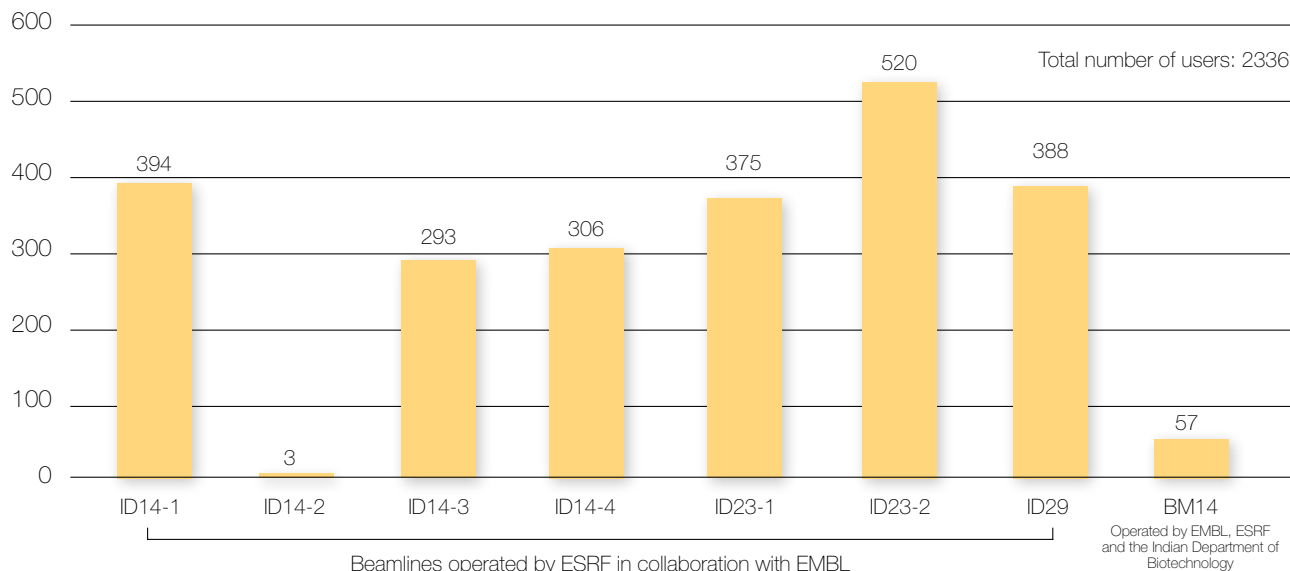
In addition to running successful research programmes in structural biology, EMBL's outstations in Grenoble and Hamburg both provide services to the scientific community by providing access to cutting-edge research infrastructures. In Hamburg they work closely with the German Synchrotron Research Centre (DESY) and in Grenoble with the European Synchrotron Radiation Facility (ESRF) to make the powerful X-ray sources available for applications in the life sciences. In 2011 EMBL Grenoble and Hamburg jointly registered 2724 users.

Since the official inauguration of the new PETRA III synchrotron in July 2010, a number of important milestones have been achieved. In May and June 2011 the first scattering patterns were recorded on the small angle X-ray scattering synchrotron beamline, BioSAXS, and the macromolecular crystallography (MX) beamline, P14. EMBL and DESY signed a collaboration agreement to make the new high-brilliance X-ray source available to external users from the life science communities. A first call for proposals for structural biology projects to be carried out at PETRA III was issued and more than 200 applications were received. After the project evaluation committee met for the first time on 24 February 2012, the first external users started their experiments on the new beamline in April 2012.

Beamline Users EMBL Hamburg 2011



Beamline Users EMBL Grenoble 2011



On 12 September 2011 EMBL Hamburg also signed a Memorandum of Understanding with the European X-Ray Free Electron Laser (XFEL), another new major research infrastructure to be constructed on the DESY campus. The agreement lays the foundations for future opportunities and collaboration. The European XFEL is unique worldwide, and is designed to generate high-intensity X-ray pulses – 27 000 times per second – with a brilliance a billion times higher than that of conventional X-ray sources. XFEL offers exciting prospects for deciphering the structure and dynamics of biomolecules. Victor Lamzin, Deputy Head of the Hamburg outstation, has submitted a proposal to XFEL suggesting ways to explore potential life science applications of the laser and to make it available to users from the biomedical research community in future.

EMBL Hamburg and EMBL Grenoble both participate in an initiative called BioStruct-X, to which structural biologists can apply for access to an integrated, transnational infrastructure of facilities and services. BioStruct-X is a collaboration of 11 European installations and offers multi-site access to structural biology applications in four key areas: X-ray scattering, macromolecular X-ray crystallography, biological X-ray imaging, and protein production and high-throughput crystallisation. The project started on 1 September 2011 and EMBL Hamburg hosted the launch meeting on 5-6 December. BioStruct-X cooperates with the Integrated Structural Biology Infrastructure (Instruct) ESFRI project in aiming to provide an integrated and coordinated technology platform. BioStruct-X is funded through the Seventh Framework Programme (FP7) of the European Commission.

In Grenoble, the ESRF will undergo a major upgrade over the coming years, which will entail the construction of eight new beamlines, the refurbishment of many existing beamlines and major new developments in synchrotron radiation instrumentation. The past few months have seen the peak activity of the upgrade, which entailed a shutdown of the synchrotron between 5 December 2011 and May 2012. This is the first time since the inauguration of the ESRF that the accelerator complex and storage ring have been shut down for such an extended period of time. In the long-term EMBL scientists and the structural biology user community will greatly benefit from the upgrade.

Core Facilities and IT Services

EMBL operates eight technical Core Facilities, which are central components of EMBL's research



network. They offer cutting-edge technology and services in the areas of advanced light microscopy, electron microscopy, genomics, proteomics, protein expression and purification, flow cytometry, chemical biology and monoclonal antibody production and are heavily used by many research groups at EMBL and, to a lesser extent, by external users from the EMBL member states. In 2011, in the context of the general reorganisation of EMBL Heidelberg, the Genomics, Flow Cytometry and Protein Expression and Purification Facilities have been relocated within the Laboratory to bring them closer to their most frequent users.

Several Core Facilities acquired new equipment in the course of the past year. The Proteomics Core Facility acquired new Orbitrap mass spectrometers that greatly improve its ability in quantitative proteome analysis. The Advanced Light Microscopy Facility (ALMF) now owns a Leica super-resolution microscope, which is an important tool to bridge the gap between high- and low-resolution imaging techniques and allows the study of biological organisation across scales. To further support this kind of research the Electron Microscopy Facility has in the past year been equipped with a Zeiss AxioObserver light microscope that allows Correlative Light and Electron Microscopy (CLEM). Also new in the ALMF are a Zeiss two photon laser scanning microscope and an Olympus bioluminescence/fluorescence microscope that allows parallel imaging of bioluminescence and fluorescence signals.

The beamline ID 14 at the ESRF in Grenoble.

In the Genomics Core Facility, there was an upgrade of its massively parallel sequencing suite, which now comprises four HiSeq 2000 Instruments. In addition, Genecore now also owns an Ion Torrent system (Life Technologies), which is based on semi-conductor and microfluidics technologies rather than conventional biochemical sequencing methods and allows much longer DNA fragments to be decoded.

In summer 2012 EMBL's Monoclonal Antibody Facility in Monterotondo will be outsourced and turned into an EMBL spin-out company. Under the lead of the current Head of the Facility, Alan Sawyer, the company will be called Paratopes Ltd. and will continue to provide services to EMBL scientists.

To cope with the vast quantities of data produced by many parts of the Laboratory, the central Information Technology (IT) Services, which operate the IT infrastructure and provide services to users in Heidelberg and Monterotondo, introduced new systems based on massively parallelised data storage access. A second newly introduced platform simplifies working together in virtual teams at EMBL and with external collaboration partners. Another major new IT project is 'Helix Nebula – the Science Cloud' that EMBL is working on as part of a consortium with the EIROforum members CERN, the particle physics organisation in Geneva, and the European Space Agency (ESA), as well as 15 leading IT industry partners. The project aims to develop a technical solution to the massive IT

requirements of European scientists and wants to launch a sustainable European cloud computing platform that will provide stable computing capacities and services to meet the demand of the European scientific community.

Training

The EMBL International Centre for Advanced Training (EICAT) had a very successful first year under its new dual leadership by Coordinator of Internal Training, Helke Hillebrandt, and newly recruited Coordinator of External Training, Andy Robertson, who joined EMBL in August 2011 from the Keystone conference organisation.

The EMBL International PhD Programme (EIPP) continues to gain in popularity as shown by the ever-increasing number of applications. Both the second yearly call in 2011 and the spring selection in 2012 attracted close to 1000 applications each. In 2012 a total of 67 positions are available for PhD students. To handle the growing demands of assessing these applications, the EMBL graduate office has introduced a new evaluation module as a first element of a new online application system, which will reduce the time and effort EMBL faculty have to spend on the selection process. Further projects for the coming year include reorganising the successful two-month core course all students attend at the beginning of their PhD studies. Rearrangements are being made in order to better accommodate the growing class size. Moreover, complemen-

Poster session in
the EMBL Advanced
Training Centre.



tary courses providing training in communication or statistics will be incorporated to provide all new students with the best possible set of tools for them to carry out their PhD successfully.

THE EIPP IN 2011

Number of PhD students in 2011: 199

New PhD students joining in EMBL in 2011: 55

Graduations in 2011: 43

Average first-author publications during PhD: 2

Like the EIPP, EMBL's Postdoctoral Programme, in particular the EMBL Interdisciplinary Postdoctoral (EIPOD) fellowships, has also attracted large numbers of applications in the past year. The EIPOD Programme was initiated to support interdisciplinary research involving two or more groups at EMBL and fellows of the Programme are members of all the groups involved. In 2011, EMBL held two calls for applications resulting in the recruitment of 33 EIPOD fellows. The EIPOD Programme has been supported by a Marie Curie Actions Cofund grant from the EU FP7 since 2009. EMBL successfully negotiated a second grant that will cofund 60 fellowships of three years duration over the next five years. EMBL offers a range of training initiatives for its postdoctoral community to prepare the currently 224 postdocs for the next stage of their career. Twice a year a workshop entitled "Preparing for the Academic Job Market" takes place and the annual 'Career Day' gives young scientists insights into non-academic careers. Postdocs from all five EMBL sites actively participated in these and other career development opportunities.

In March 2012 the EMBL Advanced Training Centre at EMBL Heidelberg celebrated its second anniversary and another year of successful training activities in the life sciences. These activities included 13 conferences, 21 courses, and many other seminars, training activities and meetings, all of which attracted over 8000 delegates, organisers, speakers and exhibitors. The three EMBO|EMBL Symposia represented the second year of collaborative programming by EMBO and EMBL. The EMBO Conference Series meetings on Chromatin and Epigenetics and on Protein Synthesis and Translational Control were held in the EMBL Advanced Training Centre and so could accommodate more participants than ever before. The Vision 2020 lecture series closed in 2011 with forward-looking lectures by three Nobel Laureates, all of

whom attracted audiences that filled the 466-seat Klaus Tschira auditorium. The EMBL Course and Conference Programme at EMBL Heidelberg continued its expansion by offering new courses on state-of-the-art research techniques.

The EMBL Course and Conference Programme has been generously supported by more than 70 organisations and particular thanks are extended to the 16 members of the Corporate Partnership Programme. Their contributions were used to support courses and conferences on diverse topics such as Chemical Biology, Electron Tomography and Metagenomics, as well as 140 fellowships for delegates from over 40 countries who would otherwise have been unable to attend.

EMBL-EBI's free e-learning resource, Train online, was launched in a beta version in September 2011, and provides short courses on the EMBL-EBI's most widely used data resources. It has attracted a total of 8000 unique users by the end of the year. Also new is the Bioinformatics Training Network (BTN), a completely open community resource that allows trainers to share, review and develop training materials and best practice. These online resources complement a dynamic face-to-face user-training programme that delivered 33 courses and workshops at EMBL-EBI, serving a total of 1003 trainees, and 24 Bioinformatics Roadshows in 18 countries that were attended by 720 trainees.

The European Learning Lab for the Life Sciences (ELLS), EMBL's teacher training initiative, took its successful LearningLab series to Barcelona in November 2011. A total of 22 high school teachers learned about the development of the fruitfly in a course that was organised in collaboration with the Institute for Research in Biomedicine (IRB Barcelona). In addition, LearningLabs have been organised at EMBL-EBI and in Monterotondo. In December the second lecture in the Insight Lecture series, which brings the latest EMBL research to secondary school teachers and students, was delivered by EMBL Heidelberg group leader Maja Köhn. 600 students and their teachers from 12 schools in 8 different European countries followed the presentation on 'Chemistry and Biology – Strong Allies in the Fight Against Cancer' either in the Klaus-Tschira Auditorium or via live internet streaming.

EMBL Alumni

The number of EMBL alumni has grown to 5341 with 193 people joining the EMBL Alumni Association in the last year, bringing the total to 2038



members. 80% of these EMBL alumni work in one of the EMBL's member and associate member states.

2011 brought new faces to the EMBL Alumni Association board following elections held in the autumn. The board now consists of 12 members who are representative of the alumni body in gender, nationality, world-wide distribution, EMBL Units and staff categories as well as current work sectors. At its December meeting in EMBL Grenoble, the board identified working groups to manage its four main areas of responsibility: communication, events, fundraising and the EMBL archive initiative.

Input for all four areas is being drawn from an alumni feedback survey that was completed by 837 alumni in December 2011. With a response rate of over a third, the survey collected extensive feedback on a broad range of topics, including the quality and delivery of EMBL and Alumni Association news, media, services, events and resource allocation. The information collected is being used for planning future alumni activities.

The John Kendrew Award, which recognises academic achievement or activities in the area of science communication of EMBL alumni, will go to two remarkable scientists in 2012. Gáspár Jékely, who was a postdoc in the groups of Pernille Rorth and Detlev Arendt at EMBL, was selected as one of the pioneers in the field of ecology evo-devo (evolution-development) and has written a book and numerous popular articles about evolutionary questions. The second awardee, Simone Weyand, a former postdoc in the lab of Manfred Weiss in Hamburg, will receive the award for her achieve-

ments in structure determination of secondary transporters and a G-protein-coupled-receptor. Simone is also highly involved in science communication at high schools and in social engagements for young terminally ill children in the UK.

2011 saw local alumni chapter meetings at Dilofo in Greece, at the EMBL summer party in Heidelberg, Germany and in collaboration with The EMBO Meeting in Vienna, Austria. The new board agreed to support the committed local chapters in Greece and Spain with a small fund to organise their 2012 chapter meetings.

Outreach

EMBL's diverse outreach activities continuously support and complement the Laboratory's scientific endeavour. In 2011, regular programmes such as the popular lab visits, the Science and Society lecture series and science communication activities, organised through EMBL's Office of Information and Public Affairs (OIPA), the EMBL-EBI Outreach and Training team and the Science and Society Programme, were complemented with some exceptional activities.

In July, EMBL Heidelberg hosted a group of 22 European science journalists, who came from 11 different countries. In the three-day programme jointly organised by EMBL and the German Cancer Research Centre (DKFZ), the journalists attended lectures given by scientists from both institutions and toured the laboratories and Core Facilities. Collaborative projects between EMBL and the DKFZ at the interface between basic science and medicine were a key focus during the event.

EMBL-EBI on the Genome Campus in Hinxton has been enjoying a lively series of debates, film and poetry about who 'owns' personal biological information, and the wider social issues surrounding personal genomics. Should research participants be given all their genomic data? If so, who will help them interpret it? What impact does this information have on individuals? Following on from these activities, the Wellcome Trust Sanger Institute is sponsoring an effort to genotype up to 1000 campus staff – including EMBL-EBI personnel – for a number of neutral traits, which were selected by a campus Working Group. The next step is to study the impact of communicating this information to individuals.

EMBL faculty, together with the European School of Molecular Medicine and the Harvard Kennedy School of Governance, organised an EMBL|EMBO Science and Society summer school for pre- and postdoctoral fellows from various disciplines and countries at EMBL Heidelberg at the beginning of August. Some of the participants, whose background ranged from natural to social science, came from countries as far-flung as the US, Mexico, Finland, Israel and Australia. The event centred around the topic of 'The Human Animal: Scientific, Social and Moral Perspectives' and addressed a number of topics ranging from 'What is it that makes us human?' to past and present quandaries regarding 'human enhancement'. The six-day programme included introductory presentations given by EMBL scientists and experts from around the world, as well as tutored sessions and an interactive programme of student presentations.

Administration

To allow EMBL scientists to focus entirely on their research and service activities, a small but efficient Administration team supports them in all relevant matters. In 2011 EMBL Administration engaged in a wealth of activities and projects, highlights of which include:

- The launch of the Risk Management project to identify and assess the major risks different parts of EMBL and the institution as a whole are facing. As a first step, interviews with key stakeholders have taken place in January and February 2012 to create a risk inventory. Funding agencies, notably the UK Research Councils, have started to make Risk Management Programmes a standard requirement for funding. Moreover, the worldwide financial crisis combined with increased organisational complexity,

and the growing awareness of the importance of reputation protection, has made it timely for EMBL to engage in a risk assessment exercise.

- The launch of Process Reengineering with an initial focus on grants. A series of administrative processes in possible need of review have been identified and will be improved over the coming years and rendered more efficient. The administration of external grants at EMBL is the first area to be reviewed. This project scrutinises all processes associated with grants at EMBL including, but not limited to, those carried out by grants services, the budget office and human resources in an effort to provide the best service possible for EMBL staff and to maximise EMBL's success in obtaining grant funding. As a first step, a training session was organised for relevant administrative and scientific staff in September 2011.
- The completion of the Administrative Systems Roadmap. Three years after the full implementation of SAP, EMBL has produced a roadmap that identifies needs for further development and integration of systems used both by the Administration and the Laboratory at large. The roadmap features priority projects, timeframes and initial cost-benefit analyses.
- The introduction of new Health and Safety training measures such as laser safety training. Many labs have been refurbished and reconstructed over the past few years, consequently EMBL's safety standards have been adjusted to meet current requirements. This has already been recognised by inspecting authorities.
- The relocation and refurbishment of several labs in a constant effort to improve rational space allocation at EMBL Heidelberg. A new cafeteria opened in Heidelberg in July 2011 and the stores and the ISG hotel have been substantially revamped. At EMBL Hamburg, work in Building 48e (PETRA III) was completed.

Finally, in January 2012, Malcolm Jolliffe joined EMBL as Head of Finance. He will take over from Keith Williamson, who after ten years of service at EMBL will retire at the end of 2012. Prior to joining EMBL, Malcolm was Project Controls Manager and then Finance Director for Alstom Power, one of the world's major providers of turnkey power plants.

Integration of European Research

Member state relations

EMBL always maintains very close relations with its member states and engages in a continuous dialogue to gather feedback about the needs of their scientific communities. In the context of preparing the new EMBL Programme and Indicative Scheme 2012-2016 this dialogue was particularly intense. This is why, over the course of the past year, representatives of EMBL's senior management visited several member state ministries and hosted national delegations to discuss plans and priorities for the next five years.

One of the long-term goals that EMBL has set itself is to encourage all European countries to join EMBL. Over the past year a lot of effort has been directed towards achieving this goal. Discussions about joining EMBL are at a very advanced stage with the Czech Republic, who is considering EMBL membership from 2013 onwards. Following a meeting between EMBL senior management and Vice-Minister for Science Ivan Wilhelm in September 2011, the Czech Ministry of Education, Youth and Sport officially requested information on EMBL accession. There has also been a range of events to stimulate scientific exchange between EMBL and the Czech Republic. EMBL participated in a workshop in Prague that was attended by key research institutes in molecular biology and related fields and in the official opening of the new Central European Institute of Technology (CEITEC) in Brno in September. In October, eight EMBL group leaders visited CEITEC to explore possibilities for future collaborations. To formalise this coopera-

Alexei R. Khokhlov,
Vice-Rector of the
Moscow State
University and
Silke Schumacher,
Director International
Relations sign a
memorandum of
understanding.



tion, EMBL and CEITEC signed a Memorandum of Understanding in March 2012.

In December 2010, EMBL and the Russian Foundation for Basic Research (RFBR) also signed a Memorandum of Understanding, with a view to developing joint cooperation towards Russian membership in EMBL. This was followed up by signing an implementation plan for the memorandum in July 2011, which foresees joint research projects and the organisation of joint workshops by EMBL and Russian scientists. Subsequently a call for joint projects was launched in October 2011. Up to six joint projects have been selected and will receive funding from the Russian Foundation for Basic Research. Moreover, EMBL was invited as a keynote speaker to the International Congress on Biotechnology in March 2011 and again in 2012.

To encourage even more European countries to consider membership of EMBL, EMBL Council established a working group in June 2011 tasked with evaluating schemes to increase the level of participation of countries from Central and Eastern Europe and the Western Balkans, including Turkey and Russia.

EMBL Partnerships

Partnership for Structural Biology

The Partnership for Structural Biology (PSB), which involves EMBL Grenoble, the ESRF, the Institut Laue-Langevin (ILL), the Institut de Biologie Structurale (IBS) and the Unit of Virus Host Cell Interactions (UVHCI), operates 14 technical platforms for integrated structural biology approaches on the Polygone Scientifique Campus in Grenoble. A 15th platform in biophysics is currently being developed. The PSB partners also carry out collaborative research projects in the areas of host-pathogen interactions, stress responses in prokaryotes and gene regulation. In future these kinds of collaborations will become even easier because the IBS, currently the only PSB member located off-site, will move onto the Campus. On 6 October 2011 a groundbreaking ceremony for the new IBS building on the campus took place in the presence of the French Minister for Science and Higher Education, Laurent Wauquiez.

The PSB was reviewed in 2011 and the evaluation panel praised it as a successful and mature organisation that adds value through its scientific activities and local platforms. The Scientific Advisory Board also pointed out that the PSB has been successful in building on and stimulating the effective collaboration of the partner institutes, and referred to the new IBS building under construction as the most visible evidence of this success.

Molecular Medicine Partnership Unit

The Molecular Medicine Partnership Unit (MMPU) brings together scientists from EMBL and the Medical Faculty of Heidelberg University. Until last year, it comprised five international research teams jointly headed by experts from both institutions. In 2011 three additional EMBL groups joined the MMPU to work on early warning signals of ageing in human stem cells, the assembly and maturation of infectious human immunodeficiency virus-1 (HIV-1), and molecular mediators of chronic pain. In September the MMPU research groups moved into a new building, the Otto Meyerhof Centre, provided by Heidelberg University on its campus. The arrangement combines the complementary expertise of basic and clinical scientists to research the molecular mechanisms that underlie common diseases all under one roof. In spring 2012 the MMPU underwent its four-yearly scientific review and received a very positive appraisal.

Nordic EMBL Partnership for Molecular Medicine

On 31 May 2011 the Laboratory for Molecular Infection Medicine Sweden (MIMS), the Swedish 'node' of the Nordic EMBL Partnership for Molecular Medicine, celebrated its official opening. MIMS, headed by Professor Bernt Eric Uhlin, is part of the Centre for Microbial Research of the University of Umeå. Established in 2007, the Nordic EMBL Partnership also includes Norway's Centre for Molecular Medicine (NCMM), affiliated with Oslo University, and the Institute for Molecular Medicine Finland (FIMM) of the University of Helsinki. Soon Denmark will also join the partnership. In November 2011 the Lundbeck Foundation, one of the largest private contributors to natural science research in Denmark, launched a call for a Danish host university for the national node of the Nordic EMBL Partnership for Molecular Medicine. The Foundation will fund the node with DKK120 million (€16 million) over 10 years. The Danish node will focus on conducting state-of-the-art re-

search in the field of neuroscience. After an evaluation of the applications the decision on where the Danish node will be hosted will be communicated later this year.

At the end of August last year the Nordic Molecular Medicine Network (NMMN) held its first annual meeting in Umeå/Lycksele with participants from EMBL, NCMM, MIMS and FIMM. The establishment of the network is an important part of the Nordic EMBL partnership and is essential to promote sustainable long-term collaboration. In the first meeting, research projects and the use and operation of core facilities were discussed.

In September 2012, EMBL and the Center for Genomic Regulation (CRG), with whom EMBL collaborates in a Partnership for Systems Biology, are organising a scientific conference on the topic of 'Perspectives in Translational Medicine'. This will be hosted by the CRG in Barcelona, Spain. Participants from EMBL and its three medically oriented partnerships with the CRG, MMPU and the three Nordic EMBL partner institutes, will present their research and discuss ways of joining the expertise of all partners by creating new and strengthening existing collaboration links.

EMBL Australia

EMBL Australia is a joint venture that has been established to oversee the implementation of the Australian associate membership to EMBL. Founding members of EMBL Australia are Monash University, the Universities of Sydney, Queensland and Western Australia, and the Commonwealth Scientific and Industrial Research Organisation (CSIRO). In the beginning of 2011 the Australian National University and the Universities of Adelaide, Melbourne and New South Wales acceded to EMBL Australia. EMBL and EMBL Australia are currently negotiating a collaboration agreement in view of effectively establishing and operating the EMBL Australia Partner Laboratory Network. The network will build on Australia's scientific strengths in cell biology, clinical research, stem cells and regenerative medicine, chemical biology and genetic epidemiology. Its hub is located at Monash University in Melbourne and nodes are planned at the Universities of Sydney, Queensland, and Western Australia.

On 21 June 2011, EMBL Australia launched a freely available service that allows Australian researchers to make the most of public bioinformatics resources. The new service is hosted by the University of Queensland, based in Brisbane. Australia's molecu-

lar biology community can now benefit from access to a wide range of data resources, including some of EMBL-EBI's core databases, software frameworks and tools, such as similarity searches, multiple sequence alignment and applications for discovering protein function. Joint EMBL-EBI Associate Director Ewan Birney provided assistance in setting up the new resource and visited Australia early in 2011 to review existing bioinformatics services. EMBL Director General Iain Mattaj also visited Melbourne and Brisbane in December last year, and met with EMBL Australian representatives and the two recently recruited EMBL Australia Partner Laboratory group leaders.

European Research Infrastructures

ELIXIR

ELIXIR, Europe's emerging infrastructure for biological information, entered the fifth and final year of its preparatory phase in November 2011. ELIXIR is a pan-European initiative to safeguard and foster data generated in life science experiments. Its core objective is to ensure that Europe can continue to handle a rapidly growing volume and variety of data from high-throughput experiments such as DNA sequencing. Proper management of this information promotes knowledge-based economic growth, and facilitates the translation of research into innovations that meet global challenges in many key areas including food security, energy and health. ELIXIR will be coordinated from its hub hosted by EMBL-EBI and its nodes will be sited at appropriate centres in participating countries throughout Europe.

In 2011, several milestones *en route* to ELIXIR's construction and operation were reached. The completion and publication of the ELIXIR Business Case in early 2011 defined the process for ELIXIR's construction and operation. During the spring and summer of 2011 ELIXIR worked on gaining the support of important stakeholders throughout. By September 2011, five countries plus EMBL had signed a Memorandum of Understanding to catalyse the implementation and construction of ELIXIR. The memorandum is the first formal – yet non-binding – step towards the implementation and construction of ELIXIR.

An ELIXIR Interim Board, the main body for negotiating the final legal and governance structure

of ELIXIR, has been convened. The first Interim Board meeting was held in London in November 2011, during which Søren Brunak of the Technical University of Denmark was welcomed in his new role as elected Chair. By then a total of ten countries and EMBL had signed the memorandum: Denmark, Estonia, Finland, the Netherlands, Norway, Slovenia, Spain, Sweden, Switzerland and the UK. Since the meeting, Israel has also signed. An important role of the Interim Board will be to establish an international consortium agreement and decide how ELIXIR will be governed and funded in the future.

By the end of 2011, funding bodies from several member states had committed a total of €117 million to the construction of both the hub and nodes of ELIXIR. A significant proportion of this – some £75 million (€90 million) – comes from the UK's Department for Business, Innovation and Skills' Large Facilities Capital Fund (LFCF) as a commitment to EMBL-EBI. This funding will allow the construction of facilities for ELIXIR's central hub at EMBL-EBI on the Wellcome Trust Genome Campus in Hinxton, Cambridge. The hub will be a nerve centre for bioinformatics in Europe, helping to coordinate the delivery of services and user training from centres of excellence Europe-wide. The hub will also establish a robust computing infrastructure that can handle the rising tide of life science data. Other significant financial contributions towards the construction of ELIXIR nodes throughout Europe have been made by Denmark, Finland, Norway, Spain, Sweden and Switzerland.

Euro-BioImaging

The mission of Euro-BioImaging is to provide access to a complete range of essential imaging technologies for every biologist and medical scientist in Europe and training in their use. From 2010 to 2013, Euro-BioImaging is engaged in its preparatory phase, aiming to define the overall framework of the research infrastructure. The project consortium comprises 39 legal partners from 15 European member states and associated countries and is coordinated by EMBL. The first year of the preparatory phase has been dedicated to consultation with the imaging community at large. In 2011, Euro-BioImaging conducted a Europe-wide survey. More than 660 participants representing imaging infrastructure users, providers, funders and industry gave valuable feedback on their needs and requirements and the existing imaging infrastructure landscape in Europe.

In 2012, 51 Euro-BioImaging proof-of-concept imaging facilities distributed over 14 countries are opening their doors to researchers free of charge. 228 scientists applied to the Euro-BioImaging open user call to conduct imaging experiments at one of these facilities. The proof-of-concept phase specifically aims to test and refine standardised execution and access protocols and to identify current community needs for access to different imaging technologies. Euro-BioImaging closely collaborates with a number of bottom-up national imaging infrastructure initiatives. To date, imaging communities from 14 different countries have organised themselves into national networks that support Euro-BioImaging principles.

In reaching out internationally, on 1 February 2012, Euro-BioImaging signed a collaboration framework with the Australian Microscopy & Microanalysis Research Facility. A first workshop on infrastructure operation, training programmes, web-tools, and sharing of best practice has already taken place and further activities (exchange of staff, common online training tools) are planned for the future.

Instruct

Instruct, the new pan-European research infrastructure for integrated structural biology, was officially launched on 23 February 2012 in Brussels in the presence of the Principal Investigators of each of the Instruct Centres, national and regional funding agencies and Robert-Jan Smits, European Commission Director-General for Research and Innovation.

Relations with the European Commission

In March 2011, EMBL signed a Memorandum of Understanding with the European Commission (EC) expressing the commitment of both institutions to cooperate to further the development of European research in the life sciences. As a first step towards the implementation of this agreement three meetings were held between EMBL and the EC in December. Representatives of both organisations explored synergies in the areas of health research, research infrastructures, technology transfer and knowledge management, human resources strategy, Marie Curie actions and international relations. The meetings were very constructive and identified areas in which EMBL and the EC will work together in future. In March 2012, a high-



level meeting between EMBL Director General Iain Mattaj and EMBL's Director of International Relations, Silke Schumacher, and the EC's Director General for Research and Innovation, Robert-Jan Smits and several of his senior colleagues, took place to plan further steps toward the implementation of the Memorandum of Understanding between EMBL and the EC.

Robert-Jan Smits,
Stephen Cusack and
David Stuart

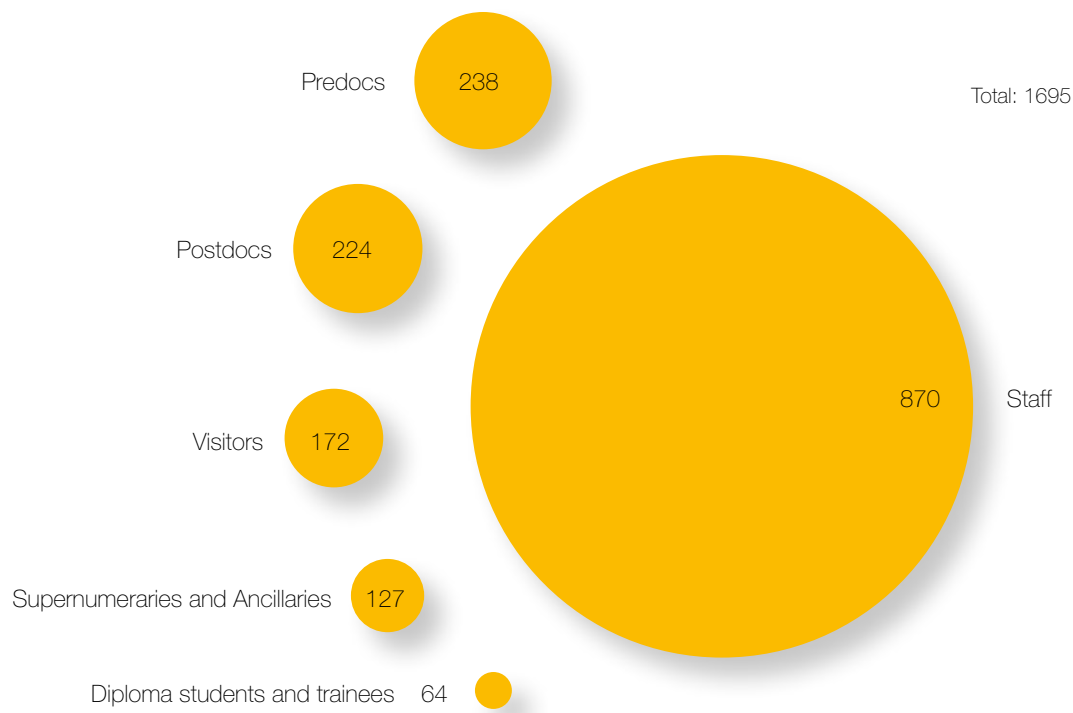
Throughout 2011 EMBL also published two important position papers in response to Green Papers by the EC that called for input into the development of the next European Framework Programme, Horizon 2020, which will run from 2014 to 2020, and on the implementation of the European Research Area (ERA). In both contexts EMBL particularly stressed the importance of basic research and world-leading research infrastructures for the future healthy development of European science.

In addition to its direct interaction with the EC, EMBL also maintains close links with the Commission as a member of EIROforum, a partnership between eight of Europe's largest inter-governmental scientific research organisations that are responsible for infrastructures and laboratories (CERN, EFDA-JET, EMBL, ESA, ESO, ESRF, European XFEL and ILL). EMBL is also a member of the Initiative for Science in Europe (ISE), an independent platform of European learned societies and scientific organisations whose aim is to support all fields of science at a European level and involve scientists in the design and implementation of European science policies.

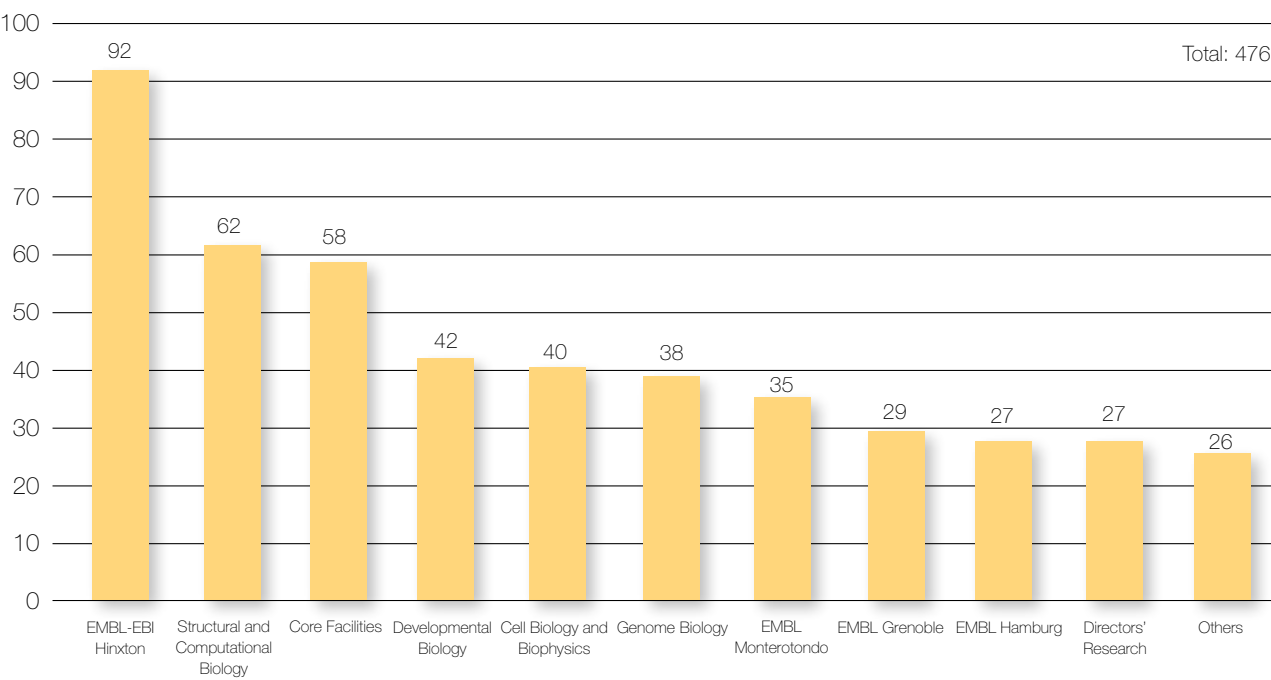
Personnel statistics

On 31 December 2011, 1695 people, including visitors, from more than 60 nations were employed by EMBL.

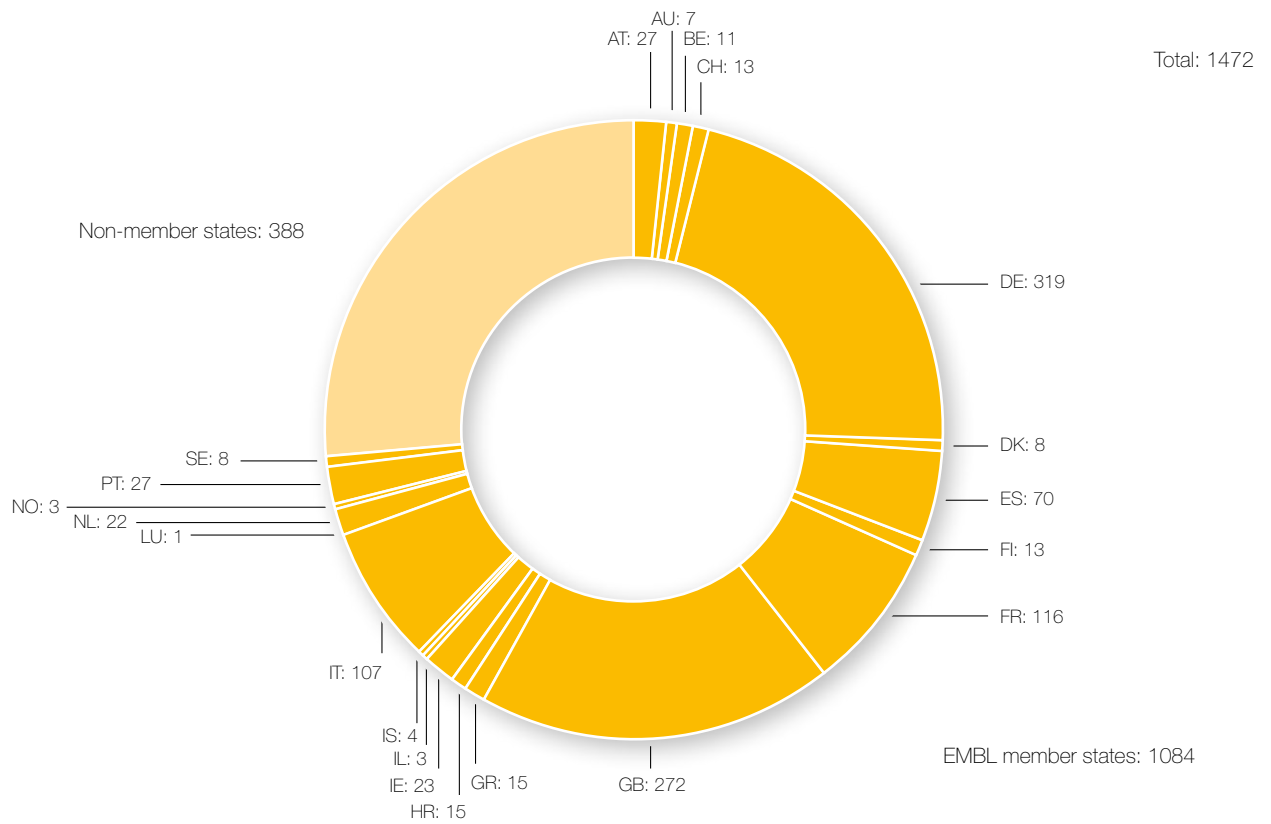
Personnel on 31 December 2011



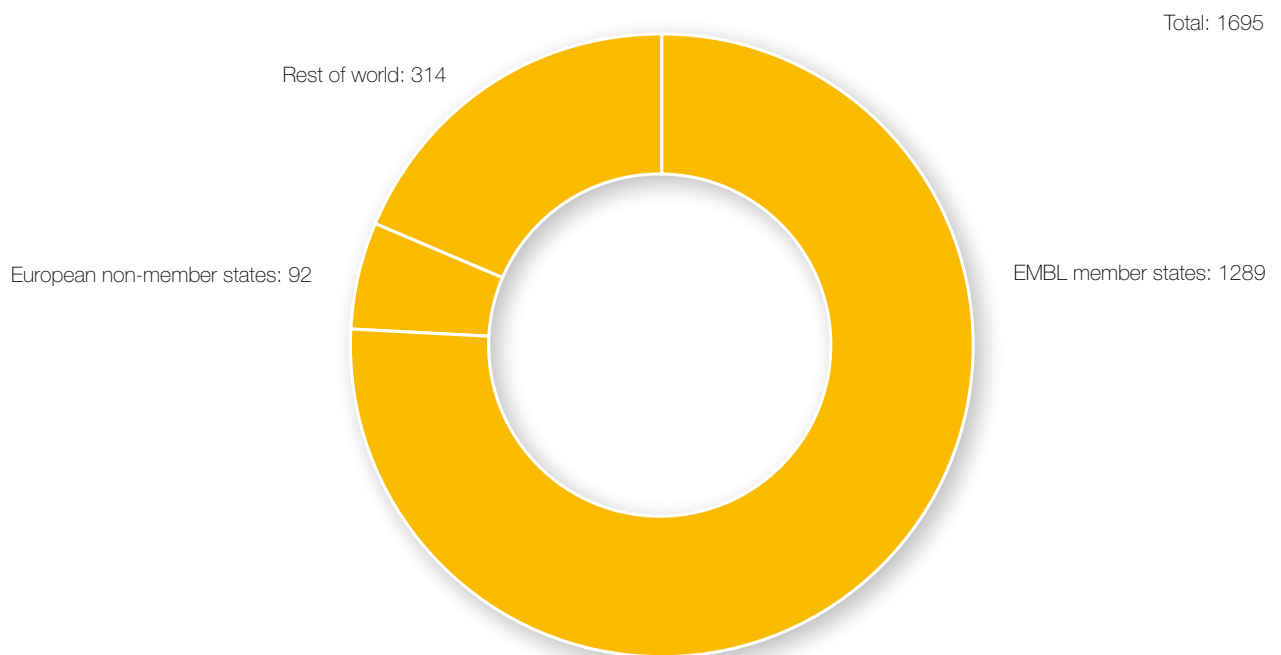
Visitors to EMBL Units during 2011



Staff nationalities – Research Areas



Staff Nationalities – All



Financial report

Income/expenditure statement

Income	2011		2010	
	€000	%	€000	%
Member state contributions				
Ordinary contributions	92,927		88,576	
Special contributions	23		23	
Associate contributions	752		2,475	
Additional contributions	600		13,866	
Internal tax	21,883		20,255	
External grant funding	37,849		33,915	
Other external funding	2,236		3,410	
Other income	15,068		20,869	
Total income	171,338		183,389	
Expenditure				
Staff costs	96,002		90,877	
Operating costs	58,969		62,742	
Capital expenditure & depreciation	13,058		24,317	
Total expenditure	168,029		177,936	
Surplus (deficit) for the year	3,309		(5,453)	

External grant funding

ANR	223	0.6	-	0.0
BBSRC	1,468	3.9	1,759	5.2
BMBF	3,461	9.1	3,644	10.7
BW	57	0.2	95	0.3
DFG	1,816	4.8	1,298	3.8
EC	13,024	34.4	12,372	36.5
ERC	330	0.9	117	0.3
FINOVI	2	0.0	-	0.0
HFSP	229	0.6	416	1.2
HUMBOLDT	17	0.0	5	0.0
MRC	173	0.5	338	1.0
NIH	8,797	23.2	6,309	18.6
VW Foundation	92	0.2	111	0.3
Wellcome Trust	5,014	13.2	5,100	15.0
Others	3,146	8.3	2,351	6.9
	37,849	100.0	33,915	100.0
Other external funding				
BIOMS	-	0.0	458	13.4
EMBL-EBI industry support	1,642	73.4	1,554	45.6
India beamline project	-	0.0	580	17.0
Other external funding	594	26.6	818	24.0
	2,236	100.0	3,410	100.0

Member state contributions

Ordinary contributions	Contributions				Pension contribution	
	2011		2010		2011	2010
	€000	%	€000	%	€000	€000
Austria	2,026	2.2	1,927	2.2	31	28
Belgium	2,500	2.7	2,352	2.7	38	34
Croatia	279	0.3	124	0.1	4	2
Denmark	1,617	1.7	1,503	1.7	24	22
Finland	1,292	1.4	1,202	1.4	19	17
France	14,766	15.9	14,091	15.9	223	206
Germany	18,948	20.4	18,201	20.5	286	266
Greece	1,682	1.8	1,865	2.1	25	27
Iceland	83	0.1	88	0.1	1	1
Ireland	1,199	1.3	1,052	1.2	18	15
Israel	966	1.0	769	0.9	15	11
Italy	11,365	12.2	11,368	12.8	171	166
Luxembourg	214	0.2	177	0.2	3	3
Netherlands	4,312	4.6	4,075	4.6	65	59
Norway	2,230	2.4	1,759	2.0	34	26
Portugal	1,078	1.2	1,079	1.2	16	16
Spain	7,657	8.2	6,833	7.7	116	100
Sweden	2,555	2.7	2,316	2.6	39	34
Switzerland	2,528	2.7	2,714	3.1	38	40
United Kingdom	15,630	16.8	15,081	17.0	236	220
	92,927	100.0	88,576	100.0	1,402	1,290

Special contributions

Croatia	23	23	-
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Associate contributions

Australia	752	2,475
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Additional contributions

Germany – to Advanced Training Centre (ATC)	-	2,663
United Kingdom – to Elixir		11,203
Germany Contribution to Infrastructure work	- 600	
		13,866

EMBL budget 2011: € 171 million

2011/2012 Reviews of EMBL Scientific Units

To ensure that its research and service activities continue to operate at the cutting edge, EMBL regularly submits them to stringent external reviews. Research and Service Units are evaluated every four years by members of the Scientific Advisory Committee and additional international experts. The following section features summaries of the scientific reviews that have taken place in the past year and presents the Director General's responses to the review reports.

Developmental Biology Unit Review

On 4 and 5 May 2011 the review of the Developmental Biology Unit took place in Heidelberg. Andrew Murray, from Harvard University, USA, chaired the panel of ten reviewers, four of whom, including Andrew, were members of EMBL's Scientific Advisory Committee (SAC).

Evaluation Summary

The research of EMBL's Developmental Biology (DB) Unit is rated as outstanding. For its size, the Unit is ranked amongst the best in the world. The strengths of the Unit are the quality of the individual groups, its strong sense of community, its ability to use the unique imaging facilities developed by EMBL, and its close interactions with the other Units at EMBL. The last of these, and especially the presence in other Units of computational biologists doing modelling and simulation studies, mitigates the modest size of the DB Unit. These interactions and the presence of groups with developmental interests in other units allow the DB Unit to achieve an excellent balance between breadth and depth.

It was the first review since Anne Ephrussi was appointed Head of Unit in 2007, replacing Stephen Cohen. Since then six group leaders have departed and five new ones have been recruited, with a sixth group leader starting later in 2011 to bring the Unit to its target size. Thus, the review took place against a background of very high staff turnover. This turnover, which is an integral part of EMBL's structure, has reoriented the DB Unit. The current Unit has a larger emphasis on genome-scale expression profiling and the use of sophisticated imaging to understand events in development and will have a substantial presence in mouse developmental biology with three groups in this area. All the groups are working on important problems and even though some work in well-populated fields, each one has a niche that should allow it to make unique contributions.

Because four of the groups in the Unit have existed for less than three years, it is premature to judge their long-term contributions to EMBL's research. Nevertheless, all four have established innovative and exciting research programmes, and are poised to make major contributions. This is illustrated by the remarkable number of three European Research Council Starting Investigator Grants and one Human Frontier Science Programme Career Development Award within the Unit.

There was universal praise for Anne Ephrussi's leadership, which has excelled in three areas: hiring of excellent and adventurous group leaders, mentoring, and the formation of an intellectual community that is cohesive and extends well beyond the formal boundaries of the DB Unit. Praise was also extended to Senior Scientist Detlev Arendt who plays an important role in the Unit. Both Anne and Detlev, in addition to their responsibilities in mentoring and leading the Unit, have taken on substantial responsibilities in EMBL's training activities: Anne heads the EMBL International Centre for Advanced Training (EICAT) and Detlev oversees the EMBL International Postdoctoral Programme (EIPOD) and acts as academic mentor to EMBL postdocs. In addition, both lead world-class research programmes in their own groups.

Response to the Panel's Recommendations

The future plans for the DB Unit point to the escalating need for collecting increasingly sophisticated quantitative data, primarily by light microscopy, and using this data to test mathematical models of developmental processes. The panel noted that achieving this aim raises two issues. The first is a need for modelling and simulation approaches for in-depth analysis of the data. We acknowledge this need and are putting structures into place to encourage the use of such methods. There are several groups with relevant expertise in the Genome Biology and Cell Biology and Biophysics Unit in Heidelberg as well as at the European Bioinformatics Institute. There are a number of ongoing collaborations between them and DB group leaders and the plan to establish three new Centres for Mathematical Modelling, Statistical Data Analysis, and Biomolecular Network Analysis, provided funding is available in the 2012-2016 indicative scheme, will help stimulate further interdisciplinary collaboration in these areas. The second issue relates to the enormous volumes of data produced by live, quantitative microscopy, and the problems they create for data storage, curation, and analysis. We are aware of these challenges and together with the leadership of other EMBL Units that face similar difficulties and with the bioinformatics service staff at the EBI, we are currently exploring possible solutions, some of them in the broader context of the emerging European Life Sciences Research Infrastructure for Biological Information (ELIXIR).

The panel pointed out that for much of its history EMBL has had a strong presence in *Drosophila* genetics. With recent departures from other units, expertise in this important area has substantially diminished and the panel recommended that EMBL consider hiring additional *Drosophila* geneticists. We appreciate this suggestion and together with the Heads of the Developmental Biology, Cell Biology and Biophysics and Genome Biology Units we will explore if and how *Drosophila* genetics fits with future plans and strategies.

While the panel strongly commended Anne Ephrussi's and Detlev Arendt's efforts in EMBL's intramural as well as extramural training programmes, they noted that it is important to make sure that these activities don't take too much time from their leadership, mentoring and research performing duties in the Unit. We very much appreciate Anne's and Detlev's continuous commitment to training at EMBL, Anne in particular has put a tremendous effort into this activity over an extended period, and are aware that both invest a lot of time into these activities. Some recent restructuring within EICAT should help take some of the administrative burden off Anne and Detlev. Helke Hillebrand, Dean of Graduate Studies, has recently been made Coordinator of Internal Training and has taken on more management responsibility for the Postdoctoral Programme in addition to her leading role as Dean of the EMBL International PhD Programme. Furthermore, we have appointed a new Coordinator of External Training, who will start at EMBL later this year, to take over the coordination of the Courses and Conference Programme. These measures are part of our ongoing efforts to ensure that these valuable activities, while remaining science-driven, obtain increasingly professional and sustainable management structures.

Finally, the panel recommended we look at the structure of EMBL Units and consider other organisational models for our research activities. This is something EMBL does regularly as part of the production of the five-year EMBL programmes. In recent times, for example, we have introduced EMBL Centres and the EIPOD programme as means of promoting interdisciplinary collaboration. We will nevertheless follow this recommendation by initiating discussion among the Heads of Units and Senior Scientists' committees.

Iain W. Mattaj
Director General
18 May 2011

EMBL Hamburg Review

The review of EMBL Hamburg took place on 12 to 14 September 2011.

Eleven international experts, including four members of EMBL's Scientific Advisory Committee, formed the panel, which was chaired by Helen Saibil from Birkbeck, University of London. The panel evaluated both the research and service activities of the outstation.

Evaluation Summary

The review took place against the backdrop of considerable change, both in terms of staff turnover and regarding the transition from DORIS to PETRA III. Major milestones have been the achievement of first light on all three EMBL-designed and -constructed PETRA beam lines and successful first test experiments on the new macromolecular crystallography (MX)2 and SAXS beam lines.

The scientific contributions of the Hamburg outstation over the past period were evaluated as being very significant in three aspects. The most outstanding in the opinion of the review panel was the substantial contribution to software development for structural biology, especially the ATSAS (for SAXS) and ARP/wARP (for MX) software package developments. The methods underlying these packages have broken new ground and created substantial improvement in the power of structural biology. The panel regarded this as an absolutely impressive performance.

The second contribution of the outstation has been in the development of synchrotron instrumentation. Here the new multilayer mirror system developed by the PETRA III Instrumentation group was considered to be particularly interesting, and it was recommended that its description should be published quickly. The power of the PETRA III beamlines is expected to contribute significantly to novel understanding of biological systems, due to their in part unique properties (very high intensity, sharp focus).

The third aspect where the outstation makes significant contributions is in addressing biological questions with structural biology approaches. Here the Wilmanns group was felt to be making important contributions not only through several Mycobacterial projects but also in projects to understand protein entry into peroxisomes and protein kinase-based regulation of signal transduction. In addition the developments from the (new) Meijers team on DSCAM, a highly variable cell surface molecule involved in the identity recognition of neurons, was also considered interesting and promising.

The panel praised the preparations for potential EMBL participation in the Centre for Structural Systems Biology (CSSB) and in the exploration of the X-ray Free Electron Laser (XFEL) projects. They considered participation in the CSSB a very welcome new direction for research activity at EMBL Hamburg because it will help attain a critical mass of structural biology research activity on the DESY campus. They also found the recent steps to explore the possibilities of the FLASH and future XFEL stations for biological applications of high energy laser technology extremely interesting and recommended that external funding should be sought to pursue this possibility further, including EMBL involvement in the provision of general access to this technology for biological users.

The leadership of the outstation by Matthias Wilmanns, Head of the outstation, and Victor Lamzin, Deputy Head, was judged as very constructive. Beyond their considerable administrative duties Wilmanns and Lamzin have maintained an impressive output of very attractive research from their own groups, continuing to showcase the possibilities of structural biology research in Hamburg. In general, other groups were also considered to have made significant scientific contributions under this leadership. The entire EMBL@PETRA III team was considered to have performed excellently under very severe constraints, both financial and in terms of personnel. Thomas Schneider, who has led this whole effort, and Stefan Fiedler, in charge of PETRA III Instrumentation, together with Christoph Hermes, long-time Head of the DORIS Instrumentation group, all received considerable praise from the reviewers.

The cooperation between the Hamburg outstation and other EMBL Units was considered very satisfactory, as it spans the whole spectrum of activities from instrumentation to research. The Grenoble outstation is a natural partner for the beamline end station construction and indeed there is an active and fruitful collaboration between the instrumentation groups at the two structural biology outstations. In summary, the panel was very pleased to see that all the possibilities offered by collaboration with other EMBL Units and outstations were being effectively exploited by EMBL Hamburg.

Response to the Panel's Recommendations

Looking toward the future and seeing the several very attractive possibilities open to EMBL Hamburg, the panel provided clear advice on what should be the priorities for the immediate future. The completion of the EMBL@PETRA III project was given highest priority, followed by participation in CSSB, in order to rebuild research activity from its current enforcedly reduced size, and finally, participation in the provision of access to the X-FEL for biological users. We will follow these priorities. We agree with the panel that the completion of the PETRA III projects still requires very significant financial outlay and the stable increase in service personnel numbers to serve the beamlines and their users (see below).

The panel noted that although the individual research projects are mostly very strong per se, the lack of a tighter overall focus of the outstation in biology might be a concern for the future. They recommended that recruitment of additional biologically oriented group leaders should be done with a view to achieving more synergy at the outstation. They felt that the construction of the CSSB also provides a good opportunity to achieve this goal. Because of the construction of the new integrated structural biology facility at PETRA III resources have been temporarily directed into technical areas at the expense of Hamburg's research activities. Once the facility is finished we plan to rebuild the research activity to the extent possible. EMBL will certainly attempt to follow the panel's advice when doing so although it must be noted that many people recruited to the outstation have a primary focus on service, making the achievement of a specific research focus even more difficult. Also, research projects at EMBL Hamburg should be seen in the overall context of EMBL. As the panel pointed out, the outstation collaborates heavily with other EMBL Units and exploits synergies across the whole institute.

Even though the panel praised the leadership of the outstation as very constructive, it voiced some concerns about some of the results of the unusual pressure on the group and team leaders in Hamburg over the last period because of the time and financial commitments necessary for PETRA III. This has resulted in short-ages of equipment and consumables for some of the research activity and to some deficiencies in day-to-day supervision of research fellows. We have noted the concerns and I am working closely with the Head of the outstation to analyse and address the situation in talks with both him and individual group leaders.

With regard to the equipment, staffing and operation of the new beamlines at PETRA III, the panel provided several detailed recommendations:

- Facilities of this type can only be fully exploited if detectors are available which are matched to the properties of the X-ray beams. This will require an investment of approximately €1.5M for each beamline with current detector models. The panel advised EMBL to consider when would be the best time to procure such detectors, taking into account future detector improvements, the commissioning schedule for the beamlines and the PETRA III shutdown and recommended that we should continue to search for external funding to help make these essential purchases possible in a timely manner.
- In terms of staff and annual running costs, it was considered important to develop a plan for the operation of the PETRA III beamlines and recommendations were provided on what the panel considered necessary.
- A suitable management structure for the operation of the beamlines should also be put in place. Again, outline suggestions were provided by the panel.
- EMBL was advised to continue to recruit specialists in software development for the new beamlines as this will be a critical aspect of keeping them state of the art.
- The panel re-emphasized the need to develop a policy on how to handle, transmit, back up and archive the large amounts of data which will be produced by the high intensity beamlines combined with high performance detectors.
- Finally, the review committee felt that an external advisory group will still be very useful over the next few years to help the beamlines reach their full potential and suggested continuing the PETRA III SAB in a modified form.

We thank the panel for their advice and will bear all these recommendations in mind in the process of equipping, staffing and operating the new beamlines.

Iain W. Mattaj
Director General
11 November 2011

Science Highlights



Beacons in the brain

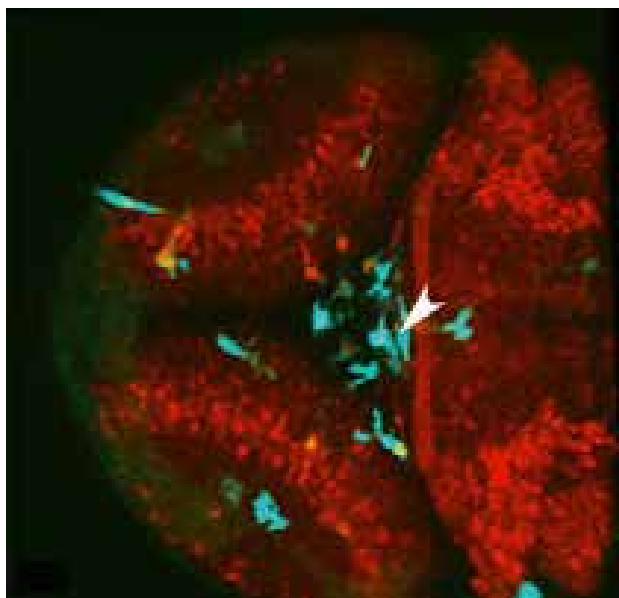
MAYDAY! MAYDAY! MAYDAY! As soon as it receives a distress call from a ship in danger, the lifeboat springs into action. By tracking the distressed ship's radio signal, the lifeboat's crew can home in on its location even if the ship is unable to send further messages. Now, it seems as though neurons in the brain are able to send out a similar kind of signal. Francesca Peri and her team at EMBL Heidelberg have discovered how injured or diseased neurons broadcast an emergency call that summons cells called microglia to clear up the mess and prevent further damage. As well as offering new insights into how the healthy brain functions, the work opens up new avenues for investigation into neurological diseases in which this process is disrupted.

Biologists have known about microglia for more than a century, but still know surprisingly little about what they do. "They have always been very enigmatic cells," says Francesca. Recently, there has been a surge of interest in microglia, as evidence has emerged implicating them in the proper development and function of the brain. "What they do is fascinating," explains Francesca. "They eat neurons."

The idea of your brain cells being eaten might sound rather alarming, but the truth is that making and running a vastly complex organ like the brain is a messy business. The developing embryonic brain creates far more neurons than it actually needs and eventually prunes the excess cells by making them commit suicide (p. 74). In the adult brain, neurons die or get diseased. All of these cells need to be cleared away, and microglia do this by sending out cellular branches like arms and hands that grab the cellular debris. If they didn't, damaged or dying cells could break up and release harmful enzymes and other molecules that could impede the function of the brain.

Microglia are also known to be involved in a number of neurological diseases, such as Alzheimer's or Parkinson's disease. However, to what extent they play a key role in causing or promoting disease, and to what extent they are merely innocent bystanders is not yet clear. Key to understanding their contribution is working out how microglia home in on damaged cells in the first place. "The big question is how they find their targets within healthy tissue," says Francesca.

Microglia move to the site of injury (arrow) to clear up debris.



But studying microglia in humans, or even mice, is no easy task. It's hard to see through opaque adult brain tissue to track migrating microglia, and the mammalian brain is encased in a hard skull that makes experiments even more difficult. So Francesca and her team work on zebrafish embryos, which are transparent, have yet to form skulls and which share enough similarities with mammals to make research in this area potentially applicable to humans.

What's more, the zebrafish embryo brain is much simpler than the adult fish brain, or indeed the human brain. The embryonic brain contains only about 30 microglia, making it easier for the team to tackle basic biological questions. So to find out how microglia home in on damaged cells, Francesca and her team decided to kill neurons in the embryonic fish brain, and study factors that might affect microglial behaviour.

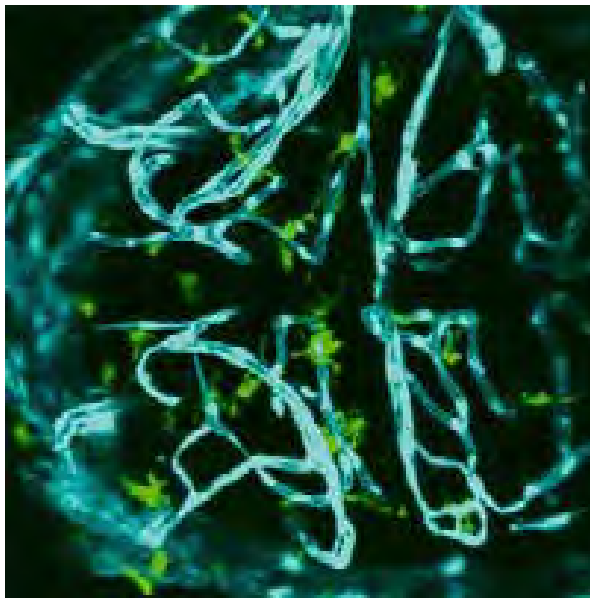
The team began by killing about 200 neurons in a specific location and saw that the microglia responded by moving towards the damage. To find out what might be drawing them there,

“If we understand how microglia get activated, we might be able to control their activity with drugs”

the team used a sensor protein that glows green whenever it encounters calcium ions. Biologists have long known that cells use calcium ions to relay information they receive from certain kinds of signals from the outside of the cell to its nucleus, its centre of command that houses its DNA.

Sure enough, the team saw waves of calcium activity spreading out from the injury site, like radio waves radiating from a distress beacon. To find out exactly what triggers the calcium ion signalling, Francesca's team needed to harmlessly inject test molecules into the brain, and then study microglial behaviour at the microscopic level in living embryos without disrupting them. This was no mean feat: “We really needed to push our setup,” says Francesca.

Help was on hand at EMBL Heidelberg from Lars Hufnagel's group and the Advanced Light Microscopy Facility, who helped with laser cutting and imaging, and from Ivo Telley in Anne Ephrussi's group, a physicist who set up a micromanipulator to work with fly embryos. Over the course of a year, Francesca's team developed a way of gently injecting molecules via the underside of the fish brain. Further experiments with the new setup confirmed the involvement of calcium ions and also showed that they made the microglia converge on the damaged cells. This raised the question of what was triggering the calcium ion waves. Other researchers had previously shown that a molecule called ATP was somehow involved in attracting microglia. At first sight, this makes sense: like all cells, neurons are full of ATP because they use it as a source of fuel. When neurons die, they break open and release ATP, which could diffuse and form a



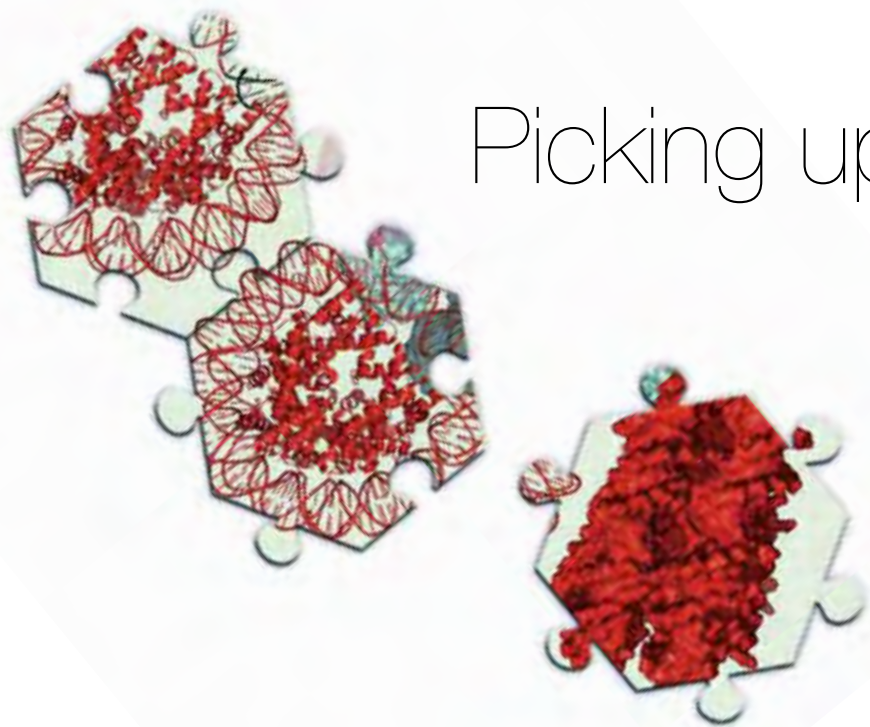
The zebrafish's transparent brain allows scientists to observe blood vessels (blue) and microglia (green).

concentration gradient to attract microglia. But there's a problem: ATP breaks down quickly outside cells and so would not hang around for long enough to form a useful signal.

To find out more, Francesca's team injected various different molecules to interfere with normal ATP signalling and found that the microglia were indeed responding directly to that molecule. But further experiments revealed that the original trigger is in fact a third molecule called glutamate. Glutamate is one of the signals neurons use to communicate with each other. Francesca's team found that glutamate triggers the waves of calcium signalling that raise the alarm in neighbouring cells. Cells receiving the calcium signal then make ATP, which acts like a radio bearing to guide microglia to the injured tissue. "The mechanism is more complicated than we expected," says Francesca. But a key advantage of this set-up is that it lets the brain create more long-lasting "beacons" that keep producing ATP.

Francesca's team is now building on this work to find out more about microglia. "If we understand how they get activated and contribute to different problems, we might be able to control their activity with drugs," says Francesca. Her team continues to collaborate with Lars to push back the experimental boundaries to studying the developing fish brain. They may be tiny, but the imaging potential of these transparent fish embryos promises to help Francesca and her team realise a hugely ambitious goal: to understand how neurons and microglia work together as a network in an entire, living brain and how this contributes to health and disease.

Sieger D, Moritz C, Ziegenhals T, Prykhodzij S, Peri F (2012) Long-range Ca^{2+} waves transmit brain damage signals to microglia. *Dev Cell*, 24 May 2012



Picking up the pieces

Epic molecular puzzle-solving by the Svergun team.

If you think completing a 1000-word jigsaw puzzle is hard going, spare a thought for Dmitri Svergun and his colleagues at EMBL Hamburg. During the past five years, they have pieced together thousands of fragments of information to get a clearer picture of how proteins called receptors interact with certain hormones and how this controls the behaviour of our genes. The work, done in collaboration with scientists in France, may also aid the development of new drugs to tackle diseases such as cancer, osteoporosis, obesity and diabetes.

Back in 2005, Dmitri and his team at Hamburg were approached by Dino Moras and colleagues at the University of Strasbourg, who were studying proteins known as nuclear hormone receptors, or NHRs. Unlike other kinds of cell receptors, they can bind directly to DNA and switch genes on or off. They do so in response to some drugs and vitamins and to particular kinds of hormones, such as the female sex hormone oestrogen. But researchers had only a piecemeal picture of the structure of the receptors and how they interacted with DNA.

The main problem is the size, complexity and flexible nature of NHRs, all of which makes studying them extremely difficult. To further complicate the picture, the receptors work in pairs, sticking to each other before binding to DNA, where they attract yet more proteins needed to switch genes on or off.

By breaking the receptors into pieces, biologists had previously been able to determine

parts of their structure by making the fragments form crystals and shining X-rays on them, a technique called X-ray crystallography. Although this gives a very detailed picture, it is often impossible to obtain crystals of large or flexible proteins.

That's when Dmitri's team came into play with another method, called small angle X-ray scattering, or SAXS. This gives a low-resolution picture of whole molecules, studied in solution. As well as avoiding the need for crystals, this means the proteins are free to bend and so behave in a more life-like manner. "We used a combination of different techniques, but the most powerful was SAXS," says Dmitri. Key to the project's success was Hamburg's robotic sample changer on the X33 beamline at the DORIS-III synchrotron. This saved time and minimised errors as the team tested hundreds of samples on various receptors by themselves, as well as receptor fragments bound to DNA or to their hormone signals.

Their hard work yielded an outline of the shape of whole NHRs bound to DNA. The team then collected other "jigsaw pieces" in the form of data from other methods, which revealed that the pairs of receptors twist in the middle when they bind to DNA – a finding that helps explain how the receptors interact with other molecules.

Using the SAXS outline – a bit like being able to use the picture on the front of a jigsaw puzzle box as a guide – the team integrated all their findings with the existing X-ray crystallography data to determine the most complete and life-like structure of NHRs to date. And now that the SAXS team has moved to the new, more powerful P12 beamline on the PETRA III synchrotron, it won't be long before even bigger protein puzzles yield their secrets.

Rochel N, Ciesielski F, Godet J, Moman E, Roessle M, Peluso-Ittis C, Moulin M, Haertlein M, Callow P, Mély Y, Svergun DI, Moras D (2011) Common architecture of nuclear receptor heterodimers on DNA direct repeat elements with different spacings. *Nat Struct Mol Biol* **18**: 564-70

When red blood cells turn green

Children aren't born doctors, artists, lawyers or scientists or any other predetermined profession. At birth they have the potential to fulfill any of these roles, as captured by the old Jesuit saying "Give me the child for the first seven years, and I will give you the man".

Many cells in the body derive from precursors that are like the Jesuit's child: they're capable of becoming many types of cell, and only commit to one profession as they mature. The most famous examples are embryonic stem cells, which can develop into any of the 200 cell types that make up the human body. Stem cells have great potential for the treatment of a variety of diseases, from diabetes to neuro-degenerative conditions, and so a detailed understanding of the factors that determine cell fate remains an important goal.

To study how stem-cell precursors diversify into mature cell types, scientists often want to track cells as they become committed to their final form. Donal O'Carroll and Kasper Rasmussen, a postdoc in Donal's group at EMBL Monterotondo, have devised a new method for doing just this.

The focus of Donal and Kasper's studies was the production of red blood cells. These derive from haematopoietic stem cells — a kind of adult stem cell — which also give rise to the two other major types of blood cells: white blood cells that fight infection, and platelets that form blood clots when we cut ourselves.

In tackling how such cell lineages arise, biologists have developed markers that flag up specific cell types, so they can be monitored as they develop. White blood cells, for example, can be tracked by looking at the expression of a protein called CD45, which is not expressed in red blood cells.

To study the development of red blood cells, Donal and Kasper have devised a new cell-

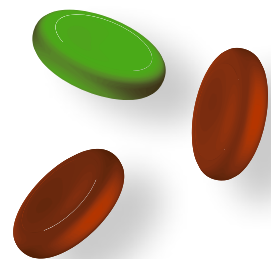
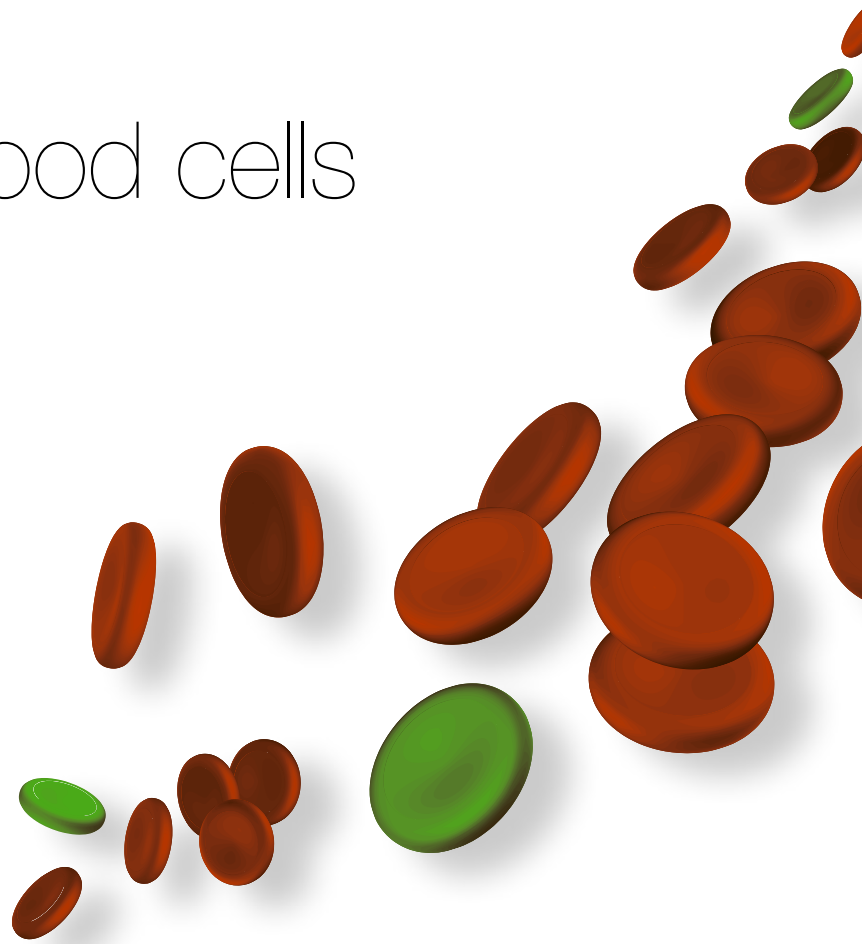
specific labelling approach. The pair inserted a gene encoding green fluorescent protein next to a gene called *miR-141/455*, which they have previously shown to be turned on only in cells that are going to become red blood cells.

This means that when precursor stem cells commit to becoming red blood cells and turn on *miR-144/451*, they glow bright green, which can then be detected through a technique called fluorescent-activated cell sorting

The new technique allows scientists to trace the origin of single red blood cell precursors in living animals. "This is a major advance," says Donal. "With this strategy we can very accurately determine the precise subpopulation of cells that express *miR-141/455* and which will become red blood cells." Using this technique, biologists will now be able to study the potential of stem cells to generate red blood cells in much greater detail – like a teacher predicting that a certain child is likely to become an artist, and another a doctor.

Making red blood cells green can help follow their fate.

Rasmussen KD, O'Carroll D (2011) The *miR-144/451*eGFP allele, a novel tool for resolving the erythroid potential of hematopoietic precursors. *Blood* **118**: 2988-2992





Paul Flicek

Party parasites

Every once in a while, you may find yourself in need of a real conversation-stopper. Perhaps you're at a party and have been cornered by a drunk and rather unattractive would-be admirer. Or you're trapped in a ghastly dinner party conversation that's veering dangerously towards the subject of religion or politics. Instead of making excuses and scuttling away to hide in the bathroom, why not draw on the discoveries of modern genetics to seize control of the situation? Just smile pleasantly and announce: "There's something you really ought to know about me. My genes are crawling with parasites. And I'm sorry to have to break it to you, but so are yours."

Use the stunned silence that follows to elaborate. Lots of people like to think of their genetic lineages as being a cut above the rest. Pristine, even. But the truth is that everyone's genomes are infested with millions of little pieces of parasitic DNA called transposable elements. They can hop around the genome either by cutting themselves loose and leaping to a new location, or by staying put, copying themselves and then letting the copy jump into a new spot. This ability to skip about chromosomes like countless genetic fleas has earned transposable elements the jolly nickname "jumping genes". Even so, they are not as innocuous as they sound. Their cutting, copying and pasting can cause such severe damage to genomes that cells have evolved a number of mechanisms to suppress their activity.

Jumping genes were initially assumed to be, at worst, selfish parasites and at best, little more than useless "junk" DNA. But over the past few years, it has started to emerge that jumping genes are not useless at all and, paradoxically, may be essential for keeping us and our genomes healthy. Now, Paul Flicek and his team at EMBL-EBI in Hinxton, together with researchers from Cancer Research UK, have uncovered new evidence showing how transposable elements could play a key role in developing the structure of our genomes and be a major driving force in our evolution. "It seems to be a big source of innovation in the genome," says Paul. "The old days of junk DNA are long gone."

Paul and his team didn't originally set out to look for jumping genes; instead, they were studying the behaviour of proteins called transcription factors. A gene's activity is regulated by stretches of DNA, some near to the gene, others scattered far away. These DNA stretches consist of particular sequences of chemical "letters", or bases, that attract and bind transcription factors, which then either switch the gene on or off. The control regions of different genes have different combinations of these sequences, and so recruit specific constellations of transcription factors, rather like the way different bank vaults need specific numerical combinations to open their locks.

This is how the body manages to be made up of hundreds of different cell types, despite the fact that almost every cell contains exactly the same DNA. Different cell types, such as a muscle cell and a nerve cell, look and behave differently because they switch on different sets of genes. And

the way they do this is by producing different sets of transcription factors to control cell-type-specific suites of genes.

When transcription-factor biology goes wrong, diseases such as cancer or birth defects can result, which is one reason why these proteins are the focus of intensive research. Another reason concerns their role in evolution. If you compare a chimpanzee's genome with our own, you will see that it is almost identical: there is more than 98 per cent similarity in the DNA sequence that codes for proteins. Yet even though our genetic make-up is so similar, humans and chimpanzees are very different.

A key to these differences is how the activity of these genes is controlled in time and space, which is where transcription factors come in. Evolution can alter their behaviour by changing the letters in the DNA sequences to which they normally bind. This alters gene activity, which in turn can alter the physical shape of an animal or change the properties of its cells. Many biologists now believe that most evolutionary change happens outside the protein-coding areas of genes, in the poorly understood “junk” DNA regions that control gene behaviour.

Together with Duncan Odom's team at the Cancer Research UK Cambridge Research Institute, Paul and his PhD student Petra Schwalie, with help from other members of Paul's group, turned their attention to a transcription factor called CTCF and studied the DNA sequences to which it binds. The function of CTCF has been the subject of research for more than 20 years, as it seems to be involved in several aspects of genome biology. One of its functions is to shut down genes by blocking their activation by other transcription factors. Another, more mysterious role seems to be the orchestration of physical interactions between different sections of DNA that control gene activity. Often, the stretches of DNA that control a gene are scattered along the linear string of the double helix. To activate a gene, these stretches and the transcription factors that bind to them need to be brought close together so that they can interact.

This involves looping the double helix back on itself to align these sequences and suggests that the three-dimensional (3D) arrangement of DNA in the cell is also important for controlling gene activity. Scientists already knew that CTCF seems to bind to DNA at the boundaries between regions of the genome that contain active genes and those that are inactive. CTCF might therefore somehow be involved in organising these enigmatic 3D genomic structures.

How to silence a party:
announce your genes are
full of parasites.



Paul and his team studied the genomes of six different mammals, including humans, to look for CTCF-binding sites. They were astonished to find that there were between 30 000 and 50 000 binding sites in each genome, and that about 5000 of these were present in all the genomes – ten times more than for the transcription factors they had previously studied.

The fact that so many of the binding sites remained unchanged over millions of years of evolution suggested to Paul and colleagues that CTCF has a key role in genome structure. “There’s probably a large-scale hierarchical structure in the genome that simply must be conserved for things to work,” says Paul. This hinted at another exciting, unexplored dimension to the control of gene activity. “I think that’s what we’re starting to get at here,” says Paul.

To find out more, the team studied the sequences of the CTCF-binding sites and found that they comprised two sections of sequence that were the same between species, separated by an intervening stretch that was different. Further investigation revealed something bizarre: the length and sequence of this intervening stretch wasn’t random, as you would expect if the binding sites had come into being via random DNA mutations being fixed by natural selection. Instead, there was a restricted set of possible sequences, which looked suspiciously similar to each other. In fact, some sets looked as though they had somehow descended from other sets. What’s more, the sets appeared in different proportions in the different mammals. It was as if the CTCF-binding site sequences had their own family tree.

This all suggested that the binding sites had been created by a copy and paste mechanism, raising Paul’s suspicions that transposable elements might be involved. Sure enough, when the team looked closely, they found the binding sites lying inside jumping genes called retrotransposons. Further study showed that there had been bursts of jumping activity in different species, perhaps helping to drive their evolution. The team even managed to trace signs of the oldest retrotransposons containing CTCF-binding sites, which hinted that these jumping genes were scattering binding sites throughout genomes even before the mammals radiated into a range of different species.

Coupled with CTCF’s role in genome regulation and structure, this has profound consequences for the way we think about evolution, says Paul. Rather than simple, gradual changes to a few letters in the DNA sequence, this is a mechanism that can dramatically reorganise a species’ genome. It might even explain why the fossil record sometimes seems to show sudden bursts of rapid evolution, says Paul. “I think it has to alter the way we think about how a species evolves if there can be fairly rapid changes over a relatively short period of time.”

So why did retrotransposons even begin to do this? Paul’s theory is that CTCF’s original function was to wrap itself around retrotransposons and stop them from spreading. Over time, CTCF gradually acquired other roles, such as controlling gene expression and genome structure. By spreading these now advantageous binding sequences, retrotransposons began to offer something useful to the cell: a trade-off that reduces the pressure on the cell to evolve measures to suppress the retrotransposons’ activity. It’s an intriguing thought that we might owe much of our evolution to these parasites and the arms race they are having with our cells. A final thought to share with your tipsy admirer or dinner party hosts – if the talk of genetic fleas hasn’t quite scared them away.

Schmidt D, Schwalie PC, Wilson MD, Ballester B, Gonçalves A, Kutter C, Brown GD, Marshall A, Flícek P, Odom DT (2012) Waves of retrotransposon expansion remodel genome organization and CTCF binding in multiple mammalian lineages. *Cell* **148**: 335-48

Magic handcuffs



New tool handcuffs interacting proteins to each other.

Criminals on probation are often forbidden to associate with other known felons, and if caught doing so they can expect to be thrown back in jail. But it's often hard to catch them in the act. The police would find the task much easier if they had magic handcuffs that could be attached to those on probation and which would automatically latch onto a felon should they be in the vicinity.

Law enforcers are unlikely to see any such technology soon, but the groups of Carsten Schultz and Christian Häring at EMBL Heidelberg have recently developed the biological equivalent: a tool to shackle together any two proteins that naturally interact within the cell.

Although some proteins do their biological work on their own, many team up with other proteins to work as a pair or as part of a larger collective. Just as efforts to crack criminal networks often start with watching suspects to see who they interact with, scientists often want to know how proteins interact with each other, and how this affects their capacity to do their jobs. Methods for sensing and manipulating protein-protein interactions are therefore important tools in unravelling the networks of protein activity in the cell.

Scientists can already link proteins together, typically by engineering them to contain extra portions that can recognise and bind to each other. The drawback is that these additional segments can affect how proteins function, and the binding is irreversible: once they have been linked, the proteins can't be pulled apart

again, which makes it impossible to study the effects of linking them at one time point, and setting them free at another.

Anna Rutkowska, an EMBL Interdisciplinary Postdoc (EIPOD) in Carsten's, Christian's and Jan Ellenberg's groups, overcame these limitations by creating a molecule called xCrAsH, which functions like a pair of molecular handcuffs. xCrAsH contains two arms, each of which is designed to bind to a specific sequence of 12 amino acids in a protein. So xCrAsH can use one arm to bind to a protein with the requisite sequence, like cuffing a person by one wrist, and then when another protein also carrying the 12-amino-acid sequence comes into close proximity, the other arm of xCrAsH locks onto it, cuffing the two proteins together. "This means we can link any interacting proteins together by adding just a short sequence of amino acids to them, though we still have to be careful that this doesn't affect their function," says Christian.

Not only does xCrAsH bind proteins, it also fluoresces once it is bound, enabling scientists to pinpoint where in a cell the proteins are handcuffed together using a light microscope. In addition, this cross-linking can be easily reversed by adding another compound to the cells, so the effects of linking and unlinking proteins can be readily explored. "We've demonstrated that this technique works inside living cells, without influencing cell viability and function," says Carsten.

Looking ahead, Christian and his group plan to use xCrAsH to study how an important protein complex called condensin, which forms a multi-subunit ring around chromosomes (see p. 20), opens and closes. By handcuffing subunits together two at a time and seeing whether the condensin ring can still open and encircle chromosomes, they'll be able to identify which subunits form the entry point of chromosomes into the condensin ring.

Rutkowska A, Häring CH, Schultz C (2011)
A FIAsh-Based Cross-Linker to Study Protein Interactions in Living Cells. *Angew Chem Int Ed Engl* 50: 12655-12658

The heat is on

Scientists at EMBL Grenoble have found an ingenious shortcut that could save researchers a lot of time when solving structures of biological molecules. The method, developed by José Márquez and his team at the High Throughput Crystallisation Facility in Grenoble, helps to determine the likelihood that a particular protein will produce the crystals needed to study its structure using a technique called X-ray crystallography.

Although X-ray crystallography is very powerful, it has one major drawback: it can be very difficult to get proteins to form crystals. Biologists therefore have to perform hundreds of experiments to find the precise conditions that encourage crystal formation. They typically spend a lot of time preparing different fragments and variants of a protein in the hope that some will give the desired result. But until now, there has been no easy way to assess which variants are more likely to produce crystals. Being able to concentrate their efforts on the samples that are most likely to crystallise would save structural biologists a lot of time and effort.

José's team realised that one way of assessing a protein's tendency to crystallise would be to measure its ability to remain stable at high temperatures. Proteins are made of chains of building blocks called amino acids, which are folded neatly into different structures. Proteins that stay folded at higher temperatures are more likely to have the kind of regular structure that forms crystals more readily. So José and his colleagues set out to find a quick and easy way of measuring the temperature at which a protein starts to unfold.

They turned to a test called the ThermoFluor assay. This involves tucking a fluorescent molecule deep inside a protein. Only when the protein unfolds will the added molecule fluoresce and its light become detectable. The team tested the thermal stability of 657 protein samples, and then tried to make crystals from the samples. They found that proteins that remained stable at about 45°C were twice as likely to form crystals as those that did not,



A simple test of temperature-resistance can help select the best proteins to crystallise.

thereby creating a simple test to help scientists select the best protein fragments to use in their crystallisation trials.

Key to the success of the work was the High Throughput Crystallisation Facility at the Partnership for Structural Biology in Grenoble. This large-scale platform run by José's team provides crystallisation screening services for scientists from all over Europe via an EC-funded programme called P-CUBE. Running such a platform provided the team with two significant advantages: first, the Facility's ability to handle a large number of samples efficiently made it possible to gather data from different types of samples very quickly; second, the highly robotised nature of the Facility allowed the team to ensure that all the conditions were constant from one experiment to the next.

As well as improving the efficiency of the Grenoble Facility, the new method is already saving scientists a lot of time by helping them to pick appropriate protein fragments. The good news is that the method consumes very small amounts of sample and can be done rapidly. "Users love it," says José. "They are really happy with it."

Dupeux F, Rower M, Seroul G, Blot D, Marquez JA (2011) A thermal stability assay can help to estimate the crystallization likelihood of biological samples. *Acta Crystallogr D Biol Crystallogr.* **67**: 915–919



A drop in the ocean

Just before dawn on a cold and windy day on the southern Atlantic Ocean, the crew working on board the 36-metre research schooner Tara were pulling on their foul weather clothing and preparing for another day of intensive sampling. As Tara's distinctive orange nose plunged in and out of the crashing waves, the team worked against the tiring conditions to record variables such as acidity, temperature and currents, and sample the water and its tiny occupants to build a picture of the biodiversity beneath their feet. The main goals of the Tara Oceans expedition are to understand more about the function, evolution, and ecology of marine species, analyse the effects of climate change on marine biodiversity, and to increase general awareness about environmental issues.

"Organisms that are at the root of the food chain are of significant interest to us because they have played a key role in the development of the Earth's atmosphere," explains EMBL's Eric Karsenti, who is the scientific coordinator of the project. "They produce around half of the oxygen we breathe and also sequester carbon dioxide through photosynthesis. It's crucial to understand how these organisms live together because we don't know how they will react to climate change, pollution, or changes in general circulation of the ocean, and it's very important to be able to monitor this in the coming years."

New science

Only one percent of life forms in the sea have been properly identified and studied. By using advanced instruments that enable extensive sampling, sorting, underwater sensing, and imaging in oceans and seas around the world, the Tara project aims to change this. Samples collected from the surface right down to more than 1000 meters below to the so-called mesopelagic region – the ocean's 'twilight zone' – will enable researchers to learn more about the billions of viruses, bacteria, and larger organisms present in each litre of seawater. This will subsequently provide crucial data on different species, their interactions, and genomes, for scientists on land working in fields as diverse as ecology, oceanography, and molecular biology. One exciting prospect is that researchers will be able to create models predicting the organisation of these species in different ecosystems, which could then help scientists to understand the carbon cycle in the oceans, and how these tiny life forms might change depending on the climate.



Ban Ki-moon, Secretary General of the United Nations, on board Tara with Eric Karsenti, André Abreu, Agnès b. and Romain Troublé.

“We will have a lot of imaging data, a lot of genomics data, and then the interesting thing is to put that together, to establish correlations between genomes and organisms,” explains Eric. “With Tara Oceans we will know much more about the global diversity of the oceans – we will nail down these numbers. We will also see how diverse ecosystems are according to the environment and learn a lot about the underlying rules of ecosystem dynamics and evolution.”

Now safely back in port after completing her mission in March, Tara has enabled researchers to collect samples from the Arctic, Atlantic, Pacific and Indian Oceans as well as many seas, travelling some 60 000 nautical miles from Lorient in France to Dubrovnik, Abu Dhabi, Mayotte, Cape Town, Rio de Janeiro, the Galapagos Islands, New York, and many places in between.

Along the way, researchers have studied a large range of marine phenomena, including: the ‘Agulhas rings’ – circular currents that form in the Mozambique Channel, which play a

key role in fertilising the Southern Atlantic Ocean with microorganisms; the plankton-sparse region in the Pacific Ocean to the east of the Marquesas Islands and the plankton-rich region to the west; and biodiversity-rich coral reefs, which many scientists believe are under threat from rising sea temperatures, ocean acidification, and invasive species. The journey has also connected with different people and cultures, providing some striking memories for those on board and on land.

“When Tara departed Lorient at the start of her journey, there were around 5000 people there to send us farewells at the edge of the harbour,” Eric recalls. “Journalists were rushing about, people were cheering and crying, it was very emotional. Gaby Gorsky, the director of the Oceanography Observatory in Villefranche, turned to me and said: ‘It’s incredible, it has happened!’ Then suddenly we were out at sea without any noise, without anybody.”

Whenever Tara docked, it was a different story, as groups such as school children hurried excitedly on board to learn about the importance of plankton in both evolution and global ecology.

“People know about the big organisms, but they are less aware of the small organisms which are so important for the ecology of our planet,” Eric says. “Each time the boat stopped in a harbour, children from local schools came on board, we explained what we were doing, showed them the boat and they really loved it. I think that it has inspired a lot of young people.”

“On the other hand, international bodies such as the United Nations are increasingly focused on the management and protection of open oceans. We have been able to reach out and com-

municate the importance of this research with policy makers throughout the journey. The expedition has shown that we need to go back to exploration in science – this is as true for the oceans as it is on land.”

Logistics

As Tara docked in many of the bustling ports she visited along the way, waiting by the water’s edge was scientific operations manager Steffi Kandels-Lewis, based at EMBL Heidelberg. Responsible for ensuring that all scientific personnel, samples, and equipment made it safely on and off the boat at the right place and time, Steffi had a far-reaching involvement in all aspects of the expedition.

“The view of Tara coming over the horizon was always very exciting,” says Steffi, who is a molecular biologist by training. “One of the major challenges of the logistics operation was working with the coordinators of different parts of the sampling effort – such as for protists, bacteria, viruses, giant viruses, and zooplankton – establishing how many and what samples they plan to take, from this working out the equipment they need, and then getting it to them.”

“Different kinds of samples need different kinds of storage and different kinds of shipping. DNA samples, for instance, are stored in liquid nitrogen or a specific kind of buffer, and must be shipped on dry ice. Other samples are preserved in fixatives such as ethanol. We could not store more than eight to ten stations’ worth of samples on board so often we had to make a compromise.”

Organising a global operation spanning 70 countries and immense distances required meticulous planning, communication and negotiation skills and the ability to make the best use of the often limited resources available.

“Sailing between Ecuador and Tahiti – a time-span of four months – it was not possible to arrange the shipment of samples back to Europe,” Steffi explains. “Daytime temperatures averaged 35°C and it was a major challenge to ensure that there was enough liquid nitrogen and refrigeration equipment to last. Some countries require import and export paperwork that must be sent weeks in advance of arrival – and determining Tara’s exact needs this far ahead of time is not an easy task. But through careful planning, a number of backup plans, and also thinking on our feet, we have ensured that the journey reached its successful completion.”



One of the thousands of zooplankton specimens captured by the expedition.

Life at sea

Tara’s journey was powered by a dedicated team of more than 200 people of 35 different nationalities and from many different professional backgrounds, including biologists, journalists, artists, and sailors. Bianca Silva, a PhD student at EMBL Monterotondo, boarded Tara in San Diego for a month-long stint as a member of the scientific crew.



Eric Karsenti separates samples in the onboard laboratory.

“At lower depths, below 500 meters, you find things that you cannot even imagine,” Bianca says. “At one station, in the last net of the night, there was an adult fish in a sample that was completely transparent. Then when we put it under the microscope, adjusting light and filters, we could see the brain and spinal chord perfectly.”

The times when the boat was travelling between locations gave the crew opportunities for exercise, relaxation and sometimes even quiet reflection. But even then, the ocean never failed to throw in a surprise or two.

“You can be at sea for weeks without seeing anything but the open ocean, the crew on the boat, and the plankton in the samples. Seeing anything else is very exciting. One day we saw whales – it was really amazing to see something so rare and they give you a feeling that they are here to visit you. Some of the days were tough – taking samples from sunrise to midnight and beyond, but the atmosphere on board the boat was fantastic. You feel at home, and in the place you want to be.”

The end?

While Tara has come to the end of her incredible voyage, the scientific journey is just beginning. Organisers and crew are sharing their experiences with scientists, the general public, and the many students who have followed the expedition. The reams of data collected will be scrutinised by researchers around the world, including groups at EMBL who will study aspects such as genomics, evolution, and imaging (see box). Ultimately, these scientists hope to understand how the microscopic life in the oceans will play its part in the Earth’s future.



Striking contrast: the enormous nets used to catch microscopic organisms.

AT EMBL

Genomics

Samples taken on the expedition were separated according to the size of organisms during collection at each station. Comprehensive sequencing of genetic material for each fraction followed. Some of the major challenges scientists have to overcome in analysing these genetic sequences include the diversity of organisms and hitherto unknown genes. Scientists in the group of Peer Bork will support and develop sophisticated bioinformatic analysis to help overcome these hurdles and make the most of the genomic data.

Evolution

Ecosystems in the oceans differ remarkably from place to place and are structured by rotating currents, or gyres, generated by changes in temperature, the rotation of the earth, and the position of the continents. The extent of mixing between surface layers and the deep ocean also varies dramatically. Scientists in Detlev Arendt's group will be studying how conditions in those different ecosystems might have influenced the evolution of marine organisms like ragworms and the lancet fish.

Imaging

Scientists led by Rainer Pepperkok are using technology to quantitatively analyse organisms between 5 and 200 microns in size, and developing methods to automatically recognise and count different species in the samples. Utilising advanced high-throughput fluorescence microscopy equipment and expertise at EMBL, scientists can pinpoint features and even molecules in the cells or organisms, and follow their every move.

ONLINE EXTRA:

Eric Karsenti and others shared their experiences of TARA Oceans on the EMBL Explore podcast: www.embl.de/explore/tara.

Sara Cuylen and
Christian Häring



All tied up

You've just bought a new home entertainment system: a top-of-the range plasma TV, Blu-Ray player, and surround-sound system, all set up on an elegant stand that takes pride of place in your living room. It looks great, except for one thing — the unsightly mass of connecting cables and power cords hanging behind the stand and running around the edge of your room. Not only do these look untidy, but they also get in the way when it comes to household chores.

There's a simple and cheap solution to these aesthetic and practical problems: small, plastic cable ties. These can be used to keep unruly wires bound together as a single, neat, easy-to-move super-cable, and if there's any slack in the wires because they're too long, this can be looped out and pinned in position with a well-placed cable tie.

Cells face a similar housekeeping challenge in keeping the long strands of their DNA neatly ordered and untangled. Now research led by Christian Häring at EMBL Heidelberg suggests that nature has hit on an analogous cable-tie solution to this knotty problem.

The studies, carried out by Sara Cuylen and Jutta Metz, from Christian's group, focused on a protein complex called condensin. "Condensin complexes were discovered in the late 1990s and found to be a major component of chromosomes," says Christian. Research in the intervening years showed that condensin is crucial for packaging and moving chromosomes during cell division. "However, the precise way in which condensin carries out these roles has remained unclear," says Christian.

Condensin is one of many proteins that are bound to DNA within the cell. In general, these proteins give the one-dimensional string of nucleotides that make up DNA an ordered three-dimensional structure: just as a pile of books dumped on the floor does not make a useful library, a tangled mass of DNA does not form a functional genome.

The DNA double helix is wound around proteins called histones, like a thread on a spool, into units called nucleosomes. This enables the long and otherwise unwieldy DNA molecule to be stored in an ordered structure so that it can be accessed as needed by the rest of the cell. This wound-up DNA-protein complex is known as chromatin; and chromatin, in turn, is twisted, folded and held in position by proteins such as condensin. Keeping DNA neatly ordered is not only important in the nucleus – so that genes can be turned on as and when they are needed – but is also central to the correct allocation of chromosomes when cells divide.

Despite the importance of condensin, the way it interacts with DNA has remained a mystery. A number of possibilities have been put forward. Some biologists have proposed that DNA wraps around condensin complexes; others have suggested that many condensin complexes bind to DNA, and then bind to each other, bending and folding the DNA in the process. Christian and



Condensin
cord-stoppers keep DNA
manageable.

colleagues wanted to explore a very different idea: that condensin forms a ring that encircles DNA, just like a cable tie around a cable.

The team tested this hypothesis in a series of experiments. First, they looked at the behaviour of condensin complexes attached to circular mini-chromosomes derived from the yeast *Saccharomyces cerevisiae*. If condensin really does function as a closed ring around DNA, then it should remain stuck on a circular chromosome once bound — unless, of course, the chromo-

some is cut. So the team did just that, and found that condensin was now able to slide off. Likewise, making a cut in the condensin ring should also cause it to fall off a circular chromosome, just as cutting a cable tie releases it from a cable. Again, this is exactly what Christian and colleagues found.

However, in both of these experiments condensin needed a little encouragement to drift away from the DNA. “We had to add salt in order for this to happen, which suggests that condensin makes some electrostatic contacts with DNA, as salt is known to interfere with such interactions,” says Christian. “And these contacts may restrict condensin rings to certain places on the genome where they’re needed.”

With these results in hand, Christian’s group turned their attention to the biological consequences of breaking open the condensin ring. In particular, they wanted to know what effect this would have on chromosome segregation, through which chromosomes are pulled to opposite ends of a dividing cell to ensure that the daughter cells receive the correct share of genetic material.

The results were unequivocal: condensin is essential for correct segregation of chromosomes. “When we opened condensin rings before the chromosomes were moving apart, chromosome segregation failed,” says Christian. “This suggests that the chromosomes have to be trapped within the condensin rings during segregation to be properly partitioned into daughter cells.”

On the basis of these results, Christian and colleagues propose a model for how condensin enables the cell to separate its chromosomes before dividing. They suggest that each condensin complex maintains chromosomes in such a way that distant regions of the chromosome are encircled within the condensin ring — much like a cable tie holding a loop of slack cable in place.

This solution to keeping chromosomes ordered within the cell is not only simple, but ancient and widespread. “The same five subunits that make up condensin complexes are found in all eukaryotes, seem to work the same way across species, and are highly conserved over evolutionary time,” says Christian. This means that natural selection must have discovered this cable-tie method billions of years ago. It works so well that species from yeast to humans have stuck with it ever since.

Cuylen S, Metz J, Haering CH (2011) Condensin structures chromosomal DNA through topological links. *Nat Struct Mol Biol* **18**: 894-901

The final frontier

When NASA decided to explore Mars, a succession of space probes armed with sensors were sent to gather information about the Red Planet. Now François Spitz and his team at EMBL Heidelberg have developed a biological equivalent of a space probe to explore terrain where no human could ever roam: the DNA in cells. Their mission? To explore the physical structure of the genome and understand how this structure controls the activity of genes. The biological probe they have developed is already helping scientists around the world address this question, and with it, gain a better understanding of how this mechanism goes wrong in disease and how evolution uses it to alter species over time.

François has been studying the control of gene activity since he was a postdoc in Denis Duboule's lab at the University of Geneva in Switzerland. Denis' group studies an enigmatic set of genes called the HOX genes, which ensure that body cells "know" where they are along an animal's head-to-tail axis.

In four-limbed animals, however, evolution has given the HOX genes an additional job: to ensure that the developing limb grows the correct number of digits in the right places. "People are intrigued to know how the genome is reusing an old gene family," says François. Differences in HOX gene activity in the developing limb result in different patterns of digits. For example, humans have five digits on each limb, whereas birds have only four. And mutations in HOX genes lead to limb malformations in humans.

"A lot of inter-species diversity lies not only in the proteins encoded by the genes themselves, but also in when and where they are expressed," explains François. "And that's mostly controlled by the large chunk of DNA where there are no genes around." This chunk consists of an enormous gene-free stretch of DNA that lies next to the HOX genes. Denis and his group knew this "gene desert" contained sections of DNA sequence that affect HOX gene activity in limbs and that the DNA

somehow had to loop around to bring these sections close to the genes they regulated. But they didn't know exactly where all these sections were and how they worked.

Until now, the technology to dissect mouse genomes to find regulatory regions has been slow and labour-intensive. After joining EMBL, François developed a new, faster method. His team engineered a piece of DNA called a transposon, which can hop in and out of the genome, with a "sensor" that produces an easily detectable protein if it encounters any regulatory regions. The transposon can hop around the genome of mice, inserting in new locations and probing for the presence of regulatory regions. What's more, it allows scientists to snip out sections of DNA so they can test their hypotheses about how gene regulation works (see Annual Report 2010/11).

François' group has used this system, called GROMIT, to make hundreds of strains of mice, each carrying transposon probes in different parts of their genome. This huge effort is made possible thanks to EMBL's Laboratory Animal Resource at EMBL Monterotondo, which cares for all the mice. In addition to using them for his own work, François has been sending mice to labs around the globe. One recipient was Denis, who used the mice to help uncover new "islands" of regulatory sequences in the gene desert that controls HOX activity in limbs. The sheer number and complexity of the interactions these islands control helps explain how evolution can make subtle changes to digit form and number, a discovery truly worthy of a high five.

How does a gene-less chunk of DNA control how many fingers a mouse grows?



Montavon T, Soshnikova N, Mascrez B, Joye E, Thevenet L, Splinter E, de Laat W, Spitz F, Duboule D (2011) A Regulatory Archipelago Controls Hox Genes Transcription in Digits. *Cell* **147**: 1132-45

Secret ciphers of the cell

“It’s a really ingenious and potentially versatile mechanism”

Producing a protein from the instructions contained in a gene is a vastly complicated process that needs to occur not only at the right time, but also at the right place within a cell. Now Anne Ephrussi and her team at EMBL Heidelberg have discovered an ingenious mechanism that targets

some of the molecules involved in protein production to their correct cellular location. This process effectively unveils a cryptic message hidden in a key molecule that flags it up to the cellular machinery for transport to the required place. “It’s a really ingenious and potentially versatile mechanism,” says Anne.

To make a protein encoded in a gene, a cell first makes a copy of that gene using a molecule called messenger RNA, or mRNA. The sequence of building blocks, or nucleotides, in the strand of mRNA spells out instructions for assembling the protein. Restricting proteins to certain areas is often essential for a cell’s func-

tion, and one way cells do this is by transporting mRNAs to specific places before the protein is made.

Anne’s team studies a protein called Oskar, which is found at the posterior end of fruit fly eggs. To localise the protein correctly, a dedicated machinery in the egg transports *oskar* mRNA to its posterior, but how it identifies the mRNAs that require transport was a mystery. Anne’s team had previously shown that one particular section of *oskar* mRNA was needed, but it clearly wasn’t the whole story. This got them thinking about another mystery that had puzzled them for a while.

A newly made mRNA molecule contains stretches of information, called exons, which code for proteins, interspersed with other stretches, called introns, which do not. So before the cell can read an mRNA, it first has to cut out the introns and paste together the exons. The mystery was that *oskar* mRNA has to undergo this editing process, called splicing, before it can be transported. Anne’s team had found that the first intron in particular



Anne Ephrussi

had to be spliced out, but they didn't know why.

So the researchers did some editing of their own. By cutting out different parts of *oskar* mRNA, they were eventually able to narrow down the sections required for transport to 10 nucleotides on one side of the first intron and 18 on the other. When the intron is spliced out, these nucleotide sequences are stitched together, causing them to form a looped structure, which the team dubbed the “Spliced *oskar* Localisation Element”, or SOLE. The SOLE sits right next to a set of proteins called the exon junction complex (EJC), and both are needed for *oskar* mRNA to reach its destination.

To see how the SOLE worked, the team tracked *oskar* mRNAs in living eggs. They found that without a working SOLE, some of the mRNAs could still move in the right direction, but couldn't keep up with the egg as it grew and so never made it to the posterior pole. This suggested a new concept: that the SOLE works by making *oskar* mRNA trans-

port more efficient. This mechanism could be more widely applicable, suggests Anne. Other mRNAs could be spliced differently, creating unique labels that target them to particular locations. More work is needed, however, to test this idea and see what other mRNAs are hiding a secret message.

Ghosh S, Marchand V, Gáspár I, Ephrussi A (2012) Control of RNP motility and localization by a splicing-dependent structure in *oskar* mRNA. *Nat Struct Mol Biol.* **19**: 441-9



Guardians of the genome

Libraries – of books
and genes – need
protection.

You may be deeply attached to the library of books stored on your iPad or Kindle, but we all carry a much more profound digital library within our cells: our genome, which comprises 23 books (chromosomes) that together contain more than 25 000 chapters (coding genes).

Yet the integrity of this library is under constant threat from genetic vandals called transposons. These are bits of DNA that can jump from location to location and insert themselves into meaningful paragraphs of genetic text, turning them into gibberish and rendering genes useless. Previous research has shown that a failure to keep transposon activity in check leads to sterility, so dealing with this threat is a biological priority. In two papers published back-to-back in *Nature* last year, three independent groups at EMBL-EBI, Monterotondo and Grenoble provided new insights into just how this is achieved.

Both libraries and cells have to protect their precious stores of information. In a traditional library, valuable books can be preserved in a number of ways. Some may be locked in secure cases to prevent anyone getting their hands on them in the first place; in addition, the library can hire guards to patrol the bookshelves to catch anyone who might wish to steal or deface their contents. The studies reported in *Nature* suggest that evolution

has come up with two analogous methods of suppressing the disruptive actions of transposons: one locks transposon DNA into an inactive state by adding chemical tags called methyl groups so that they cannot jump around, whereas the other silences any rogue transposons that might still roam the cell.

These biological solutions for policing genomic vandals might sound simple, but their implementation is not — not surprising given the complexity of how transposons wreak genomic havoc in the first place.

One method they use is a form of ‘copy and paste’ that, like normal gene expression, begins with the process of transcription, in which the DNA of a transposon is used as a template to produce a long RNA molecule. In ordinary gene expression, this RNA would contain the instructions for assembling a protein, but transposon RNAs do not serve this purpose. Instead, they are used by an enzyme called reverse transcriptase to recreate the original DNA molecule, after which it can be pasted into another part of the genome.

The new experiments focused on this intricate copy-and-paste variety of transposon, and the ways in which their activity is regulated. Previous research in fruit flies had suggested a complex picture of transposon regulation. In the first step, enzymes called Piwi proteins use their endonuclease or ‘slicer’ activity to cut

up long transposon RNAs into small pieces. These snippets of RNA — called Piwi-interacting RNAs (piRNAs) — then attach to Piwi proteins, which use the piRNA fragments to seek out other transposons that are then also chopped into pieces. In other words, a few ‘primary piRNA’ fragments lead to an amplification process in which Piwi proteins generate many more ‘secondary piRNAs’ from transposon RNAs. This slicing up of transposon RNAs prevents the transposon DNA from being reverse-engineered and pasted back into the genome.

Yet the precise roles of specific Piwi proteins in species other than fruit flies, and the contribution of their slicer activity to transposon silencing, remained unclear. These were the questions addressed by the EMBL groups.

The first paper, from a team led by Anton Enright at EMBL-EBI and Donal O’Carroll at EMBL Monterotondo, explored how two important Piwi proteins — Mili and Miwi2 — shut down transposon activity in mice. From studies in fruit flies, it was thought that Mili and Miwi2 work together, taking turns to generate secondary piRNAs from primary piRNA templates in what has been called a ‘ping-pong’ model. “It was thought that to get amplification and silencing you needed these two proteins acting together,” says Anton. “Our data, however, suggest a new model.”

Anton and Donal began by genetically engineering mice so that both Mili and Miwi2 lacked slicer activity. The Mili mice were defective in piRNA amplification and were sterile, confirming that Mili’s slicer activity is crucial for the cycles of piRNA amplification that lead to effective transposon silencing — a finding in line with earlier models. Anton and Donal also found that mice whose Miwi2 could not slice RNA nevertheless successfully silenced their transposons, showed normal piRNA amplification, but remained sterile. “This suggests that the main role of Miwi2 is not in the amplification step, as previously thought, but in switching off transposon genes,” says Anton. So in contrast to earlier models in which Mili and Miwi2 act in concert to amplify piRNAs, these new results show Mili alone amplifies piRNAs.

In the revised model proposed by Anton and Donal, piRNAs generated by Mili are loaded onto Miwi2, and guide the piRNA–Miwi2 complex to areas of the genome harbouring

transposons; here, the complex somehow indirectly targets methyl groups to the DNA, which makes the transposons unreadable and therefore inactive. “Mili is essential for amplifying the piRNAs that primes Miwi2 to initiate methylation of transposon genes,” says Donal.

Shutting down transposon genes at the source through piRNA amplification followed by DNA methylation is comparable to locking up precious books in a secure case. But like any security system, it is not fail-safe. “You need to have a surveillance system that can continually monitor the cell for any escaped transposons,” says Ramesh Pillai from EMBL Grenoble, lead author on the second *Nature* paper.

Ramesh’s studies suggest that a third Piwi protein called Miwi serves this surveillance role, like a guard patrolling a library. As in the previous study, Ramesh and colleagues created mice carrying a mutation in Miwi that removed its slicer activity. This led to defects in sperm production and infertility in male mice, showing that the function of Miwi depends mostly on its slicer activity.

However, Miwi — unlike Mili — is not implicated in piRNA amplification, or shutting down the transcription of transposons. Instead, it patrols the cell looking for escaped transposon RNAs, and then destroys them, Ramesh and his group found.

Although a library’s two-pronged security strategy of protecting books and hiring patrolling guards operate in parallel, nature’s analogous solutions for keeping transposons in check may function at different times. Earlier research has shown that Mili and Miwi2 are active during embryonic development, whereas Miwi is turned on after birth. Ramesh says that these findings suggest that during early development, Mili amplifies piRNAs, which Miwi2 uses to shut transposons down; then, after birth, Miwi acts as a backup system to silence transposons that have evaded the Mili/Miwi2 system. In this way, the precious information in the genome is protected throughout life so that it can be passed on intact to future generations.

De Fazio S *et al* (2011) The endonuclease activity of Mili fuels piRNA amplification that silences LINE1 elements. *Nature* **480**: 259–263

Reuter M *et al* (2011) Miwi catalysis is required for piRNA amplification-independent LINE1 transposon silencing. *Nature* **480**: 264–267

Paul Kersey



Spanning taxonomic space

An interview with Paul Kersey

Paul Kersey leads the Non-Vertebrate Genomics team at EMBL-EBI, which develops Ensembl Genomes. Together with colleagues at Rothamsted Research in the UK, Paul and his team launched a new resource for agri-food researchers called PhytoPath in 2011. PhytoPath integrates genome-scale data from plant pathogens with high-quality information about host infection. We asked Paul to tell us about the growing importance of bioinformatics in agri-food research today.

How does bioinformatics help agri-food research?

Bioinformatics impacts on agriculture and food research in many ways, perhaps most notably in the area of plant breeding. There is now enormous pressure on land availability and, consequently, on food supplies – clearly, doing nothing to improve our crops will mean malnutrition or starvation for many people in the world. It is absolutely essential to identify the causes of natural variation so that we can speed up the development of crops that are more resistant to pathogens, drought and other stresses.

Bioinformatics lets us do what plant breeders have always done, but in a more targeted way: use information to breed useful crops in much shorter timescales than were previously possible. But the challenge is more complex than simply making better-armed plants; using sequencing technology we can potentially sample a field and find out exactly what is living there, then select the best crops for it.

Science has also made great strides in recent years in understanding how pathogenic organisms, such as fungi, interact with host plants. There is a constant struggle to protect plants from fungi, which can be frustratingly adaptable to new disease-resistant strains or fungicides. They produce ‘effector’ molecules that hijack plant pathways, and can also trigger immune responses – there’s an arms race between the pathogen and the plant in evolutionary time, and it’s a rapid one. Bioinformatics lets us look closely at the evolution of mechanisms that spur on this process. We’re also interested in the genomes of insect vectors – the insects that carry crop pests or animal diseases.



Sequencing genomes is useful for animal welfare as well. It's not just about breeding 'better' livestock but understanding and managing how they interact with the life forms that populate the rather unnatural environments in which they live.

Bioinformatics is also being used to understand the microbes that reside in the human gut, and how our systems react to the food we eat. Currently there is a lot of interest in the variability of gut microflora profiles between individuals, and their effect on how we metabolise food.

Resources like PhytoPath make it easier to study the pests that plague staple crops.

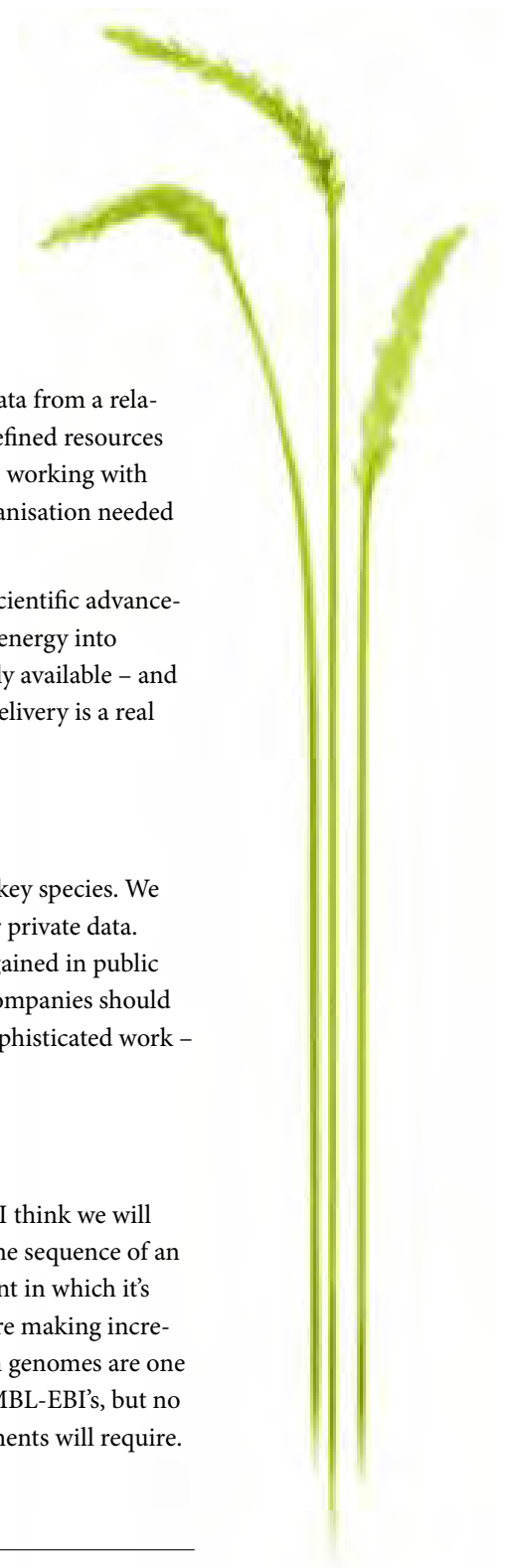
What activities are you focusing on now?

One genome we're really interested in is wheat. The 'post-genomic sequence era' has been slow in coming for plants. In some ways the wheat genome is the last significant frontier when it comes to primary reference genomes. In the past couple of years, many crop species of economic importance have been sequenced, and it's very likely that within two to three years we will have a fairly decent draft of the major cereal genomes as well.

We're working on a project on salmon lice with researchers in Bergen, Norway. Farmed salmon live in close quarters and problems can arise – the environment is very favourable to parasites. Sea lice can be controlled with chemicals but these need to be more targeted to protect the fish themselves and the rest of the living environment. The sea louse is an interesting creature because once it bites the salmon it stays where it is, shuts down its brain and just eats – it only moves to find a mate. We are studying the biology of this lifecycle change, for example looking for shifts in gene expression. Because it's a bug, my team gets to look into it!

What kind of resources does EMBL-EBI offer to people working in this field?

Ensembl Genomes is the primary resource at EMBL-EBI for analysing non-vertebrate genomes such as plants, fungi and bacteria; and of course, it supplements the coverage of farm-animal genomes in Ensembl. Recently, we partnered with Rothamsted Research in the UK to develop a plant pathogen resource. PhytoPath lets us serve this scientific community much better than before, because it makes it easier to ask questions about pathogenic phenotypes and genotypes at the same time. Thanks to my colleagues Sarah Hunter and Guy Cochrane, we now have a metagenomics resource that supports researchers looking at the microbiome and the results of environmental sequencing.



What are some of the major challenges in this area?

When you're trying to understand a plant genome you're trying to leverage data from a relatively small number of well-characterised genes. The absence of really well-defined resources for metabolic pathways in plants and fungi is a big challenge. And often we're working with relatively small communities, so it can be hard to obtain the funding and organisation needed to generate these resources.

But on a more practical level, there is a disconnection between the speed of scientific advancement and the time it takes to get a new crop to the field. You can put a lot of energy into developing a disease-resistant crop but it may be years before it's commercially available – and during that time the pathogen has had plenty of time to evolve. So speed of delivery is a real issue.

How closely do you work with the agri-food industry?

Companies use sequencing to drive Research and Development progress for key species. We help companies use public data – which we look after – as an anchor for their private data. Commercial organisations will largely be responsible for taking the insights gained in public programmes to develop seeds that people can buy and use. We believe that companies should share the basic information gained from nature so they can focus on more sophisticated work – this partnership can benefit both sides.

How do you see this area growing at the EBI?

The reference genome is a useful concept if you're studying pure biology, but I think we will need to concentrate on developing a more multidimensional view in which the sequence of an individual fungus or plant strain can be seen in the context of the environment in which it's found – for example, to support the study of host-pathogen interactions. We're making incremental progress from a world of static reference genomes to a world in which genomes are one component of dynamic environmental systems – this task isn't exclusively EMBL-EBI's, but no one else is as well-equipped to manage the reference data that these developments will require.

RESOURCES

Ensembl Genomes, for analysing non-vertebrate genomes: www.ensemblgenomes.org

The plant pathogen resource, PhytoPath: www.phytopathdb.org

Trans-national infrastructure for plant genomic science, transPLANT: www.transplantdb.eu

Logic gates of the genome





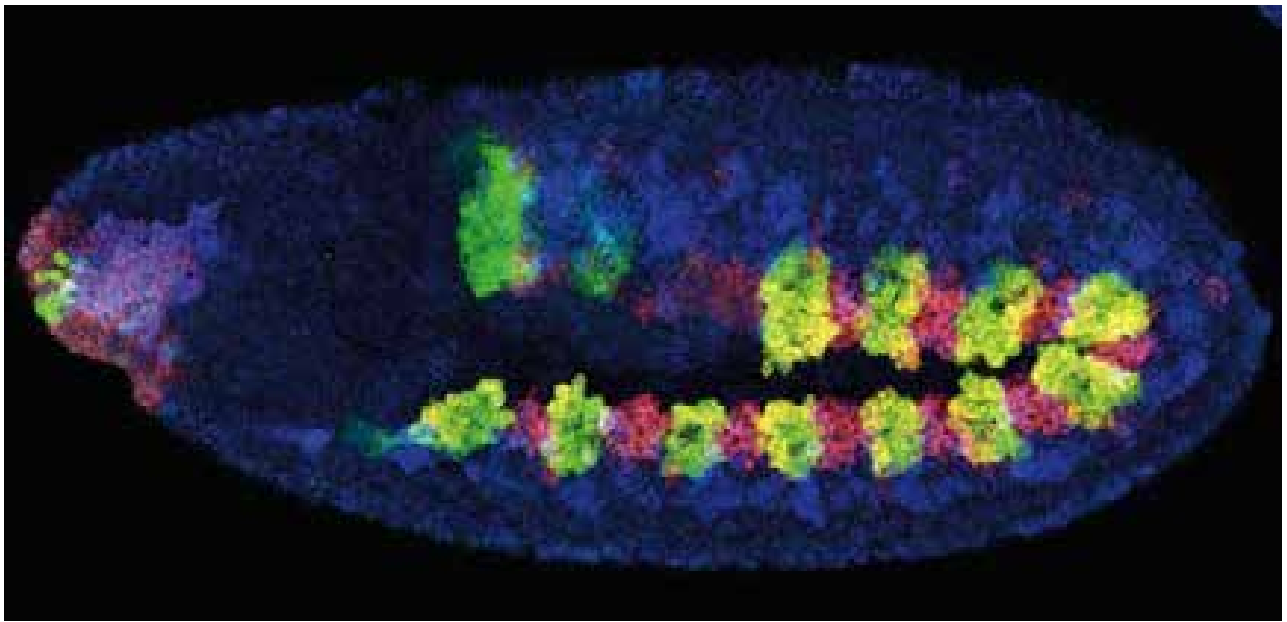
From supercomputers to the machine on your desk, computers today use electrical circuits to perform logical calculations, thanks to so-called logic gates. These are simple switches that can integrate electrical signals representing a binary code of ones and zeros, and use these inputs to create an output signal that can feed into further logic gates. Yet as simple as logic gates are, when you put millions of them together into an integrated circuit they can work wonders: in fact, every complex task that today's computers carry out is broken down into a series of simple steps that are performed by these tiny switches. At EMBL, scientists are combining computers and lab experiments to work out how cells use their own logic gates to control gene activity.

Since the 1960s, scientists have known that genomes contain a biological version of logic gates called promoters, which are located at the start of genes and determine when and where these genes are switched on and off. The input signal for promoters are proteins called transcription factors (TFs) that can bind to promoter DNA: when the right combination of TFs is in place, the neighbouring gene is switched on; at other times, specialised repressor proteins sit on the promoter, preventing TFs assembling and ensuring that the gene remains switched off. Then, in the 1980s, biologists discovered a new kind of biological logic gate dubbed an enhancer. Like promoters, enhancers can be bound by TFs and regulate whether other genes are active or not. However, unlike promoters, enhancers do not need to be near the gene that they affect. This 'action at a distance' is achieved by looping out the DNA separating an enhancer from the gene it regulates, so that they can be brought close together.

Although the existence of enhancers has been known for 30 years, the details about precisely how they regulate gene expression remain unclear. New answers, however, are being generated by the laboratories of Eileen Furlong, Head of the Genome Biology Unit at EMBL Heidelberg, and Ewan Birney, Associate Director of EMBL-EBI. In research published this year in the journal *Cell*, they revealed new insights into the subtle, complex world of enhancers.

Eileen's group studies the genetic networks that guide the development of muscle cells in the fruit fly *Drosophila*. Muscle cells come in a variety of specialised types: some, called cardiac mesoderm cells, function in the heart, whereas others function in the gut – the visceral mesoderm. Both cardiac and visceral mesoderm cells are derived from precursor mesoderm cells, and they become different by activating different sets of genes. As such, the process by which they differentiate provides an excellent model for understanding how TFs and enhancers work together to ensure that certain sets of genes are expressed and therefore to drive cells to one fate or another.

The major goal of the new research was to explore in more detail how TFs congregate at enhancers to regulate the expression of genes that cause cells to become cardiac tissue. "We knew from genetic studies that the TFs involved act cooperatively to regulate heart development, and we therefore expected the enhancers they interact with to have specific DNA sequence features that would reflect this," Eileen explains. For example, the 'enhanceosome' model of enhancer activity describes extensive cooperativity between TFs, which bind to enhancers in a strict order that is determined by the DNA sequence within the enhancer and which forms a scaffold for the attachment of TFs. The specific genetic motifs that TFs recognise, and the order in which they are lined up and spaced out along an enhancer, have been suggested to form a 'motif grammar' for enhancer function. This constrains where TFs can bind to the enhancer, which ensures that they do so in the correct order and in the right orientation. This is impor-



The ancestors of a fruit fly's gut (green/yellow) and heart (red) muscle cells.

tant, because the enhanceosome model also proposes that the multiple TFs that bind to an enhancer do so 'cooperatively', meaning that the binding of one TF requires interactions with the other TFs so that they all bind as a single complex in an all-or-nothing fashion. According to this view, enhancers are relatively inflexible on-off switches, in which even small changes to the motif grammar cause the system to break down.

There is also an alternative 'billboard' model of enhancer function, in which TFs bind to enhancers independently, and do not require a rigid motif grammar. Rather, enhancers function like billboards: they can display different messages using a common set of 'letters' (sequences that TFs can bind). This makes the enhancer more flexible, and means that parts of the enhancer can be altered without having catastrophic effects on enhancer function.

Yet both of these models are based on just a few examples of each mode of action. "We wanted to assess them on a more global, genome-wide scale," says Eileen. To do this, her team focused on five TFs known to be important in driving mesoderm cells towards a cardiac fate. These TFs bind to many enhancers in the genome, and so looking at all of them would shed light on whether enhancers function like enhanceosomes or billboards.

The first step in these studies involved isolating the thousands of sections of DNA to which these TFs bind. This enabled the team to study the DNA sequences required for binding, and to see whether they followed a motif grammar or not. In addition, Eileen and colleagues looked at which cardiogenic TFs were bound to enhancers to see whether they bound collectively, as expected from the enhanceosome model, or independently, as proposed by the billboard model.

In fact, neither model explained how cardiogenic TFs operate. Most of the enhancers regulating early stages of cardiac mesoderm development tended to have all five TFs bound to them, yet they achieved this in the absence of a strict motif grammar, in accordance with the billboard model. At the same time, the fact that these TFs were typically found to bind simultaneously suggests that their binding is cooperative, in line with the enhanceosome model.

In light of these findings, Eileen and colleagues propose a new mode of enhancer function that they call a 'TF collective'. In this model, a subset of TFs bind to the enhancer directly, and then recruit further TFs until the entire collective is in place, at which point the enhancer becomes active. Unlike the enhanceosome model, this does not require that the genetic motifs to which TFs bind are rigidly organised in the enhancer. This model provides a mechanism by which enhancers can tolerate extensive turnover in sequence motifs between species during evolution, with very little effect on the enhancer's function.

transcription
factors can prime
the genome, setting
the stage for cells
to differentiate

Along with suggesting a new model of how TFs assemble on enhancers, these studies have produced another intriguing yet unexpected finding. Eileen and colleagues found that the set of TFs bound to enhancers in a cell not only explains why the cell takes on the role of heart or gut muscle, but provides clues to the cell's developmental history.

Cardiac and visceral mesoderm both derive from the same population of mesoderm precursor cells. Both kinds of cell contain the same set of enhancers, but use them differently: in cardiac mesoderm, heart enhancers are bound by cardiogenic TFs and are therefore active, whereas in visceral mesoderm a different set of TFs activate gut-muscle enhancers.

So far, so good. The surprise came when Eileen and colleagues discovered that gut-muscle enhancers are also bound by TFs in cardiac mesoderm, but in an inactive or dormant state. In fact, the activation of gut-muscle enhancers has to be actively repressed in cardiac cells by a protein that binds to these enhancers and interferes with the function of the bound TFs.

Based on their TF collective model — in which the binding of one or more TFs sets the stage for the binding of further TFs — Eileen and colleagues suggest that during development, certain TFs bind to an enhancer to make it easier for other TFs to bind to that same enhancer at a later stage. “We think that TFs in precursor cells bind to a whole range of regulatory regions, so that the offspring of these cells contain enhancers that are in a ‘primed’ state. The TFs bound to a particular set of enhancers are required by visceral mesoderm, but in cardiac mesoderm they’re just a relic of developmental history, so they have to be actively shut down with a repressor,” says Eileen.

In the past, many biologists have assumed that TFs only bind to enhancers to promote gene expression at that point in time. The results of these studies, however, show how TFs can prime the genome, setting the stage for cells to differentiate into various specialised types. In addition, the discovery of dormant enhancers occupied by TFs opens up the possibility of using patterns of TF occupancy to trace the developmental origins of cell lineages — a tool that will help biologists tease out the pathway from a single fertilised egg to the billions of cells that make up adult organisms.

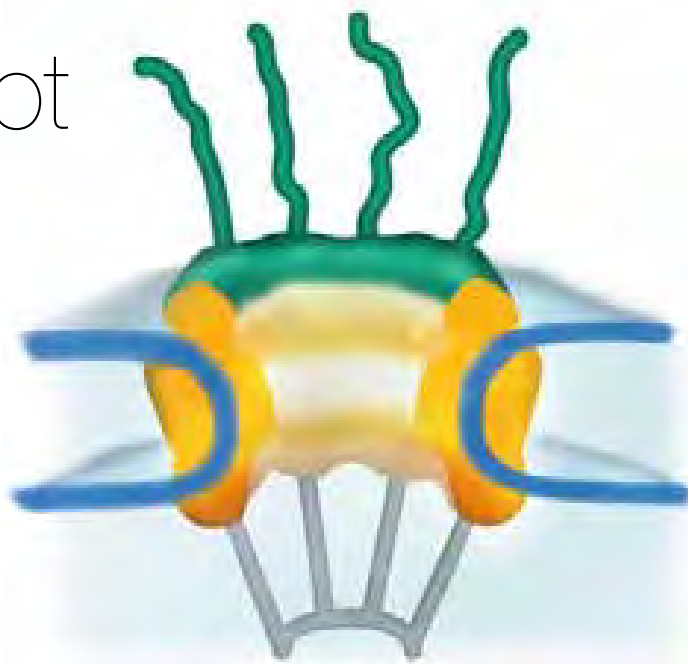
Junion G, Spivakov M, Girardot C, Braun M, Gustafson EH, Birney E, Furlong EE (2012) A transcription factor collective defines cardiac cell fate and reflects the developmental history of this cell lineage. *Cell* **148**: 473-86

Some like it hot

A steaming heap of dung sounds like an unlikely place to look for new clues about how our cells work. But this is exactly where you'll find a creature that is set to transform biologists' ability to understand how large, complex proteins fit together, thanks to a collaboration between EMBL's Peer Bork and researchers at Heidelberg University. As well as creating a valuable new resource for the global scientific community, the work has allowed biologists to get their first peek at the structure of an important piece of cellular machinery that has so far proved elusive.

The creature in question is a fungus called *Chaetomium thermophilum*. As its Latin name suggests, *Chaetomium* is a "thermophile" – a lover of heat – and so possesses the unusual ability to survive at 60°C. Most organisms can't withstand this relatively high temperature, which is why public health advisers tell you to wash your bed linen at 60°C to kill bacteria and dust mites. They die because their proteins unravel as the temperature rises beyond 40°C, a process you can see happening to a raw egg white as you cook it. But *Chaetomium* can withstand the sweltering environment of a rotting dung or compost heap, which can easily reach 60°C or higher, because its proteins have evolved to be much more stable. This allows the fungus to access nutrients in the environment with little competition from other organisms.

Chaetomium's proteins are therefore extremely attractive to biologists, whose experiments are often thwarted by proteins unfolding once extracted from cells. Researchers have already investigated proteins from other thermophiles but these have all been bacteria and archaea, whose cells lack many of the features of more complex, or "eukaryotic", cells like our own. As a fungus, *Chaetomium* is a eukaryote, so studying its proteins will give better insights into what happens in humans and other animals. "It's a goldmine for structural biology," says Peer.



Details of the nuclear pore's inner ring emerged thanks to a heat-loving fungus.

Ed Hurt, an EMBL alumnus and now structural biologist at Heidelberg University, contacted Peer because he wanted to work with *Chaetomium*, but needed help with analysing its genome to find his proteins of interest. Together with Peer's bioinformatics team at EMBL Heidelberg, Ed's team identified the *Chaetomium* proteins that build the nuclear pore – a massive, intricate, gate-like structure that guards access to the cell's brain centre, its nucleus. In creatures that live at everyday temperatures, including us, these proteins are too big and too bendy to keep their shape in lab experiments. But thanks to the stability of the fungus' proteins, the scientists were able to see how they assemble the all-important inner ring of the pore, which dictates which molecules may enter or exit the nucleus. The new structure reveals how the pore is able to mould itself to accommodate different-sized cargoes.

"It's a proof of principle," says Peer, whose group continues to study *Chaetomium* and provide resources for other researchers who want to do the same. Peer's team has made the genome sequence and analysis tools publicly available, and Ed's team sends out DNA samples to structural biologists around the world. For all its humble origins, *Chaetomium* looks set to become biology's next hot property.

Amlacher S, Sarges P, Flemming D, van Noort V, Kunze R, Devos DP, Arumugam M, Bork P, Hurt E (2011) Insight into Structure and Assembly of the Nuclear Pore Complex by Utilizing the Genome of a Eukaryotic Thermophile. *Cell* **146**: 277-89



Small wonders

In the 1980s, a toy sensation swept through the US and Europe: MicroMachines. These scale models of everything from monster trucks to sports cars packed an incredible amount of detail into about the size of a teaspoon. Scientists are now performing an even greater feat of miniaturisation, by creating a whole laboratory that fits in the palm of your hand.

The novel approach, called microfluidics, is a quickly growing, highly interdisciplinary field that combines physics, engineering, chemistry and biology. A microfluidics chip is essentially a plastic maze of tiny channels into which scientists inject either a continuous flow of the liquid they're studying, or individual droplets that serve as microscopic test tubes. At EMBL Heidelberg, Christoph Merten leads a group dedicated to developing and using microfluidics techniques for molecular biology. In this interview, he shares what drew him to the field, and how it is being used at EMBL to answer an incredible array of biological questions.

How did you personally get involved in microfluidics?

My primary training is in biochemistry; I did my diploma thesis and PhD thesis on directed evolution, in which you genetically engineer many, many different versions of a protein and then screen them for a particular trait – so quite far away from microfluidics, but there is a little overlap. The high-throughput aspect was already there, but it was limited to things you can do with cells. Then I joined Andrew Griffiths' lab in the UK, and at that time he already used droplets as individual compartments. He generated those droplets with conventional equipment, though – the idea was visionary, but in practice there were very, very strict limitations. So soon after I joined his lab, I did an internship in Dave Weitz' lab at Harvard – Weitz was a pioneer in droplet microfluidics. I touched it for the first time, and thought: "that's my future".

What are the advantages of microfluidics?

One big benefit is that you can use samples that are not available in big quantities – primary cells, patient material, these kind of things. In a conventional set-up it's often difficult to get the sample amounts you need for assays. But because the sample amounts that we need are much smaller, we can think of addressing issues that conventional lab techniques just can't contemplate.

Another advantage of miniaturisation is that you can get massively increased throughput: when using droplets we can process something like 500 samples per second.

What limitations does microfluidics face?

One big limitation at the moment is integration. Many groups have developed individual modules: a device that creates droplets, a device that fuses droplets, a device that sorts droplets and



Lab-on-a-chip:
a microfluidics
experiment in action.

so on. Then you want to do a biology application, and you see that for your application you need 3,4, or 5 modules, but they are not totally compatible with each other – even if people try to sell them like this. Sometimes you have to re-engineer a module completely to make it compatible with the rest.

You don't just design and produce microfluidics chips, though. You also carry out your own experiments. That must mean your group has people with many different areas of expertise.

Absolutely, there's a lot of diversity. We have people with a physics background, with a programming or engineering background, biologists and even a synthetic chemist. Even within our group we are totally interdisciplinary, and I think that makes it attractive for the students, as well as for me. I can get new input from people within the group, and that's nice.

What are you working on, in terms of technology development?

We're focusing on designing integrated chips. Also, we're starting to combine valve and droplet technology, which is not something many labs do, but it allows you to have much better control over the droplets. People who use valves generate a few thousand chambers by pinching off different regions of the channel. When we use droplets as vessels we can generate millions of them. So just in terms of the number, it's much better than the valves. But without valves, we cannot control the droplets very well. If you combine the two, you no longer need thousands of valves because you already have millions of droplets, but you can use the valves, for example, to guide the droplets through the chip.

In your research projects, you collaborate with many different EMBL groups, don't you?

Yes, one of the great advantages of working at EMBL is that we can tap into all the biological expertise in the building, and that makes for great collaborations. We have shared EMBL Interdisciplinary Postdocs (EIPODs) with Marcus Heisler and Eileen Furlong here in Heidelberg, for embryonic development and transcriptional studies. With Marcus, the idea is to take individual embryos and work on single cell transcriptomics, to get a blueprint of each individual cell during development. This will tell us, for example, what happened to the cell, what's the ultimate cell fate, why one cell differentiates into cell type A and another into cell type B. This EIPOD is also shared with Paul Bertone from EMBL-EBI – once we've generated the data, Paul will help with the analysis.

With Eileen we aim to do single-embryo chromatin immunoprecipitation (ChiP) studies. Normally you do these on large populations of embryos, and you have to try to synchronise them so that they're all in more or less the same developmental stage. That means your time window isn't very precise, because you're averaging out over the different embryos. But if you push that to the single-embryo level, you have exact stages, and you can say 'this embryo is at exactly this stage, now we'll do some transcription factor-binding measurements', and we know that exactly at that stage the transcription machinery is on or off. So you can see things that you wouldn't

see in a vial, but you have to be aware that the variation will be greater than if you were averaging over a whole population. On the other hand, this means we might be able to start looking at just how much variation there is within a population.

We're also collaborating with Detlev Arendt here in Heidelberg. We have designed a microfluidic aquarium where marine ragworm larvae can move around freely, and in different parts of that microfluidics chip we can create different chemical environments. For example, we've done an experiment in which we have a stable pH gradient inside the aquarium, and then the larvae can choose which pH they like most. This is something you can't easily do in a conventional lab. You can put the animals in a Petri dish at a certain pH, and they either survive or they die – they can never tell you which they prefer. They are either in Petri dish A or in Petri dish B, they cannot be in Petri dish B and tell you 'I would like to be in Petri dish A', right? This set-up also allows us to really track when an embryo gets certain capabilities: we can determine at what stage they start to sense pH or salinity, or whatever.

And with Carsten Schultz and Maja Köhn's groups at EMBL Heidelberg we have started doing synthetic chemistry on a microfluidics chip. We're doing it mainly because we have massively increased throughput, and it's also worth mentioning that this has huge cost advantages. When we use droplets, assay volumes can be roughly a million times smaller. So if a screen would normally cost €100 000, with a microfluidics chip the cost would be 10 cents. So there's also potential for commercialising some microfluidics technologies.

Alongside all this, we also have our own independent research projects. We're starting to do many assays involving primary cells, for example screening for therapeutic antibodies. We've also just started to analyse exactly what triggers the differentiation of stem cells, and how we can trigger their differentiation into a particular lineage. Another project based on primary cells is that we want to analyse cellular senescence, and analyse the cross-talk between different pathways.

Does working on all these different scales and with such a variety of organisms pose challenges?

In the end, we optimise everything for a certain project and a certain organism. There may be some chips that we can use as a starting point for another project, but for instance the embryo chips we use on the project with Eileen look different from the ones we're using for the work with Marcus' group. But that's also nice – I think I get so much inspiration from all these different people, I start thinking about things that I'd never think about before, and that's very, very nice. You're pushed to think about things that otherwise you would not even have contemplated.

MICROFLUIDICS CONFERENCE

25-27 July, EMBL Advanced Training Centre, Heidelberg

Microfluidics experts and biologists come together to exchange ideas, foster collaboration and present the latest Lab-on-a-Chip technologies & applications.

<http://www.embl.de/training/events/2012/MCF12-01>

Lord of the rings

a ring that,
although not magic,
has yielded a new
understanding

In 1949, American mythologist Joseph Campbell noted that although human cultures differ, the structure of their stories is universal. A hero or heroine is forced to step outside their normal world and embark on an unexpected quest and, with the help of friends, and sometimes also ancient swords

and magic rings, they hunt down an elusive goal before returning with a “boon” – that is, treasure or knowledge to benefit their world.

A basic drive behind this narrative, said Campbell, was to explore the nature and wonder of the Universe, something storytelling has in common with science. So it is perhaps no surprise that Christoph Müller and his team at EMBL Heidelberg recently found themselves undertaking a heroic quest of their own. They were called away from their usual avenue of research by the surprise discovery of a ring that, although not magic, has yielded a new un-

derstanding of how cells ensure they produce proteins correctly. The ring is in fact made of a set of proteins, and the discovery of its structure by Christoph and his colleagues has shed new light on the function of the proteins involved. “The ring-like arrangement of these proteins we saw in the crystal was completely unexpected, but the moment we saw it, it gave us plenty of ideas to test to work out its function,” says Christoph.

Christoph’s team set out to understand more about a complex – an interacting group of proteins – known as the Elongator complex. They had long been interested in a process called transcription, one of the first steps in turning the information coded in a gene into a protein. During this process, the cell makes a copy, or transcript, of a gene using RNA, which is a molecular relative of DNA. The cell uses this transcript as a guide for making a protein in a process called translation.

The work began as part of an EU project called 3D-Repertoire, which was established



to study large protein complexes. As part of this project, Christoph's team identified the Elongator complex as being of interest. The complex seemed to be involved in transcription, but no-one knew exactly how it functioned.

Working in collaboration with scientists at the Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC) in Illkirch, France, Christoph's team began by working on the yeast version of the complex, Elp456, that consists of three interacting proteins and determined part of its structure with a technique called X-ray crystallography. This revealed that two sets of protein trios slotted together to form a ring – a finding the team then confirmed by electron microscopy.

The ring-like structure was uncannily similar to rings formed by a family of proteins known as helicases, which interact with DNA and RNA. So the team tested the complex's ability to bind to these molecules, and found that it bound to a set of molecules called trans-

fer RNAs, which are involved in translation. What's more, they showed that it uses up energy to control their binding as a first step to introduce chemical changes to these RNAs. Such changes were already known to improve the accuracy of translation, and the recent results now explain how this part of the Elongator complex is involved in this process.

The team plans to continue their quest by studying the structure of the entire Elongator complex, which is sure to yield further knowledge boons to benefit humankind.

Sebastian Glatt and
Christoph Müller

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making
sense of **MENTAL**
ILLNESS

biology, medicine & society

Making sense of mental illness

When someone suffers from flu or breaks a bone in their leg, the condition is usually relatively straightforward to identify. Yet when the complex neuronal networks in the brain malfunction, it can take months or even years to recognise that there is a problem. This is because mental illness is often caused by diverse and interconnecting factors in both the environment and the body; acknowledging this is crucial in addressing conditions that the World Health Organisation estimates affect around one in four people during their lifetimes, participants at this year's EMBO|EMBL Science and Society conference heard.

Defining mental illness

Giving the keynote introduction at 'Making Sense of Mental Illness: Biology, Medicine and Society', sociologist Nicholas Rose said that in addressing the causes and consequences of mental illness, it is important to begin by asking what is mental illness? "Where should one draw the boundaries between a condition that is appropriate for diagnosis and treatment and a condition that is part of everyday life and that people need to accept? Where does childhood bad behaviour end and attention deficit hyperactivity disorder (ADHD) begin? Normal sadness end and depression begin? Age-related memory loss end and mild cognitive impairment (MCI) begin? Many individual and social consequences flow from the way we draw these boundaries," he warned. "The question then is: what kind of factors does one consider in order to make these decisions?"

The causes of mental illness, he said, cannot easily be asserted. "Factors can be found in the relationship between individuals, the social circumstances of those individuals, and in situations of stress, anxiety, and conflict." Rather than an explanatory system that begins with the brain, Nicholas suggested that: "a more effective way of thinking about it would be to ask in what way, and through which neural pathways, do these social factors shape neurobiology?"

This year's event, held at EMBL Heidelberg, was the 12th in an annual series of two-day conferences, which provide a platform for members of the public and scientists to debate the impact that research is having on society, and to engage in a shared understanding of science. Nicholas' talk, which raised other concerns such as the tendency to overstate new scientific findings, set the scene for a lively, engaging, and forward-looking programme.

Connecting with end users

Donna Franceschild, a television writer and dramatist, provided one of the meeting's most striking talks, in which she called on researchers to remember the end user in developing ways to address mental illness. Donna suffers from bipolar disorder, an illness that has severe effects such as unusual shifts in mood, energy, activity levels, and the ability to carry out day-to-day tasks. She called for patients to be empowered through active involvement in their treatment: "I think there is a resistance from sufferers to the idea of what's happening to the individual and what feels very personal to the individual being reduced to some genes that one is unlucky enough to inherit. When I speak to my psychiatrist, I feel like we are in collaboration. I feel empowered by being actively involved in tackling my own illness, and in the medicine I take – this is absolutely the way it should be."

One challenge involves overcoming stigma. Wulf Rössler, from the University of Zurich, described a "vicious cycle of discrimination", which can lead to social isolation, unemployment, homelessness, and drug abuse. "One of the reasons why depression is less stigmatised is because a lot of people are aware of what depression is," he said.

Hans-Ulrich Wittchen, from the Technical University of Dresden, warned participants that the majority of cases of mental illness remain untreated (less than 10% of sufferers receive adequate treatment) and emphasised early intervention as a means to reduce the problem. "We need to focus on primary prevention and prevent recurrence, slow down disease progression, and reduce the associated psychological impairment. European countries need to invest in research – not just pharmacological treatment, but psychological treatments as well," he told participants.

Understanding causes

Michael Rutter from Kings College, London, followed up this point, and said that there is increasing evidence that factors such as experience, education, and social interactions can have a crucial impact on treatment. "Scientists and geneticists say, with good evidence, that genetic influences are very important. But the identified genes that we know about have a terribly small effect and the question is: how does that all come together? We know from researching the field of cancer that the genes that protect us are as important as the genes that put us at risk. The same will probably apply in the field of mental health disorders."

Important too is a greater understanding of different social environments, attendees heard. Steven Rose, Emeritus Professor of the Open University, delivered a blunt assessment of the current research: "It is time to move on from genetic studies and stop hunting for genetic factors," he said. Instead, he argued, research should start with epidemiology and ask questions relating to why certain groups such as women, poor people, ethnic minorities, and people living in inner city areas are more badly affected by illnesses such as depression than others.

Towards better treatment

Another issue that drew impassioned responses from speakers and the audience was obstacles to drug development, particularly in response to an alarming rise in costs of bringing new drugs to market. Andreas Meyer-Lindenberg, Director of the Central Institute of Mental Health in Mannheim, described this and the resulting fall in investment by pharmaceutical

companies as a “drug deadlock”. “The reason this has happened is that the success rate has been low, and we need to think about how we address this,” he said, reflecting on the reality that between 2008 and 2011 the average cost to get a drug on the market spiralled from US\$0.8bn to around US\$4bn. “We need to speed up and focus the development process, think differently about how we develop new targets, or take drugs that might already be available and offer them to people that can benefit.”

Mathias Berger from the University Medical Centre in Freiburg cautioned that competing theories and assumptions were creating problems, but also highlighted positive developments such as new disorder-tailored therapies, which are having a positive impact on illnesses such as chronic depression, anxiety, obsessive-compulsive disorders, and substance abuse. He also applauded neuroscientists and psychiatrists who use combinations of psychotherapy and drugs to increase the effectiveness of both. “Schools of thought that have been enemies for decades are beginning to come together in this more scientific, evidence-based behaviour,” he explained. “We are in a very good position to profit from the neuroscience revolution of the past 20 years and increase the effectiveness of psychological methods.”

A multidisciplinary approach is also a central part of the ongoing revision of the Diagnostic and Statistic Manual of Mental Disorders, DSM-5, explained David Kupfer, who is chair of the task force in charge of the changes to the standard classification of mental disorders. David’s talk on the “diagnostic bible”, which is used worldwide by clinicians to assist diagnosis and treatment, provoked questions from the audience relating to culture, suitability, and misdiagnoses that spilled over into lively panel discussions, and reflected the significance that the issues relating to mental illness have in everyday life.

Beyond the plenary hall

More than 350 participants from around the world attended the event, including members of the public, journalists, and students, and talks were streamed live on the web, ensuring that the discussion extended far beyond the plenary hall. “Mental illness is an issue that touches the lives of every one of us,” said Sandra Bendiscioli, the main conference organiser from the EMBO Science Policy Programme. “The range of talks, from implications of scientific and technical developments to real-life experiences of patients, has given participants important and challenging questions to reflect on and discuss.”

13TH EMBL/EMBO SCIENCE AND SOCIETY CONFERENCE

Biodiversity in the Balance: Causes and Consequences

9-10 November, EMBL Advanced Training Centre, Heidelberg

<http://www.embl.de/training/events/2012/SNS12-01>

Jan Korbelt and
Stefan Pfister



Broken necklace syndrome

It's a scene that has been played out in many a household: the whole family on their hands and knees chasing coloured beads, as a distraught child stands wide-eyed, holding the remnants of a favourite necklace. Once most of the beads have been collected, a kind adult threads them onto a new cord, and the crisis is over. Unless, of course, the child is not satisfied with anything less than an exact replica of the original necklace: getting all the beads – including those under the sofa or behind the cupboard – and threading them back onto the cord in the right order can be a tricky business.

At Heidelberg University Hospital, Andreas Kulozik encountered a family with a much more serious problem. First a little girl and then her brother had highly aggressive tumours. In initial genetic tests, Andreas found that the two siblings had the same mutation in the gene p53. They had the mutation in all their cells, not just the cancerous ones, which meant that it was inherited from their parents rather than acquired later by the tumour cells. When Jan Korbel, a group leader at EMBL Heidelberg and EMBL-EBI, and Stefan Pfister and Peter Lichter from the German Cancer Research Centre (DKFZ) teamed up to look at the genetics of childhood brain tumours, this family connection seemed like a good place to start. As part of the International Cancer Genome Consortium, Jan, Stefan and Peter were the first researchers to sequence the whole genome of cells from a particular paediatric tumour called a medulloblastoma. This is the most common of all malignant paediatric brain cancers, which are the most fatal cancers in children and the second most common cause of childhood deaths in developed countries after road accidents.

“When we got the DNA sequence data back, we saw a chaos in the girl's genome that we couldn't really explain at first,” says Tobias Rausch from Jan's group, who led the data analysis. “Then we saw a paper by another group, describing a new phenomenon they called chromothripsis, and it clicked,” adds fellow group member Adrian Stütz. The scientists realised they were seeing the cellular equivalent of the broken necklace scenario: a chromosome had somehow exploded into countless small pieces, and had then been put back together with some pieces missing and others in the wrong order. As they analysed more samples, they realised this happened in all medulloblastoma patients who carried an inherited p53 mutation, but not in the patients with normal p53. “This makes us suspect that these three events are connected,” says Jan. “We believe that the p53 mutation may cause chromosomes to explode, or possibly prevent the cell from reacting properly when they do. This somehow then leads to highly aggressive forms of cancer.”

So how could the mutation in p53 cause chromosomes to explode, and how could that lead to cancer? Scientists know that p53 helps to prevent chromosomes from fraying at the ends by protecting telomeres, which are the caps that keep the ends of chromosomes together. If p53 is faulty, Jan and colleagues speculate, the telomeres could be compromised, and chromosomes could stick to each other. In such a scenario, when the cell came to divide, chromosomes that were stuck together could run into problems. They would be pulled in opposite directions. At some point the strain would be too much and, like the bead necklace that's pulled too hard, the chromosomes would shatter, sending fragments of DNA flying. As the cell's machinery races to put the chromosomes back together, bits of genetic material might be left out, and others re-assembled in the wrong order.

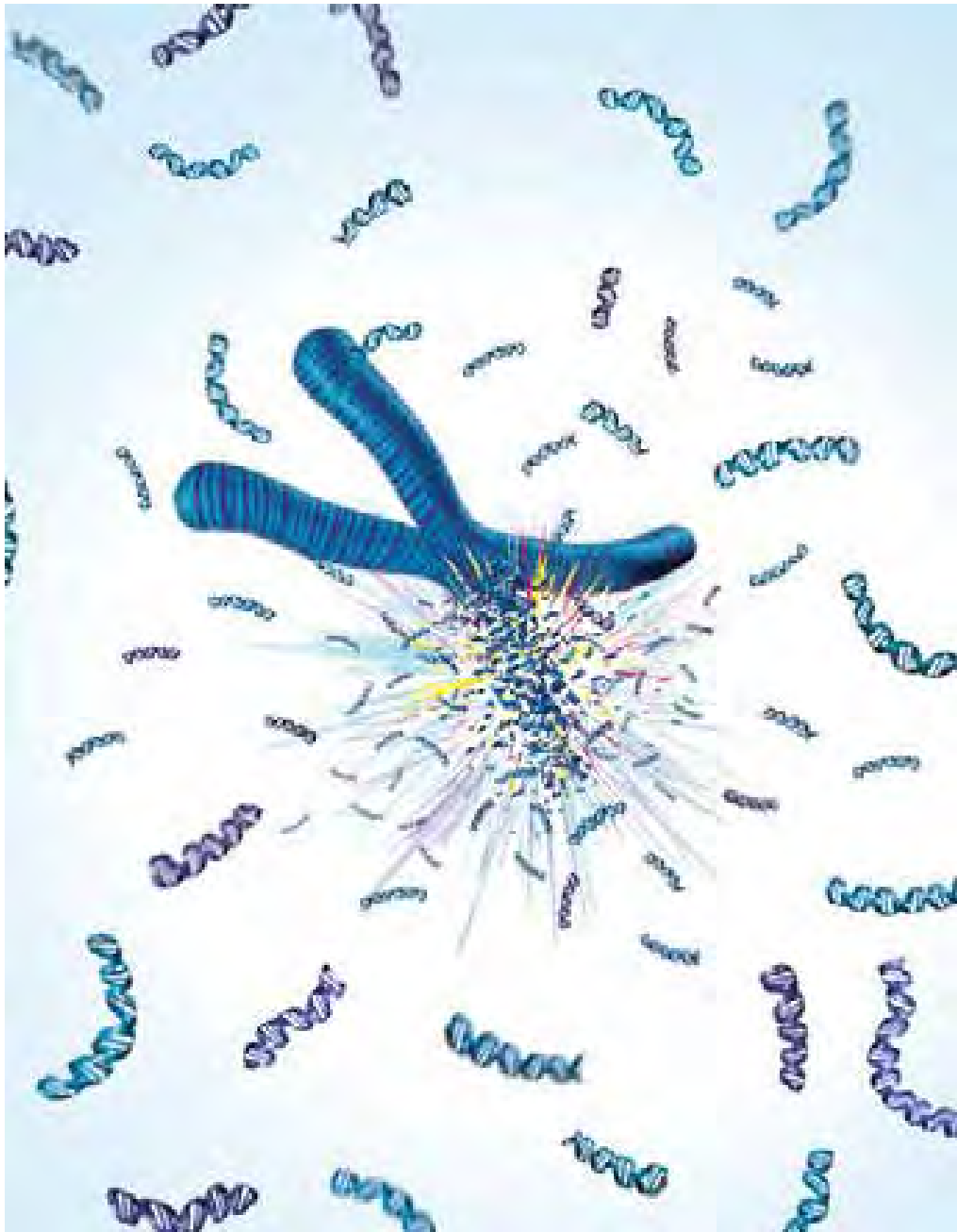
In addition, p53 plays a key role in inspecting our DNA for damage. If this guardian of the genome finds too many mistakes, it can push the cell into a programmed suicide (apoptosis) or into the cellular equivalent of old age (senescence), to prevent the cell from dividing and passing on the genetic defects. But if p53 is mutated, extensive DNA damage – such as a badly reassembled chromosome after chromothripsis – could go unnoticed regardless of whether

like the bead necklace
that's pulled too hard,
the chromosomes
would shatter

p53 was involved in causing the chromosome explosion or not. As a result, oncogenes – genes that lead to cancer – could be activated, and the cell could start dividing and dividing, unchecked, creating a tumour. Jan, Stefan and Peter speculate that both effects of faulty p53 may combine to lead to cancer in these patients, and would now like to investigate exactly how this is happening at each step.

In the meantime, their findings are already having repercussions for clinicians like Andreas and Stefan, and their patients. “If a patient's tumour cells show signs of chromothripsis, we now know that we should look for an inherited p53 mutation,” Stefan says. And this is important, because having an inherited p53 mutation could make the most commonly used cancer treatments backfire. Many chemo- and radiotherapy treatments kill cancer cells by damaging their DNA, but they also affect other cells in the body. This can lead to painful side effects although in most patients, there is little long-term harm. But for someone with an inherited p53 mutation, all of their cells, including the healthy ones, will have trouble reacting to DNA damage. So treatments that target DNA could actually make healthy cells turn cancerous, causing so-called secondary and tertiary tumours – “something we often see in patients with inherited p53 mutations,” says Stefan. For these patients, it may be preferable to prescribe less intensive treatments using agents that do less damage to DNA. Furthermore, if a patient has an inherited p53 mutation, then that person's immediate family need to be tested. If any healthy family members carry the mutation, they should be regularly screened, as they are very likely to develop tumours at some point in their lives. “And the best chances of fighting cancer – especially the aggressive, early-onset types of cancer that seem to be associated with chromothripsis – are if it is diagnosed early,” Jan points out.

In fact, scientists think that 2-3% of all cancers are probably caused by chromothripsis, so Jan's group are now investigating whether p53 mutations play a role in similar chromosome explosions in other tumours besides medulloblastoma. They have already found evidence for the same link between chromothripsis and inherited p53 mutations in acute myeloid leukaemia. In this aggressive type of blood cancer in adults, Jan and colleagues discovered that patients with both a non-inherited p53 mutation (that is, a p53 mutation only in tumour cells) *and* evidence of chromothripsis tend to be elderly. The scientists point out that this makes sense in the light



An inherited mutation likely causes chromosome 'explosions' linked to cancer.

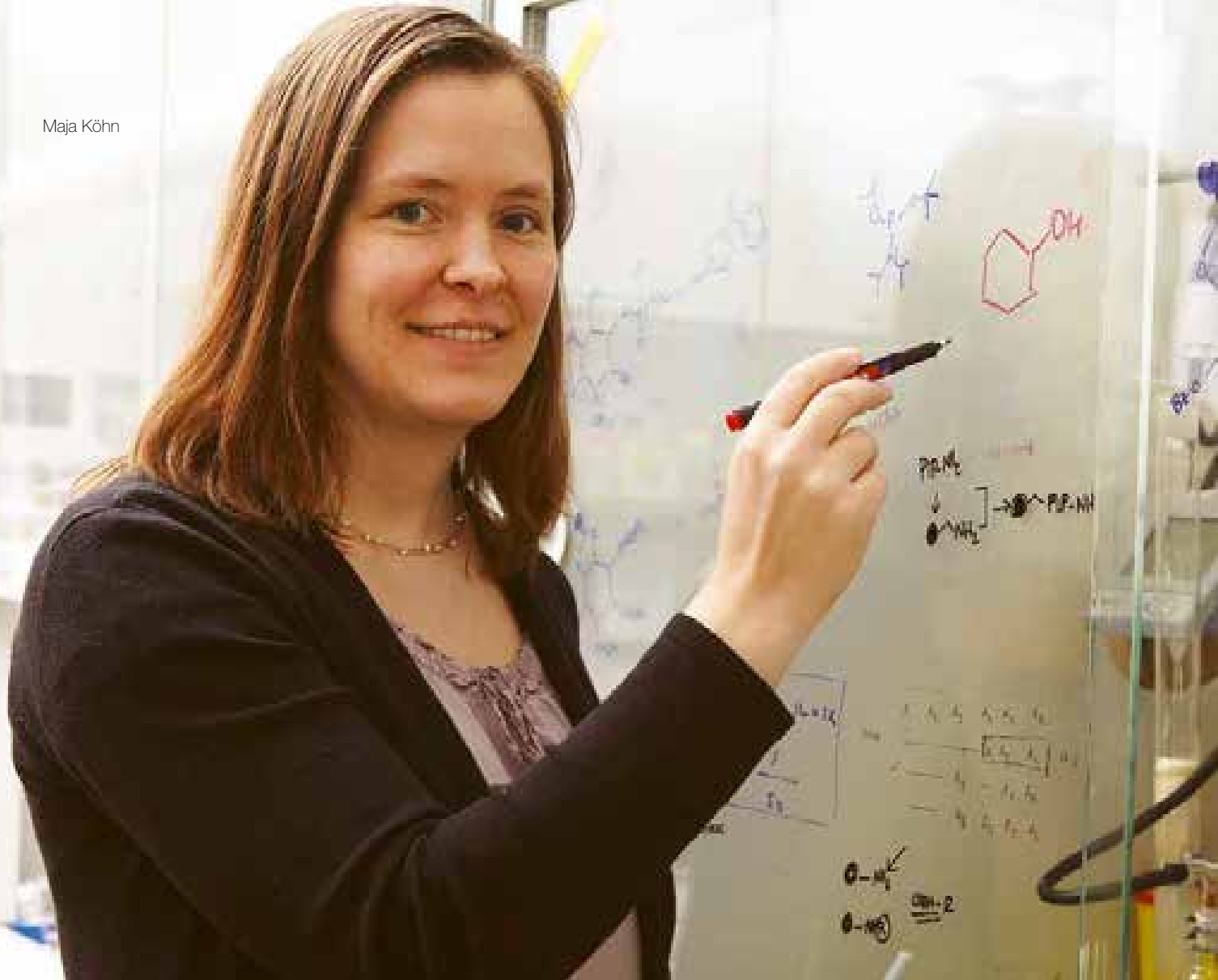
of p53's role in telomere integrity. Our chromosome caps naturally shorten as we age, making chromosome ends more likely to stick to each other if p53 goes awry. This in turn makes chromothripsis – and the ensuing cancer – more likely, the scientists suspect.

Jan's group is continuing to explore these issues in brain, blood and other cancers, to unravel just how faulty versions of p53 are linked to chromosomes exploding like broken necklaces, as well as investigating which other aspects of the cell's housekeeping efforts are involved in cancer.

ONLINE EXTRA:

Jan is interviewed about this study on the EMBL YouTube Channel:
<http://youtu.be/LimNx0m227U>

Rausch T *et al* (2012) Genome Sequencing of Pediatric Medulloblastoma Links Catastrophic DNA Rearrangements with TP53 Mutations in Cancer. *Cell* **148**: 59-71



Shape changer

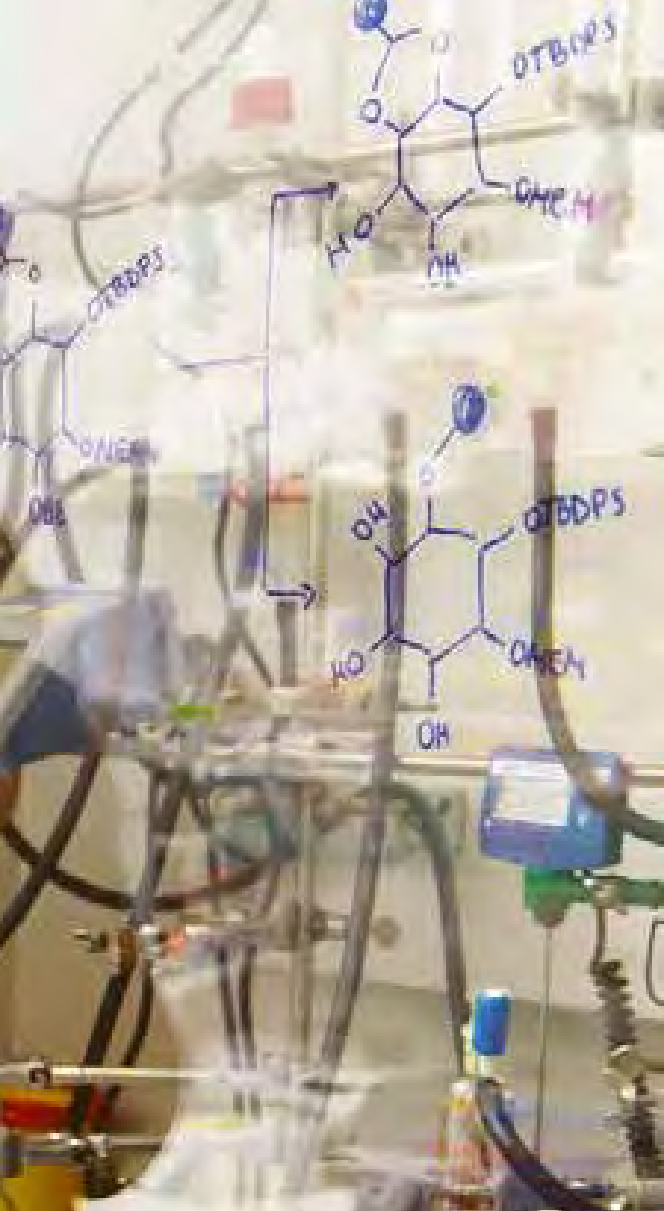
Cancer can be caused by many things, from smoking or a diet rich in red meat to viral infections or exposure to radiation. Yet all these factors give rise to cancer in the same way: by mutating and altering the activity of ‘oncogenes’, which leads to the growth, proliferation and migration of cells around the body to new sites – the hallmark of cancer.

Oncogenes function in a wide variety of tasks. In some cases, mutations lead to an oncogene being active at particularly high or low levels, or being expressed in inappropriate cells; in other cases, a mutation can alter the function of the protein that the gene encodes. Crucially, these changes in gene expression or protein function can provide a marker for specific

cancer types, which can have a big impact on treatment choices for cancer patients.

Despite the enormous amount that has been learnt about oncogenes in the past 40 years, the functional role of many of them remains unknown. But last year one of the gaps in our knowledge was plugged when Maja Köhn, a group leader at EMBL Heidelberg, in collaboration with Janet Thornton’s group at EMBL-EBI, published their studies on an oncogene called *phosphatase of regenerating liver-3* (*PRL-3*) in the journal *Biochemistry*.

When Maja and colleagues began their study, *PRL-3* was known to promote cancer metastasis, which is the ability of cancerous cells to spread from one part of the body to another. The protein encoded by *PRL-3* had also been proposed as a marker for several cancer types — for example, it is frequently over-expressed in colorectal cancers — and as a target for treating them.



CHEMICAL BIOLOGY

Living systems, from small molecules to entire ecosystems, are subject to the laws of physics, mathematics and chemistry. Likewise, the study of biology and the life sciences is influenced and enriched by other disciplines.

Chemistry is particularly well-placed to increase our understanding of, and ability to intervene in, biological processes. Like Maja, many EMBL scientists are working at the interface of biology and chemistry, using chemical tools to provide unique insight into biological functions and mechanisms. Ideas can flow in both directions, creating a melting pot of collaboration and creativity.

Using diverse experimental and theoretical techniques, chemical biologists at EMBL tackle challenging questions, like pinpointing when molecules interact inside a cell (p. 12) or using artificial amino acids to overcome problems in protein labelling (p. 84). And at events such as the *Chemical Biology 2012* conference, to be held at the EMBL Advanced Training Centre in Heidelberg in September, biologists, chemists, biochemists and chemical biologists are able to join forces, in what is emerging as an important discipline in its own right.

Chemical Biology 2012 conference:

<http://www.embl.de/training/events/2012/CHB12-01>

ONLINE EXTRA:

Listen to EMBL scientists discuss the advantages, challenges and future of chemical biology on the EMBL Explore podcast: www.embl.de/explore/chemical_biology

Like other phosphatases, PRL-3 removes chemical tags called phosphate groups from other molecules, which can affect their function. However, the target molecules – or substrates – of PRL-3 were unknown, so Maja and colleagues set about finding out. “Knowing the substrate of an oncogene provides clues about which pathways it functions in, which in turn can help in developing inhibitors of the oncogene for cancer treatments,” says Maja.

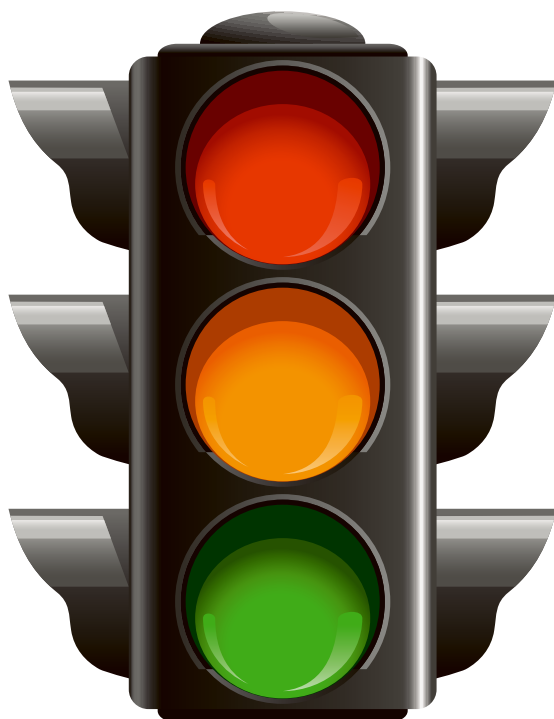
Two possibilities for the nature of the PRL-3 substrate stood out: proteins, or a kind of fat molecule called phosphoinositides (PIPs). In a series of test-tube experiments, Maja and colleagues found that PRL-3 did not remove phosphate groups from peptides, suggesting that proteins are not the natural substrate for PRL3. However, PRL-3 did remove phosphate tags from a PIP molecule called PI(4,5)P₂, suggesting that this is a target of PRL-3.

These findings, combined with previous knowledge of PI(4,5)P₂, point to a possible mechanism by which too much PRL-3 can promote cancer. PI(4,5)P₂ is a known component of cell membranes, where it interacts with the internal protein scaffolding that maintains the cell's overall shape. “We think that an excess of PRL-3 depletes phosphate groups from PI(4,5)P₂, which disrupts the mechanical support of the cell membrane and allows cells to change shape — a skill that cancer cells need in order to squeeze into the bloodstream and spread to other parts of the body,” says Maja.

The next step for Maja's group is to confirm that PRL-3 removes phosphate groups from PIPs in living cells as it does in the test tube.

McParland V, Varsano G, Li X, Thornton J, Baby J, Aravind A, Meyer C, Pavic K, Rios P, Köhn M (2011) The Metastasis-Promoting Phosphatase PRL-3 Shows Activity toward Phosphoinositides. *Biochemistry* **50**: 7579-7590

Stop and go



A 'traffic policeman' protein drives a crucial step of cell division.

A traffic policeman standing at a busy intersection directing the flow of vehicles may be a rare sight these days, but a similar scene frequently plays out in our cells every time they divide. Some of our tissues, like skin, are constantly being renewed, with dying cells being replaced by new ones. For a tissue to regenerate like this, or an embryo to grow, its cells must divide. And when one of our cells divides to give rise to two, the membrane that surrounds the cell's nucleus – the nuclear envelope – has to be broken down, the chromosomes dragged apart, and the nuclear envelope rebuilt.

Iain Mattaj, Director General of EMBL, has long been interested in how the nuclear envelope is put back together at the end of cell division. To investigate the process, Claudio Asencio and Mátyás Gorjánác in Iain's group scanned the worm *Caenorhabditis elegans* for proteins that might drive the reassembly

process. Among the proteins identified in the screen was a new discovery, which seemed to be equivalent to a human protein known as Lem4. Together with fellow group member Iain Davidson, Claudio and Mátyás found that, although the sequences of amino acids that make up the two proteins are quite different, the worm and human versions of Lem4 both seem to carry out the same task inside the cell.

For the nuclear envelope to reassemble at the end of cell division, chemical tags called phosphates must be removed from a protein called BAF. Whether a protein like BAF has phosphate tags attached or not is known as its phosphorylation state, and the cell has different sets of molecular machinery at its disposal to alter that state. There are proteins that add phosphate and proteins that remove phosphate and the EMBL scientists discovered that Lem4 controls both sets of cellular machinery. Like a policeman stopping one lane of traffic and waving another through, Lem4 stops one protein from adding phosphate tags to BAF and brings in another protein to remove the tags already attached.

"Since this happens in both human cells and in the worm *C. elegans*, it seems to be a strategy which evolved long ago," says Iain. He and his group suspect that this tactic – having a single molecule that prevents tags being added and simultaneously promotes their removal – could be employed in the many cellular processes that involve phosphate tags, such as cell growth and division or the relaying of signals from the environment into cells.

It would now be interesting to investigate just what prompts the dual action of Lem4 at precisely the moment when the nuclear envelope needs to be reassembled, the scientists say.

Asencio C, Davidson IF; Santarella-Mellwig R; Ly-Hartig TB; Mall M; Wallenfang MR; Mattaj IW; Gorjánác M (2012) Lem4 coordinates mitotic signaling on BAF to enable its essential function in nuclear envelope reassembly. *Cell*, in press

Life in sharp focus

Researchers at EMBL Hamburg have established a new record for the most detailed picture of a biological molecule. The development pushes the technological boundaries of structural biology and paves the way for helping biologists build a better understanding of the relationship between a molecule's structure and its function. "Hamburg has really pushed the limits of macromolecular crystallography," says Victor Lamzin, whose team performed the work at the outstation.

Victor's team wanted to see whether they could increase the level of detail, or resolution, of the structures of proteins. Increasing the resolution would allow scientists to study the arrangement of subatomic particles called electrons in a protein in more detail. Electrons are the key players in chemical reactions, which take place when atoms or compounds give, take or share electrons with each other. Electrons also carry a negative electric charge, and the way they are distributed across a protein dictates how it can stick to other molecules via a kind of static electrical attraction.

Victor and his colleagues made the breakthrough by studying the plant toxin crambin, using a technique called X-ray crystallography. This involves getting a protein to form crystals and deducing its structure from the way the atoms inside it scatter X-rays. Crambin made an ideal subject because the molecules in the crystals it forms pack tightly together in a regular way. Victor's colleague Martha Teeter from Boston College in Massachusetts grew stable crystals, which allowed the strongest X-ray scatter patterns ever to be measured.

"Having a detailed picture of how the electrons in a protein are behaving would give biologists valuable new insights into exactly how it interacts with other proteins, for example, how it might speed up or slow down chemical re-



actions in the cell, or how it binds to potential new drugs. We wanted to explore," says Victor.

This exploration was made possible thanks to a beamline built on the former PETRA II synchrotron ring at Hamburg by Victor's colleague and DESY director Edgar Wecker. The high intensity and short wavelength of the X-rays on this beamline, dubbed PETRA 1, allowed the team to resolve points in the structure a mere 0.48 Ångstroms, or just under 20 billionths of a metre, apart. To give an idea of scale, this is about one million times thinner than the width of a human hair and it allowed the team to determine the structure to a sharper level than any done before.

Pushing back boundaries, however, is never easy. One issue is that the computing software used to interpret the X-ray data is not yet able to fully resolve the finest details of the structures. Victor's team has placed their data in a publicly available database in the hope that it will stimulate developers to create new software. Another challenge is that the detectors used to sense the scattered X-rays are only just able to sense the short wavelengths used in Victor's experiments, meaning the team had to expose the crystals to damaging radiation for long periods. Nonetheless, the team plans to move forward with the work. "The experiments were not straightforward," says Victor, "but I am very glad to have accomplished it."

The Lamzin team obtained the sharpest look ever at a biological molecule.

Schmidt A, Teeter M, Weckert E, Lamzin VS (2011) Crystal structure of small protein crambin at 0.48 Å resolution. *Acta Crystallogr Sect F Struct Biol Cryst Commun.* **67**: 424-8

Treasure islands

“This is one of the frontiers in modern biology.”

Cursed by Zeus for his crew's sacrilege, the Greek hero Odysseus was shipwrecked on the mythical island of Ogygia on his long voyage home from the Trojan war. There, seduced by the island's nymph Calypso, Odysseus remained distracted from his true destination for seven long years, until the Gods intervened and set him back on course. In a strange case of life mim-

icking art, Matthias Hentze and his team at EMBL Heidelberg have discovered the biological equivalent of Calypso's island hidden in about half of all our genes. These “islands” are key to fine-tuning the production of proteins in our cells and are also implicated in a number of serious diseases including cancer and psychiatric illnesses.

The process they help to control is called translation, which is the final step in turning the information encoded in genes into proteins. To do this, a cell first makes a copy of a

gene using a molecule called RNA. The next step is translation, in which complex molecular machines called ribosomes read along the strand of RNA, decoding its instructions for assembling a protein. Only the middle part of the RNA, known as the main open reading frame, or ORF, codes for the protein. At either end are control regions containing instructions for when and where the protein should be made. These control regions are now the focus of intense research as scientists try to uncover their roles in health and disease. “We still have a rather incomplete understanding of them,” says Jan Medenbach, a postdoc in Matthias' lab. “This is one of the frontiers in modern biology.”

Like Odysseus, Jan came upon these “islands” thanks to a twist of fate. He was studying how the translation of a fruit fly protein called msl-2 was regulated. He focused on one of the control regions of its RNA and was surprised to find that it contained a short island-like stretch that appeared to code for protein. Such



Jan Medenbach and
Matthias Hentze

stretches, called upstream ORFs (uORFs), had previously been seen in other RNAs. But unlike other uORFs, the msl-2 uORF didn't require the ribosome to read it all the way through for it to work. "This told me I was working on a completely novel mechanism," says Jan.

Further experiments revealed that when a protein called SXL binds close to the msl-2 uORF, it stops the cell from producing msl-2 protein. It does this partly by distracting the ribosome away from its "true" destination of the main ORF and confining it to the uORF, the genomic equivalent of the island of Ogygia and from where SXL then appears to dispel it into the depths of the ocean.

There are still many questions to answer, says Matthias, and his team believes that this mechanism may be at work in genes in many other species, including humans. A number of mutations in uORFs are associated with human diseases, including psychiatric conditions such as schizophrenia. "We think there

is a broad applicability in biology for controlling whether a protein is made or not in this way, which is why we are so excited about it," says Jan. Doubtless there remain more gems to be unearthed in our genomic Ogygias.

Medenbach J, Seiler M, Hentze MW (2011)
Translational Control via Protein-Regulated Upstream
Open Reading Frames. *Cell* **145**: 902-13

Remote control for genes

The human genome contains some 26 000 genes. Yet these are not all switched on in every part of the body, all of the time. Instead, their activity is tightly choreographed by many so-called regulatory regions of the genome, which act as remote controls for when and where a given gene should be turned on or off.

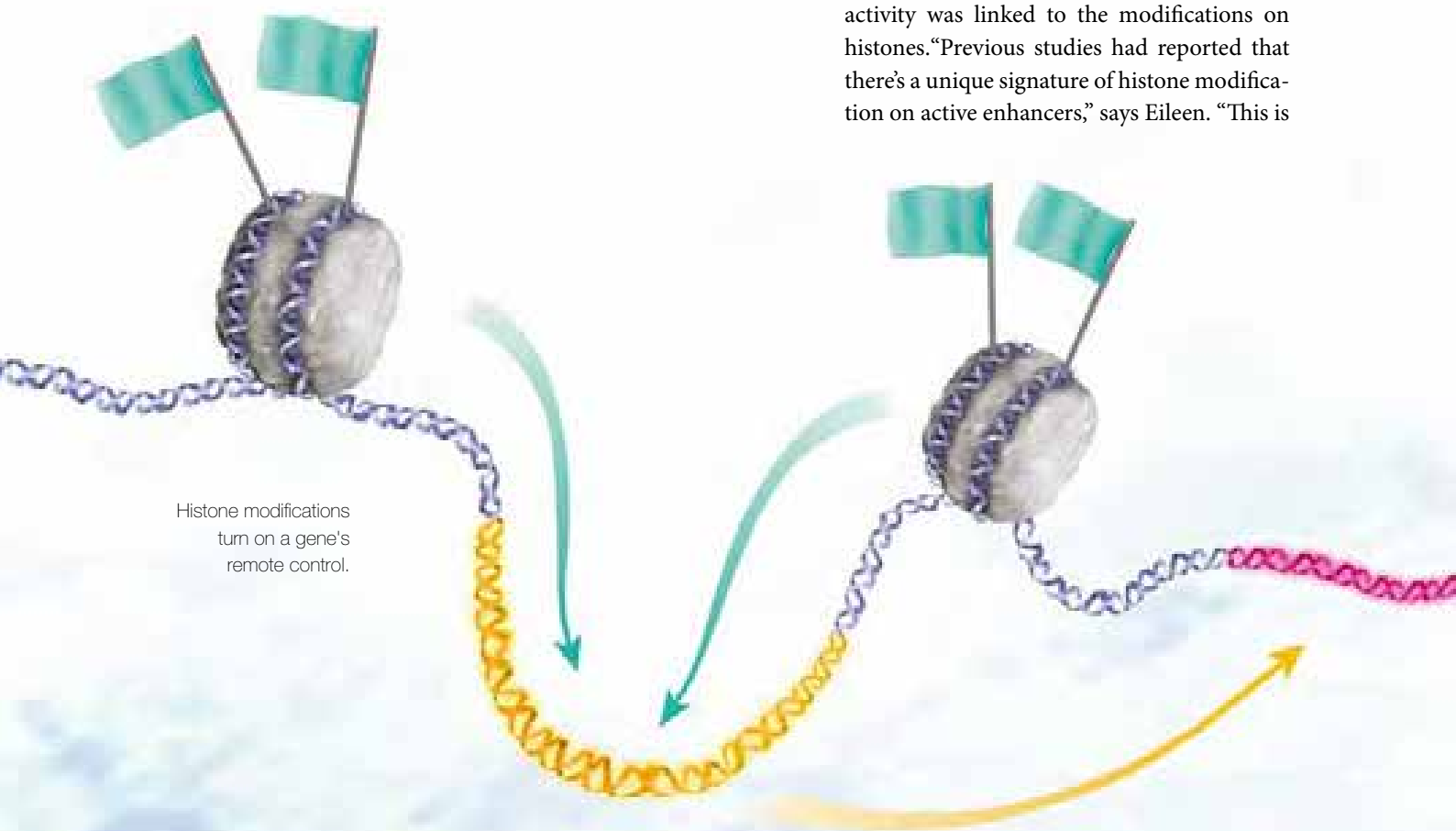
In the 1980s, biologists discovered a new type of regulatory region dubbed enhancers. These are stretches of DNA that can be recognised by proteins called transcription factors that, when bound to an enhancer, switch a gene on. However, recent research from the group of Eileen Furlong, Head of the Genome Biology Unit in Heidelberg, has shown that transcription-factor binding is not the only sign that an enhancer is active.

These new studies focused on proteins called histones that act like spools around which DNA can be wound, packaging the cell's genetic material into a more compact, manageable state known as chromatin. Histones,

however, do more than just help store DNA — they also regulate the function of the DNA to which they are attached, such as enhancers.

By attaching and removing chemical tags called methyl and acetyl groups from histones, cells can influence when enhancers turn genes on or off. To explore how histone modifications affect enhancer activity, Eileen and colleagues developed a new method for extracting histones and the DNA they are attached to from specific tissues within a complex multicellular embryo — in this case, cells that give rise to muscle in the fruit fly *Drosophila*. Together with Andrew Ridell and Alexis Gonzalez in the Flow Cytometry Core Facility, Eileen's group modified a technique called fluorescence-activated cell sorting (FACS; see box) to pick out the nuclei of the cells they were interested in and gain access to the histones contained within.

Using this technique, the team were able to look at enhancers that control muscle-related genes, and to see whether their activity was linked to the modifications on histones. "Previous studies had reported that there's a unique signature of histone modification on active enhancers," says Eileen. "This is



Histone modifications
turn on a gene's
remote control.

GLOWING WITH THE FLOW

Looking at how cells acquire their identity during development, Eileen and her group wanted to know exactly which histone modifications they carry at a specific time in the embryo's development. An embryo's cells normally like to stay together – separating them causes a stress response that could include altered gene activity, muddying the waters. To avoid this, before the cells are isolated, the embryo can be frozen in time through a fixing technique that links cells to each other. But the downside to this approach is that because the cells are linked together, scientists can't extract the ones they are interested in without breaking them. However, Eileen's group wasn't interested in whole cells, only in the DNA and histones inside their nuclei. So the solution was to extract not the entire cells, but rather intact nuclei.

Separating the nuclei of cells that would give rise to muscle from the thousands of other nuclei in the embryo posed a new challenge to Andrew Riddell and Alexis Gonzalez in the Flow Cytometry Core Facility. They redesigned their fluorescence-activated cell sorter (FACS) to improve its resolution for sorting nuclei, instead of cells. The histones inside the nuclei of the cell type Eileen's group was interested in were labelled with a fluorescent tag, allowing the instrument to identify them and sort them into a pool of similarly fluorescing nuclei. Eileen and her team were then able to get a detailed picture of which genes and regulatory elements carried specific histone modifications, and thereby learn about gene regulation in this specific cell type during an embryo's development.

not what we found —instead, we saw diverse combinations of modifications along active enhancers. Yet despite the diversity, we could still use histone modifications as a very accurate read out of enhancer activity. This has exciting possibilities, as it means that when combined with a time-course, these signatures can reveal dynamic enhancer usage driving developmental progression.”

These findings raised the possibility that patterns of histone modifications on enhancers could be used to *predict* whether an enhancer is active or not. So Eileen and colleagues fed their data into a specially designed computer program capable of learning about the links between histone modification and enhancer activity, and then presented it with histone/enhancer combinations it hadn't encountered before. In almost 90% of cases, the program correctly identified enhancers that were active in the predicted cell type and at the appropriate stage of development.

This adds to the group's previous research showing that an enhancer's spatial and temporal activity can be predicted on the basis of whether transcription factors are bound. “We can now predict whether an enhancer is active or not by looking at these histone modifications as well,” says Eileen. The next challenge is to merge insights into histone modification and enhancer activity with emerging details on how other gene-regulatory elements, such as promoters, orchestrate the intricate patterns of gene expression that underlie the development of complex organisms.

Tissue-specific analysis of chromatin state identifies temporal signatures of enhancer activity during embryonic development

Bonn S, Zinzen RP, Girardot C, Gustafson EH, Gonzalez AP, Delhomme N, Ghavi-Helm Y, Wilczynski B, Riddell A and Furlong EEM (2012) Tissue specific analysis of chromatin state reveals temporal signatures of enhancer activity during embryonic development. *Nature Genetics* **44**: 148-165





Let's stretch

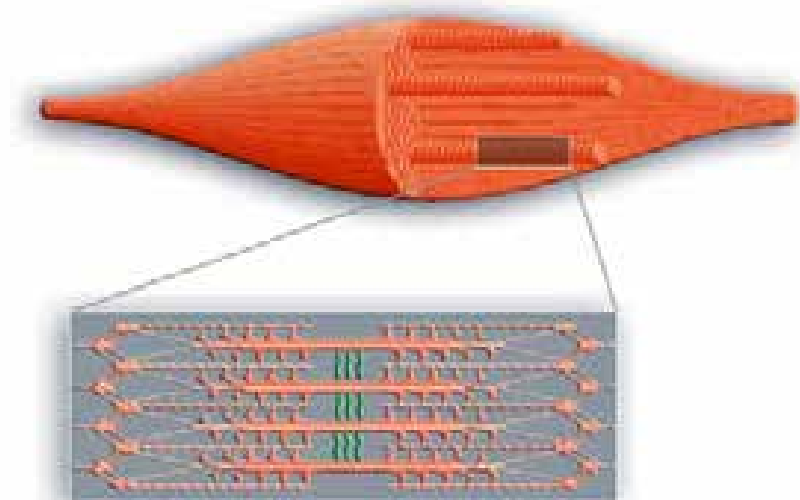
San Francisco's Golden Gate Bridge is hailed as one of the seven wonders of the modern world. At more than two and a half kilometres long, its structure and the mechanical properties of its materials enable the bridge to withstand the titanic forces that act upon it. Movable gaps called expansion joints, for example, allow the bridge to expand by more than a metre in length in warm weather, without buckling under the strain.

But you don't have to travel all the way to San Francisco to witness such a miracle of mechanical engineering. Matthias Wilmanns and his team at EMBL Hamburg have uncovered new insights into how the cells of our muscles employ similar engineering strategies. They have deduced the structure of part of a muscle cell's equivalent of an expansion joint, which it uses to stay intact and elastic under physical stress. As well as increasing our understanding of how the body works, such research also promises to shed new light on muscle wasting diseases such as muscular dystrophy and on heart disease caused by defective heart muscle contractions.

The next time you raise a mug of coffee or a tankard of beer, look down at your biceps and watch them flex. Inside your muscle, millions of cells are contracting to exert the force needed to lift your mug or glass. Now remember how tiny these cells are and how big and heavy your glass is by comparison. Imagine the stresses and strains these cells are experiencing, and how they keep experiencing them day in, day out, for years on end. Suddenly, the stress on the Golden Gate Bridge seems to pale in comparison.

Under an electron microscope, muscle cells appear to be made up of a series of stripy blocks called sarcomeres. At either end of the sarcomere are stiff disc-shaped structures that anchor thick cable-like molecules, which extend towards the middle of the sarcomere. These molecular cables, consisting of proteins called actin and myosin, ratchet past each other to make a muscle contract. Running down the middle of the sarcomere is a structure called the M-line. Its function is similar to that of an expansion joint, as it helps the sarcomere to absorb the stresses created during muscle contraction.

"A fundamental property of the sarcomere is to be elastic and respond to external forces," says Matthias. "I became fascinated by the molecular mechanisms behind this elasticity." So Matthias and his colleagues turned their attention to myomesin, a protein found in the M-line. Researchers knew that myomesin binds to many other sarcomere proteins, but its role in muscle elasticity was somewhat mysterious. Myomesin's main job seems to be as a bridge both between muscle filaments and between these filaments and important structural proteins in the sarcomere. Although myomesin's flexibility is good news for cells, it makes life difficult for structural biologists. When researchers want to study a protein's structure, they often use a technique called X-ray crystallography, which involves forming crystals of a protein. By shining X-rays on the crystals, scientists can deduce the structure of the protein from the way its atoms



Myomesin (green) helps make your muscles stronger than the Golden Gate Bridge.

scatter the X-rays. But if a protein is large, flexible or – as in myomesin’s case – both, producing crystals is not easy. What’s more, an X-ray scatter picture of a crystal doesn’t let scientists see how a flexible protein naturally bends.

So instead of trying to crystallise the whole protein, Matthias’s team studied its tail end by breaking it into pieces. “It was a divide

and conquer approach,” he recalls. The X-rays revealed that the tail comprises two intertwining myomesin filaments. Each filament is made up of a string of globule-shaped structures linked together by thin, spring-like protein helices. In collaboration with scientists at the Institute of Cancer Research in the UK, Matthias’ team double-checked their findings with an electron microscope, which confirmed they had the overall shape of the myomesin tails correct.

With the help of Hamburg colleague Dmitri Svergun, they also checked the structure using a technique called small angle X-ray scattering, or SAXS. This lets scientists study proteins in solution rather than crystals, and yields low-resolution, but more life-like, information about a molecule’s shape. So as well as confirming myomesin’s overall structure, SAXS allowed the team to gauge how flexible the molecule might be. “We are unique in having the opportunity to undertake such cross-disciplinary collaborations here at Hamburg,” says Matthias.

The results suggested a mechanism in which the helical protein springs of myomesin are responsible for its flexibility. To find out more, Matthias’s team paired up with colleagues at the Technical University of Munich to use a device called an atomic force microscope to physically pull on individual myomesin molecules to see how stretchy they are. It turns out that myomesin tails can stretch to two and a half times their original length and then rebound back to normal when the strain is released. By studying the mechanics of the stretching, Matthias and his team were able to confirm that this remarkable elasticity was down to the helices uncoiling and then springing back to their original shape. “It was nice to see the predictions confirmed with atomic force microscopy,” says Matthias.

This is the first time a helical-shaped link has been seen in a protein, says Matthias. Normally, helices are packed into more complex protein structures called domains, but in myomesin they are free. “From the structure, we now have a clue about the molecular origin of the elasticity,” says Matthias, “but the next step is to look at its function in living cells.” The team hopes to do this by studying mutations that affect myomesin, including those already associated with heart muscle problems. The sarcomere is not as vast or as grand as the Golden Gate Bridge, but perhaps it is time to start thinking of it and the muscle proteins it contains as being among the greatest engineering wonders of the world.

Pinotsis N, Chatziefthimiou SD, Berkemeier F, Beuron F, Mavridis IM, Konarev PV, Svergun DI, Morris E, Rief M, Wilmanns M (2012) Superhelical architecture of the myosin filament-linking protein myomesin with unusual elastic properties. *PLoS Biol* **10**: e1001261

ONLINE EXTRA:

Watch myomesin stretch to 2.5 times its original size: <http://youtu.be/hkkahEONoxQ>

Setting the standard

Enormous quantities of data about the biological properties of medicines, pesticides and food additives are produced every day by commercial and academic research groups. But only a fraction of this information is made available to the research community. The result is needless repetition of work that wastes time, energy and resources.

A deeper understanding of what makes successful drugs work can be gained by pooling this vast volume of 'lost' information about bioactive molecules. But it's not a straightforward process: to analyse these data properly, they need to be available and comparable. Crucial data are often missing from the published literature or are reported in an unstructured format. In other words, progress has been hampered by missing and messy data.

In 2011, considerable headway was gained when EMBL-EBI's Industry Programme brought together pharmaceutical companies, public and commercial data providers and academic groups to agree on the basics for describing the effect of a compound on a biological entity. The Minimum Information about a Bioactive Entity (MIABE) standard is paving the way for the effective interchange of data on drug-discovery success and attrition.

"Considering all the restructuring going on in industry and the growing interest in drug-discovery research in the academic sector, data availability has become a major limiting factor," explains John Overington, head of the Chemogenomics team (ChEMBL) at EMBL-EBI. "Information is missing about what compounds are active, what they are active against and who is working on them. One of the great benefits of the MIABE standard is that it gives

us an accessible, consistent and standard format that everyone can use, so everyone can play by the same rules in terms of data provision."

"One of the exciting things about this standard is that it was developed by key players in many areas of biology – not just chemistry," says Sandra Orchard of EMBL-EBI's Proteomics Services team. "It makes it easier to combine data, for example on small molecules and biological pathways. This gives a substantially higher return on the investments made in this work – in terms of both effort and funding."

Creating a standard is all very well, but will people use it?

"I'm absolutely sure it will be adopted very quickly because it's the right standard at the right time," states Christoph Steinbeck, head of Cheminformatics and Metabolism at EMBL-EBI. "This type of data is produced everywhere, in open as well as closed environments. It's become absolutely necessary to channel this information into our public databases so that people can use it."

"One of the strongest potential future applications of MIABE is in the output of EU OpenScreen, a pan-European platform for screening small molecules to discover leads for new drugs against interesting targets," adds John. "We hope and trust that similar screening initiatives in the US will again embrace these standards with the same vigour and enthusiasm that we have here."

Orchard S et al (2011) Minimum Information About a Bioactive Entity (MIABE). *Nat Rev Drug Discov* **10**: 661-669

EMINENT ELECTIONS

Funding curiosity-driven research with scientific excellence as the sole criterion, European Research Council (ERC) grants come as a breath of fresh air to many top scientists looking for resources to pursue innovative research projects. Recently nine EMBL scientists were awarded such a prestigious ERC grant to further their research. Six received a 'Starting Grant' aimed at the most promising young talents and three of EMBL's established group leaders were awarded an 'Advanced Grant'. The scientists work in areas as diverse as evolutionary biology, oncogenesis, and neurobiology, but all share a strong focus on interdisciplinarity, combining data from molecular genetics, live imaging, modelling and other techniques to achieve more meaningful results.

The 'frontier research' supported by ERC grants holds the promise of fascinating discoveries, as well as laying strong foundations for building Europe's knowledge-based economy. Such scientific pursuits might, in the short term, have few practical applications, but in the long term they hold the potential to bring about revolutionary scientific discoveries.

Launched in 2007 in an effort by the European Commission to retain the best basic science researchers in Europe, ERC grants have since supported more than 2500 projects. Proposals are peer reviewed and successful candidates can receive up to €2.5 million over five years, depending on their seniority.

DETLEV ARENDT – HEIDELBERG, ADVANCED GRANT

Research interests: origin and evolution of the central nervous system in vertebrate and insects' brains

Platynereis dumerilii is a marine worm considered to be a living fossil, because it has changed very little in the past six million years, and is therefore thought to be very similar to the common evolutionary ancestor of vertebrates and insects. Detlev's BrainEvoDevo project aims to describe the neuronal map of this worm's brain, and to dissect its chemo-sensory circuits using the whole molecular biology, cellular biology, and genetics arsenal of techniques. Results are expected to give researchers an unprecedented insight into brain development by unveiling a simple evolutionary blueprint for the olfactory circuits of more developed animals such as mice and flies.

PEER BORK – HEIDELBERG, ADVANCED GRANT

Research interests: towards understanding the function and evolution of biological systems by comparative and integrative data analysis

The human intestinal system holds approximately 2 kg of bacteria that may have a much more important role than just digesting the food we eat. The Cancerbiome project will look deeply into their potential relationship with cervical cancer, oral squamous cell carcinoma, and colorectal cancer. Using only non-invasive samples (vaginal, oral and faecal), Peer will assess whether cancer patients have distinctive bacterial populations in their gut. These results might lead to totally new markers for cancer and cancer progression, and fundamental knowledge about the relationships between cancer cells and their environment.

MARCUS HEISLER – HEIDELBERG

Research interests: plant development

Depending on where they are placed in developing plant organs, cells express different sets of genes that specify their dorsal (top) or ventral (bottom) identity. By using cutting-edge techniques to image growing plant tissues, in combination with powerful perturbation tools, Marcus aims to describe and understand the complexity of dorso-ventral patterning and, as a consequence, the mechanisms by which these cell types influence plant morphogenesis. How are the boundaries between these cell types positioned? How do these boundaries regulate cell polarity patterns and growth? What is their involvement in wound repair and how are they influenced by the plant hormone auxin?

MATTHIAS HENTZE – HEIDELBERG, ADVANCED GRANT

Research interests: Discovering hidden connections between nature and nurture

One of the biggest mysteries in biology is how genes and metabolism communicate with each other. Matthias has discovered that some enzymes, the workhorses of metabolism, have a “secret night job” regulating gene expression. His research aims to determine whether enzymes could be the hidden connection between genes and metabolism. In the current context of exploding metabolic diseases, this project might have very large consequences in the long run

TAKASHI HIIRAGI – HEIDELBERG

Research interests: embryo development in mammals

At a very early stage in embryonic development, cells in the blastocyte start differentiating along different axes, thus defining the front, back, left, right and other directions of the future organism. Although fundamental, the mechanisms involved are largely unexplained. Takashi studies them from the molecular level up to the embryonic level using techniques from molecular genetics, live imaging, cell physics, and modelling. He aims to identify the parameters that induce the fundamental break of symmetry, as well as to understand the mechanisms leading to the establishment of the first cell lineage and, subsequently, to the embryo’s development.

FRANCESCA PERI – HEIDELBERG

Research interests: neuron-microglia interactions

Microglia are specialised phagocytes that clear the brain of dying neurons to prevent the diffusion of damaging degradation products. As these cells have crucial roles in many neuronal diseases, there is a strong need to better understand their close relationship with neurons. Francesca uses the transparent zebrafish embryo as an *in vivo* model in which to apply genetic engineering and quantitative imaging techniques to determine how microglia are attracted both in space and time to apoptotic, injured and sick neurons.

RAMESH PILLAI – GRENOBLE

Research interests: piRNA biogenesis and function

piRNAs are small non-coding RNAs that silence genes that cause instability in germline cells. Little is known about their origin or the extent of their role in living organisms, so Ramesh has taken up the task of filling this gap. Using complex purification techniques he aims to identify the various factors involved in the genesis of piRNA, and to define the regions of the genome from which they originate. The study of how piRNAs assemble and function *in vivo* will rely mostly on live-cell imaging techniques.

CHRISTIANE SCHAFFITZEL – GRENOBLE

Research interests: ribosome-protein complexes in protein targeting and mRNA quality control

Complexes formed by ribosomes and proteins determine the fate of the various proteins in a cell by ensuring the integrity of the mRNA and that newly synthesised proteins are transported to their correct localisation in the cell. Despite the crucial role of these complexes, their molecular mechanism is still not fully understood. For example, the decay of messenger RNA with a premature stop codon during protein synthesis is one of these processes, as is the targeting and folding of membrane proteins. Christiane Schaffitzel will tackle these questions using molecular biology, biochemistry, and cryo-electron microscopy.

ROCIO SOTILLO – MONTEROTONDO

Research interests: cancer initiation and chromosome instability

Using genetics and 3D cell culture systems, Rocio tries to explain the mechanisms leading to chromosomal instability, and the connections between this instability and tumour initiation, relapse and suppression. What comes first: chromosomal instability or the tumour? And how do they influence each other? Answering such fundamental questions might have therapeutic consequences in the future as drugs targeting various stages of cell division are already being developed as part of the fight against cancer.

Stephen Cusack, Thomas Lunardi,
Eva Kowalinski, Andrew McCarthy



The cell's guard dogs

If you want an alarm system to protect your house, there's nothing quite like a guard dog. Sleeping most of the day quietly in the corner, its sensitive ears and nose can pick up the first signs of a dangerous intruder and its hysterical barking can rouse an entire neighbourhood – perhaps even seeing off a burglar before anything is even stolen. It may sound odd, but the cells of your body have an army of guard dogs of their own, all able to sniff out the first signs of any invading viruses or bacteria. Now Stephen Cusack and his team at EMBL Grenoble have determined the structure of one of these molecular “guard dogs” and have provided important new insights into how this little-known division of our immune system raises the alarm.

When people speak of the immune system, they usually think of white blood cells and antibodies, which are highly specific cells and proteins that home in exclusively on one particular component of a bacterium or virus. This part of the immune system is called the adaptive immune system, which not only clears the host of pathogens but also forms a “memory” of a particular disease-causing organism. The next time the host encounters the same invader it can mount a swifter response, a feature exploited by vaccines. The adaptive immune system is a relatively recent evolutionary innovation found only in animals with a backbone.

There is, however, another far more ancient arm of the immune system called innate immunity. Unlike adaptive immunity, the innate immune system is less discerning, and reacts to general classes of microbial invaders rather than individual species of bacteria or viruses. Although innate immunity is less specific, it triggers an immediate response to infection as well as stimulating the adaptive immune response, which can take several days to lumber into action. “The innate immune system is critical in viral infections, many of which remain subclinical because they are dealt with at this very early stage,” explains Stephen. His group has long been interested in the influenza virus, particularly in how it copies its genome to make more viruses. Unlike our genome, which is made of DNA, influenza's genome is made of a closely related nucleic acid called RNA. Although our own cells also contain RNA, viral RNA often has distinctive features, notably a particular chemical modification at the end of the RNA molecule that marks it out as being alien to the cell.

If this viral RNA appears inside the cell, it is detected by a molecular guard dog called RIG-I, which raises the alarm by causing the cell to produce another protein called interferon. This summons other proteins and cells to come and interfere with viral replication, destroy infected cells and warn surrounding cells about the danger. Scientists had hypothesised that, in the absence of viral RNA, RIG-I lies dormant, but when viral RNA is present in the cell, it binds to RIG-I, changing its shape so that it can interact with other molecules that eventually trigger the production of interferon. But the nature of this shape change and how it worked was a mystery. “We really needed the atomic structure of RIG-I to clarify what was going on,” says Stephen.

To determine RIG-I's structure, Stephen's group turned to a technique called X-ray crystallography. This is a technique in which scientists form crystals of a protein and then shine powerful X-ray beams on to them. The way the atoms in the crystal scatter the X-rays reveals how they are arranged within the protein, thus enabling its three-dimensional structure to be determined. But the team immediately hit a stumbling block: the human RIG-I protein simply would not form crystals. Stephen's PhD student, Eva Kowalinski, who performed the lion's share of the work on the project, struggled with the problem for almost four years. "It was very tough going," says Stephen "and for a period we gave up."

Eventually Eva and Stephen came up with the idea of using avian RIG-I from the mallard duck: as birds have a higher body temperature, they suspected this protein might be more thermally stable. Proteins with higher thermal stabilities form crystals more readily, so the duck RIG-I offered a potential solution to the problem. José Márquez and his team of the Grenoble outstation high-throughput crystallisation platform, which systematically measures the thermal stability of all proteins submitted for crystallisation, confirmed Stephen and Eva's hunch (p. 12). Their gamble paid off, as Eva could crystallise both the 'dormant' duck RIG-I as well the activated form with bound RNA. From then on, the project moved very quickly. Technician Thomas Lunardi helped to produce large quantities of the protein, whereas EMBL team leader Andrew McCarthy tested many crystals on the ID14 EH4 beamline at the nearby European Synchrotron Radiation Facility (ESRF) to find the ones that gave the best quality data. Finally the structures were solved, giving the first snapshots of RIG-I in both the inactive "sleeping" and the activated conformation. The different atomic structures obtained by Stephen's group fitted with other published results like pieces of a puzzle to give a complete picture of how the protein "guard dog" is woken by a viral RNA intruder.

The structure revealed that the areas, or domains, of RIG-I that sound the alarm are normally locked inside the main body of the protein, a bit like a guard dog being locked inside its kennel. When viral RNA is present in the cell, it binds to another RIG-I domain – the "door" of the kennel – and unlocks it. This releases the guard dog domain, on the end of a chain-like tether, so that it can bind to other molecules. Studies in living cells in the laboratory of collaborator Denis Gerlier at Lyon University confirmed the results and also showed that the knowledge gained on duck RIG-I is transferrable to humans.

"Although the details of this mechanism are likely to be unique to RIG-I, the general principle of keeping a molecular guard dog kennelled until released by the presence of a foreign molecular intruder almost certainly applies to other receptors of the innate immune system," says Stephen. The work could also offer useful insights into the tricks viruses have evolved to evade cellular alarm systems like RIG-I. Some influenza viruses, for example, produce a protein that can block the cell's ability to trigger interferon signalling. "This is part of the constant molecular warfare that goes on between viruses and host," says Stephen.

Insights such as this could help researchers find ways to artificially stimulate the innate immune system, or to design vaccines that are better able to stimulate a lasting response from the adaptive immune system. There's an old proverb that says the dog is a man's best friend: this applies to the watchdogs of our cells as well.

Kowalinski E, Lunardi T, McCarthy AA, Louber J, Brunel J, Grigorov B, Gerlier D, Cusack S (2011) Structural basis for the activation of innate immune pattern recognition receptor RIG-I by viral RNA. *Cell* **147**: 423-435

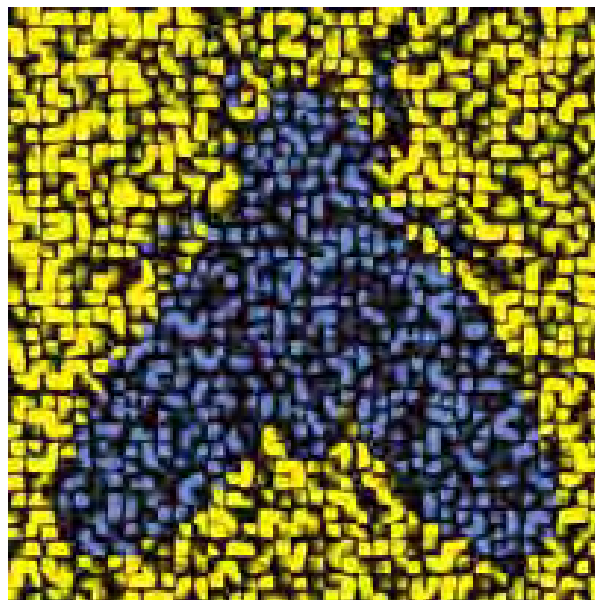
Genes are social too

Two-and-a-half thousand years ago Aristotle argued that humans are quintessentially social beings, a view vividly confirmed by modern social networking sites like Facebook and Google+. Genes are much the same as, like people, they function in complex social networks with their peers. In other words, most traits, from height to disease susceptibility, are governed by the interaction of many genes, and most genes have effects on more than one trait — just as many human endeavours are undertaken by social networks of people, and individuals take part in many social circles.

This complexity makes it difficult to work out how an individual's genetic makeup (or genotype) maps on to their physical and behavioural characteristics (phenotype) — a challenge complicated by the fact that variations in interacting genes sometimes amplify each other's effects, and in other cases cancel each other out. To date, the interactions between genes have often been worked out on a case-by-case basis, but ideally biologists would like to be able to study patterns of genetic interaction across entire genomes.

In research published in *Nature Methods*, Wolfgang Huber and colleagues at EMBL Heidelberg together with Michael Boutros from the German Cancer Research Centre (DKFZ) have described a new method for studying such genetic interactions on a large scale. "This technique enables us to gain new insights into the underlying biology of how genotype leads to phenotype," says Wolfgang.

The approach developed by Wolfgang and colleagues draws on a tool called RNA interference (RNAi), which enables researchers to silence specific genes. The researchers began with 93 genes known to be involved in communicating biological signals within cells of the fruit fly *Drosophila*, and developed RNAi molecules that would inhibit each of them.



These genes were first silenced one by one to examine their individual effects on the phenotypes of *Drosophila* cells, such as their growth rate or size. Next, the team inhibited every pairwise combination of these genes (A and B, A and C and so on), and observed the phenotypic consequences. Finally, the effects of silencing these 93 genes individually and in pairs were compared so that interactions could be spotted, according to a simple logic: if the effect of shutting down two genes simultaneously was greater (or less) than the sum of their individual effects, this signalled an interaction.

Overall, Wolfgang and colleagues identified more than 600 interactions that affected *Drosophila* cell phenotypes, which led to novel biological insights — for example, they identified a new component in the Ras cell-signalling network, which is known to regulate cellular proliferation and often goes awry in tumour cells.

Wolfgang and colleagues also borrowed a technique from social network analysis to identify genes that function in related pathways. If you have 500 friends on Facebook, and 50 of these are also friends with someone else, Facebook may suggest this person as a friend, as it looks like you're both part of a common social circle. The same logic can be applied to genetic networks. "Even if two genes do not directly interact with each other, if they have overlapping interaction profiles they tend to work together in a biological process," says Wolfgang.

Tell me who a gene's friends are and I'll tell you what it does.

Horn T, Sandmann T, Fischer B, Axelsson E, Huber W, Boutros M (2011) Mapping of Signalling Networks through Synthetic Genetic Interaction Analysis by RNAi. *Nat Methods* 8: 341-6



Sloppy fishing

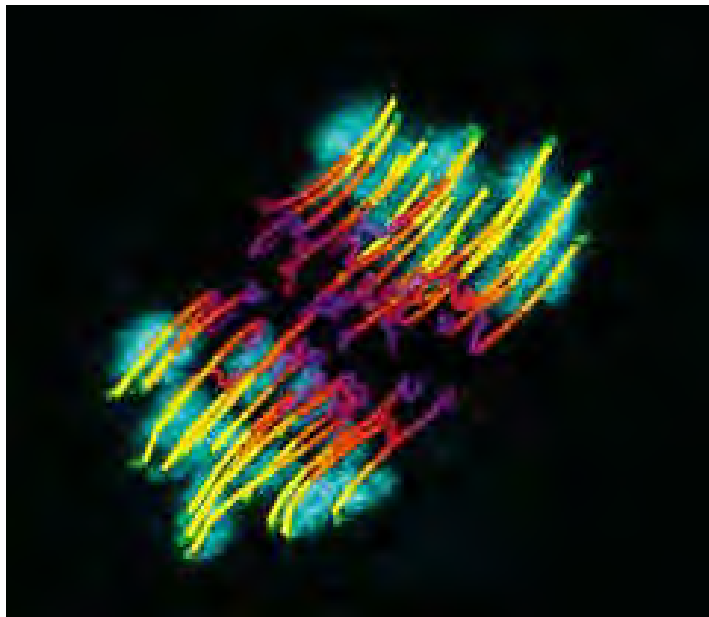
At a village fair, between the bumper-cars and the candyfloss, a child's forehead creases in concentration. She's doing her best to snag a wooden fish out of a plastic pond, with the magnet on the end of her fishing rod. Freeze the scene, rewind all the way back through the child's development, past the point when the sperm fertilised the egg, to when the egg cell itself was formed, and you'll find a similar fishing game in action. The difference, scientists at EMBL Heidelberg have found, is that magnet-wielding children are probably more successful fishermen than the egg cell's machinery.

As an egg cell, or oocyte, matures inside a woman's ovary, it undergoes a type of cell division called meiosis, during which the pairs of chromosomes inside it are lined up and fished apart, and half of them are expelled. The chromosomes are brought together from all over the cell (see p. 86) and fished apart by protein rods called microtubules. Like the child's rod pulling a toy fish by its magnet, a microtubule catches a chromosome by its kinetochore – a conglomerate of protein and genetic material at the centre of the chromosome's X shape. Tomoya Kitajima, or Tomo as he is known in Jan Ellenberg's lab, was the first to track the movements of all of the kinetochores in an egg cell throughout the whole of cell division – all 10 hours of it. "We were able, for the first time, to keep track of all the kinetochores throughout cell division – so there's not a single time-point where it's ambiguous where that part of the chromosome is – and that's really a breakthrough in the field, achieving this in these very large and light-sensitive cells," says Jan, who heads the Cell Biology and Biophysics Unit at EMBL Heidelberg.

Tomo used software that had been previously developed in Jan's lab, and which allowed him to programme a laser scanning microscope to find the chromosomes in the egg cell's vast inner space, and then film them throughout cell division. "The oocyte is a big cell, but the chromosomes sit in only a small part of that cell, and that's what we were interested in. So basically we just made our microscopes smart enough that they can recognise where the chromosomes are and then zoom-in in space and time just on that region," Jan explains. By focusing the microscope only on the part of the cell that contains the chromosomes, Tomo was able to obtain high-resolution images at short, one-and-a-half minute intervals, which gave him a very clear picture of the process. And, because the microscope was only firing light at that small region of the oocyte, it did less damage to the cell, which enabled the scientists to perform the imaging for the whole 10 hours of cell division (for more on smart microscopy, see box).

Back at the fishing pond, tempers can flare and voices rise in shrill accusations: "That's cheating! You can't push the fish with your rod!" Thanks to Jan and Tomo's work, the accused could argue that, in her defence, her cells were already 'cheating' like this before she was even born. When they analysed the videos, the EMBL scientists found that before microtubules attach to kinetochores, they nudge chromosomes into a favourable position, like a child repositioning

No mean feat: tracking all kinetochores over 10 hours of cell division.



a fish with the end of her rod. The microtubules nudge the chromosome arms, arranging the chromosomes in a ring from which they can then fish them out more easily.

“But even with this pre-positioning, it still doesn’t work very well,” says Jan. “We saw that 90% of kinetochore connections were initially wrongly established, and the microtubules had to release the chromosome and try again – on average, this had to be done three times per chromosome.”

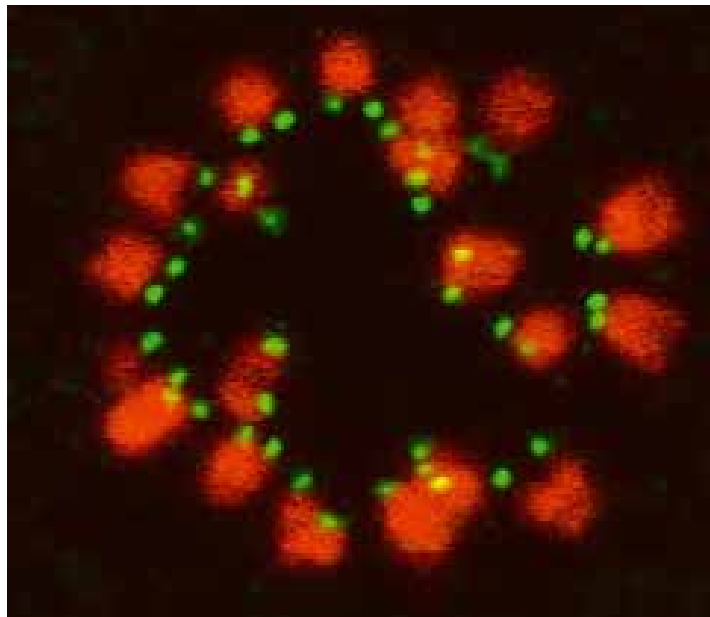
Scientists in the USA have now shown that the same ‘cheating’ also happens in the other type of cell division, which our cells undergo when we grow or when tissues like our skin regenerate. In this second type of cell division, called mitosis, a cell divides into two daughter cells each with the same amount of genetic material as the ‘mother’, instead of only half the genetic material like in meiosis. But Jan and Tomo’s findings highlight that the fishing of chromosomes

is much more inaccurate in meiosis than in mitosis. This could, the scientists believe, be due to a fundamental difference in how microtubules fish chromosomes apart in the two types of cell division. During mitosis, the microtubule rods start forming at two opposite points in the cell and come together in a lemon-shaped structure – the spindle – that then pulls each chromosome in a pair to one side, or pole. But in meiosis, Jan’s group discovered a few years ago (see AR2007/08) that the spindle’s microtubules initially converge from as many as 80 different points, and only later arrange into a two-poled structure. “So when microtubules are first attaching to chromosomes, it’s hard to know if they’re going to

end up pulling them in opposite directions or not,” Jan explains. This, along with the fact that the egg cell is a much larger expanse across which microtubules have to find and drag chromosomes – a human egg cell is more than four times larger than a skin cell – could explain why chromosome fishing is so much more error-prone in egg cell division.

These findings also provide scientists with a more concrete place to look when studying female infertility and conditions like Down Syndrome, which largely stem from egg cells with an abnormal number of chromosomes. By determining that such errors most likely occur because microtubules fail to make the right connections to fish chromosomes apart properly, Tomo and Jan have provided a focus for future studies. In fact, Tomo is now going on to study why this trial-and-error process is even more error-prone in old egg cells. If he and others can determine at what point the error-correction mechanisms fail in older cells, it could one day be a

“But even with this pre-positioning, chromosome separation still doesn’t work very well”



Chromosomes are nudged into place before being fished apart.

starting point for medical procedures to help microtubules improve their fishing technique. Perhaps the secret to countering age-related infertility is to make microtubules as successful at fishing as children are with their toy magnets.

MAKING MICROSCOPES SMARTER

The software Tomo used to find and film chromosomes throughout cell division was a prelude of things to come.

In collaboration with Rainer Pepperkok's team at the Advanced Light Microscopy Core Facility, Jan's lab has, in the meantime, developed a more complex programme, capable of even greater feats of automation. Called Micropilot, the new software analyses low-resolution images taken by a microscope and finds not just chromosomes but whatever structure the scientist has taught it to look for.

Once Micropilot has identified the cell or structure the scientists are interested in, it automatically instructs the microscope to start an experiment. This can be as simple as recording high-resolution time-lapse videos or as complex as using lasers to interfere with fluorescently tagged proteins and recording the results. The software is a boon to systems biology studies, as it generates more data, faster. Thanks to its high throughput, Micropilot can easily and quickly generate enough data to obtain statistically reliable results, allowing scientists to probe the role of hundreds of different proteins in a particular biological process.

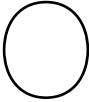
Watch it in action on the EMBL YouTube Channel: <http://youtu.be/PoHy37LAMIQ>

ONLINE EXTRA:

Watch microtubules nudge chromosomes into position on the EMBL YouTube Channel: http://youtu.be/LH9wl7_B-4g



Etch-a-sketch

 In the streets of 16th century Rome, an ageing Leonardo da Vinci could sometimes be seen studying a person with a particularly interesting face, taking note of facial proportions, the angle of the nose, the shape of the eyes. Five hundred years later and 20km away, scientists have discovered that our brains, too, employ artists' techniques as they develop. While sketching, artists like Leonardo draw guiding lines to help them measure out proportions and align features. As he fleshes out the drawing with stronger strokes, highlights and shadows, the artist erases the guidelines. Like lines on an artist's canvas, connections between brain cells are sketched out, highlighted and erased as the brain develops, Cornelius Gross from EMBL Monterotondo has discovered.

As a healthy brain develops, each neuron first reaches out to one other neuron. Later, neurons start branching out, making lots and lots of connections – or synapses – with many neurons in different parts of the brain. These connections are important to serve as guidelines for neurons to find the partners with which they will need to share information. But if all these neural links stayed in place, they'd interfere with each other. Just as a jumble of guidelines could prevent the completed work of art from shining through, a tangle of neural connections could have dire consequences for the brain's owner. So at some point in brain development, the web of criss-crossing synapses has to be thinned out, and the guidelines that are no longer needed erased, so that the crucial neural connections can be strengthened. Even after such guidelines have been removed, some neurons in the fully developed brain still end up connected to trillions of others. Half a millennium after Leonardo's famous anatomy studies, the picture of how this brain wiring is correctly established – that is, how the excess synapses are identified and removed – remains surprisingly fuzzy.

Looking at the brains of healthy newborn mice under the microscope, Rosa Paolicelli, a PhD student in Cornelius' group, noticed that cells called microglia had proteins from synapses inside them. Could these cells be the erasers that eliminated superfluous connections? Known as the brain's cleaning crew, microglia are related to the white blood cells that engulf and 'eat up' the invaders that make us ill, and scientists know that they play a crucial role in eating up dead and dying neurons (see p. 2). "But almost all studies have focused on brain injury," says Cornelius. "It was really not clear what microglia do in the healthy brain."

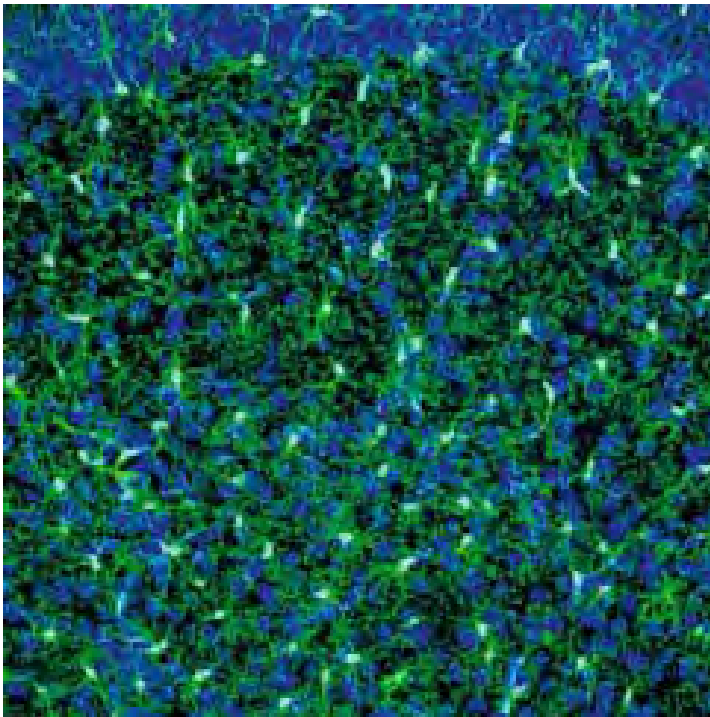
To investigate, Cornelius and Rosa teamed up with Davide Ragozzino from the University of Rome and Maurizio Giustetto and Patrizia Panzanelli from the University of Turin, to see what happened to the jumble of neuron connections if there were fewer microglia in the brain. To do so, the EMBL scientists accomplished some nifty genetic engineering. "We were able to make a very subtle change," says Rosa: "we introduced a mutation that decreased the number of microglia in the brain – but the ones that were there still looked and behaved normally."

The mutation prevented microglia from producing a molecule called the fractalkine receptor. As its name suggests, this antenna-like molecule enables microglia to detect fractalkine, a protein which neurons produce at synapses. At the start of brain development, neurons make almost no fractalkine, but they ramp up its production when the web of neural connections is being sketched out. According to the EMBL scientists' results, it seems fractalkine is a sign for microglia to enter the brain and start erasing synapses. But how do microglia know which connections to erase, and which to leave? One idea that Cornelius and Rosa are looking into is that fractalkine may also play a role in marking 'superfluous' connections for deletion. However, it doesn't function alone, so Cornelius' group is now trying to identify other 'eat me' signalling molecules that highlight synapses that need to be erased.

What the scientists do know is that when they stifled the production of the fractalkine receptor, the number of microglia in the brain dropped. And sure enough, with fewer microglia roaming

the brain, Cornelius and Rosa found many more connections between neurons. Moreover, these neural connections were immature. "This was very exciting, because it shows that microglia are crucial to get the brain's connectivity right," Cornelius says. "It was also interesting because what we saw was actually similar to what others have seen in the brains of people with autism." One explanation that has been put forward for many autistic people's heightened sensitivity to input from their senses is that the brain is getting cross-wired, possibly because it has too many neural connections. Genes usually turned on in microglia have also been linked to autism. Coupled with Cornelius and Rosa's findings, this means these eraser cells may be interesting to look at in the context of this and other conditions that involve altered brain wiring.

The microglia-reducing mutation introduced by the EMBL scientists has only temporary effects. Eventually, microglia with the mutation are



Microglia (green),
the developing
brain's erasers.

able catch up and enter the mouse's brain, connections are erased, and the normal number of synapses is established. But this happens later than normal, so the researchers would now like to know what effects this delay has on the animal's development, and whether – and how – it affects the mice's behaviour, particularly autism-related behaviours like prolonged grooming or a lack of interest in social stimuli.

Thanks to Cornelius' work, we now have some idea of what microglia do while the healthy brain is developing. Yet to be unveiled, however, is their role in the adult brain. It may be a while yet before we have a clear picture of what these cellular erasers might have been doing in Leonardo's head as he wandered the streets of Rome, sketching everything from living beings to flying machines.

Paolicelli RC et al (2011) Synaptic pruning by microglia is necessary for normal brain development. *Science* **333**: 1456-8

Visual biochemistry

A flock of starlings is surely one of nature's great live shows. Hundreds or thousands of birds come together to form what looks like an amorphous super-organism expanding, contracting, twisting and folding in the sky. The effect is mesmerising.

Sometimes, however, you may want to look at just one or two birds in such a fabulous display of aeronautical agility. A pair of binoculars is the obvious tool for the job, but they have drawbacks. If the birds are far away it may be hard to focus on individual birds and keep them in view, as the smallest hand movement can shift your field of vision dramatically. So you may need the stabilising support of a tripod and, if you're taking photos, to use a fast shutter speed to get a crisp image.

Biologists face comparable challenges in watching the dynamic flocks of molecules within living cells. At present, most techniques for doing this are based on confocal microscopy, but this has numerous limitations — for example, only isolated spots can be examined within a cell.

To overcome these limitations, Malte Wachsmuth and former group leader Michael Knop at EMBL Heidelberg set out to create a new kind of microscope for visualising the molecular swarms inside cells, and their resulting invention was described last year in *Nature Biotechnology*.

Whereas confocal microscopy typically focuses light onto a small area of a sample, the technique developed by Malte and colleagues uses a light sheet to illuminate an entire sample - a whole cell, for example. This is then viewed with a sophisticated camera that divides the sample under study into a series of tiny pixels, each of which captures an 'observation volume' a million times smaller than a millionth of a millilitre. "This is so small that it contains just a few molecules of the many thousands or millions present in the whole cell," says Malte.

Crucially, the camera in this new microscope is extremely sensitive to light and is able to



take snapshots at intervals of less than a millisecond. Tagging molecules of interest with fluorescent probes enabled Malte and colleagues to watch the moment-to-moment flux of molecules within each pixel by simultaneously measuring fluctuations in the amount of light emitted by each one. "It's a very sensitive detection method," says Malte.

The measurements obtained from each pixel can then be brought together to provide a broad picture of molecular dynamics across the whole sample — like building up a picture of how a flock of starlings behaves by assembling lots of snapshots of different parts of the flock.

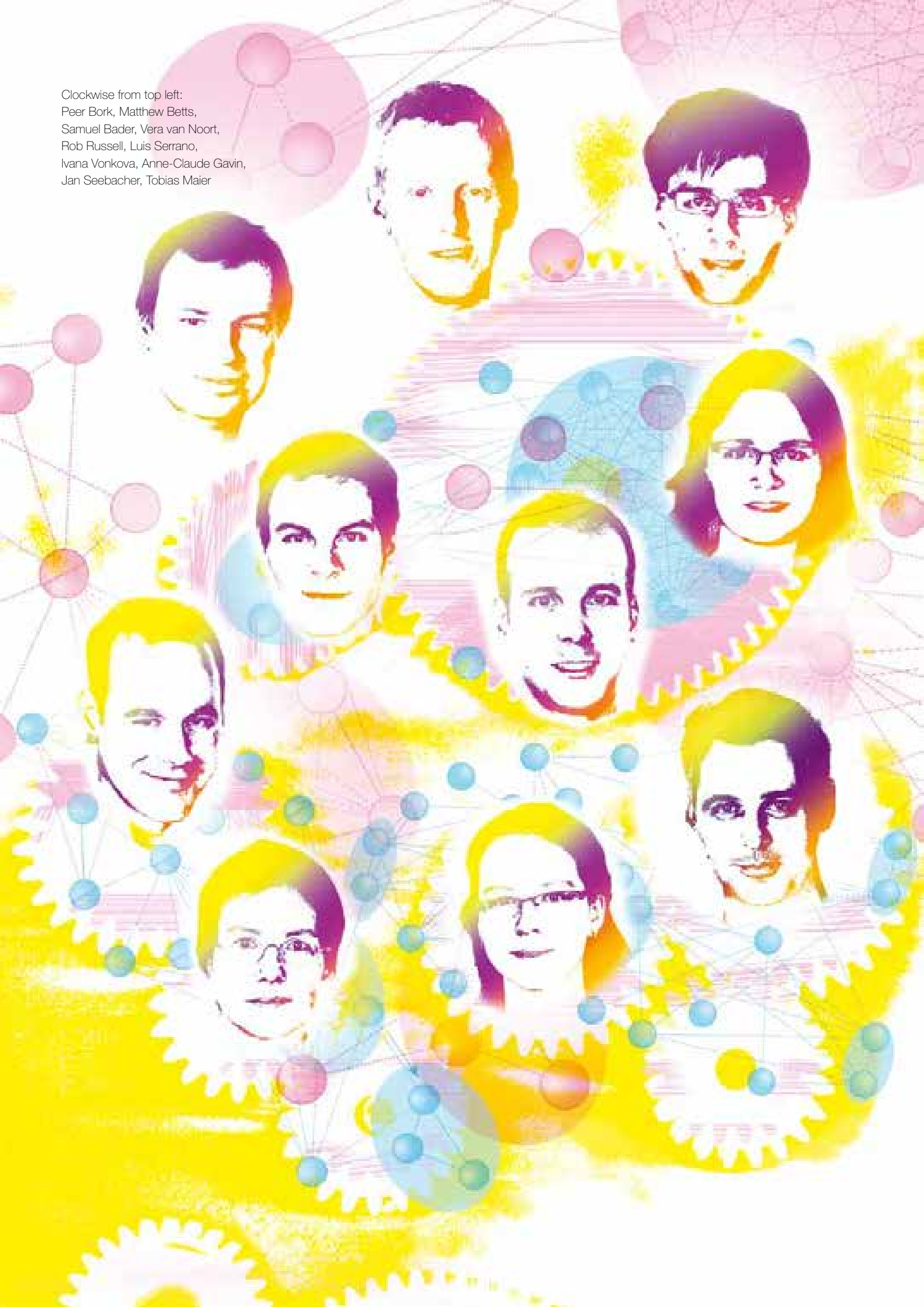
To demonstrate the power of this approach, Malte's team used it to study the dynamics of proteins that associate with DNA to form a complex called chromatin. Previous research had shown that chromatin exists in two states — tightly or loosely packed — but the new microscope revealed a previously hidden intermediate state.

In the future, the ability to visualise fast-moving molecules inside living cells will help researchers to study many biological processes, such as the signalling pathways involved in cancer, in much greater detail than ever before.

New microscope follows single molecules by the millisecond.

Capoulade J, Wachsmuth M, Hufnagel L, Knop M (2011) Quantitative fluorescence imaging of protein diffusion and interaction in living cells. *Nat Biotechnol.* **29**: 835-839

Clockwise from top left:
Peer Bork, Matthew Betts,
Samuel Bader, Vera van Noort,
Rob Russell, Luis Serrano,
Ivana Vonkova, Anne-Claude Gavin,
Jan Seebacher, Tobias Maier



The logic of life

A bug that causes pneumonia might sound like an unlikely candidate for a biological celebrity, but *Mycoplasma pneumoniae* is not your typical pathogen. With only 691 genes, this tiny bacterium has one of the smallest genomes of any free-living organism, which makes it ideal for studying the minimal set of biological building blocks required for a cell to live an independent existence.

M. pneumoniae, however, provides biologists with an opportunity to do more than just catalogue the minimal repertoire of genes required for life. Increasingly, researchers are turning to this bacterium as a model organism for teasing apart the complex genetic and biochemical networks that constitute the fundamental cellular processes of all living creatures — in short, to uncover the basic logic of life.

Much of this research falls under the rubric of a burgeoning paradigm called systems biology. Historically, biologists have tended to study single genes or the proteins they encode on a case-by-case basis. Systems biology, on the other hand, takes a more global perspective, looking at all the genes and proteins that comprise biological systems.

In recent years Anne-Claude Gavin and Peer Bork, group leaders at EMBL Heidelberg, have been applying systems biology to *M. pneumoniae*, and in 2009 were co-authors on three landmark papers published back-to-back in *Science* (see Annual Report 2009/10). These studies, involving many groups in the Structural and Computational Biology Unit, revealed unexpected levels of complexity in the biology of this miniscule microbe. Take its genes, for example. In both prokaryotes and eukaryotes, genes encode proteins in roughly the same way: the DNA is read or ‘transcribed’, creating an intermediary molecule called messenger RNA (mRNA) that serves as a template for constructing the encoded protein in a step called translation.

Despite these similarities, eukaryotes are renowned for having more complex genetic tricks up their sleeves. Many of their genes can be read in more than one way, generating variant mRNA transcripts that encode different proteins with different functions. Yet the studies reported in *Science* showed that even an organism as humble as *M. pneumoniae* uses similar tricks to make the best use of the information in its genome.

In the years since these major papers in *Science*, Anne-Claude’s and Peer’s labs have continued to collaborate with EMBL alumni Luis Serrano at the CRG in Barcelona and Rob Russell at the University of Heidelberg, to push forward systems-biology studies of *M. pneumoniae*, as exemplified by two papers published in *Molecular Systems Biology* in the past year.

The first paper had two key goals. “First we wanted to quantify how *M. pneumoniae* responds to adverse environmental conditions, such as high temperature, by changing the number of copies of individual proteins within the cell,” says Anne-Claude. “Then we set out to relate these fluctuations to changes in the number of mRNA transcripts encoding these proteins.”

Anne-Claude, Luis and colleagues found that stressors such as heat or a DNA-damaging chemical led to changes in the abundance of certain proteins: some became more abundant, whereas others became more scarce.

They also found that mRNA levels only loosely correlated with protein levels in *M. pneumoniae*, suggesting that in this bacterium, as in other more complex organisms, the regulation of protein levels depends on more than just how much mRNA the cell produces.

The team considered two means by which this regulation could be achieved: first, the bacterium could dismantle existing proteins in the cell to reduce protein levels; or second, the speed or efficiency at which mRNAs are translated into proteins could be altered. The scientists found evidence for both mechanisms in *M. pneumoniae*. For example, shocking cells with heat initially caused an increase in the number of stress proteins, but these were specifically and rapidly degraded as the cells recovered, which points to the existence of a dedicated protein-destroying system to regulate the levels of these proteins. At the same time, modifying how efficiently mRNA is translated into proteins was also found to be extremely important in regulating protein abundance — in fact, the team estimate that the influence of translation efficiency on protein abundance is 40% higher than that of protein destruction.

“The extent of these modifications suggests that they are as important in *M. pneumoniae* as they are in eukaryotes”

Anne-Claude says that these ways of regulating protein abundance complement gene silencing at the transcriptional level. “They enable rapid fine-tuning of protein composition within the cell so they can respond quickly to changing conditions.”

Altering the abundance of proteins within the cell is, however, just one means of regulating their activity. Another method is to add chemical groups to the amino acids that make up proteins in a way that affects their function and stability, and also their capacity to interact with other proteins in the cell. Two kinds of chemical modification are particularly important: adding acetyl groups to the amino acid lysine, and adding phosphate groups to a variety of amino acids. Some scientists have postulated that the addition of diverse chemical groups to proteins constitutes a ‘protein modification code’, in which different patterns of modifications on the same molecule ‘encode’ different functions for that protein — enabling the cell to generate a greater diversity of protein function without the need for additional genes. “It’s another way to create biological complexity with a limited number of building blocks, and just the kind of trick that a bacterium with few protein-coding genes might take advantage of,” says Anne-Claude.

In the second paper in *Molecular Systems Biology*, Anne-Claude, Peer and colleagues set out to measure phosphorylation and lysine acetylation in *M. pneumoniae*, and to search for links between these two protein-regulation tactics. Both kinds of chemical tags are known to be important for controlling protein activity in eukaryotes — organisms made up of cells containing a nucleus, which includes nearly all multicellular life on earth. However, whether simpler prokaryotes such as *M. pneumoniae* make use of these chemical tags to the same extent was not clear.

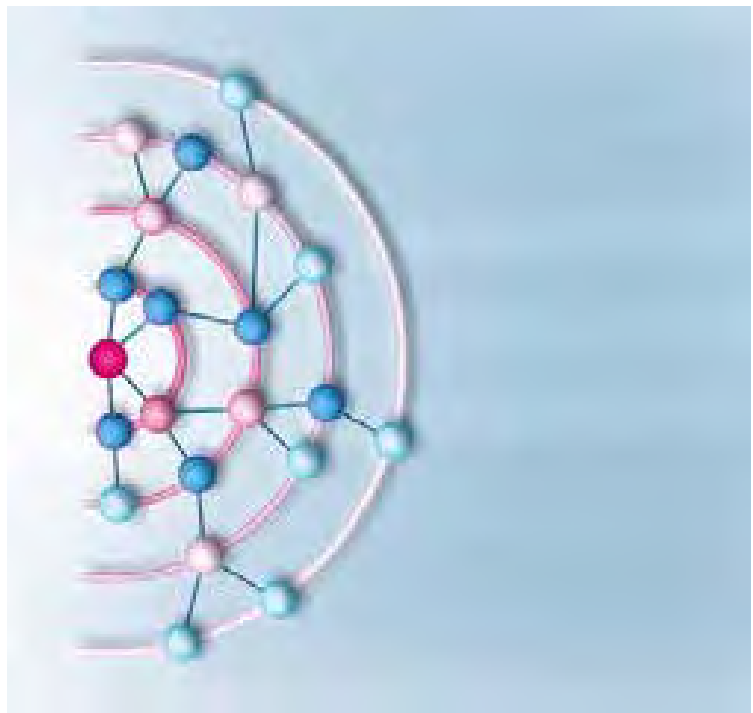
The relative simplicity of prokaryotes nonetheless makes global analyses of protein activity much easier than in eukaryotes. “The beauty of *M. pneumoniae* is that it only has 691 genes, which means we were able to look at more than 90% of expressed proteins,” says Peer. Out of

564 proteins that the team looked at, 241, or 42.7%, were modified by one or both of these chemical groups, including 93 phosphorylation sites on 72 proteins and 719 acetylation sites on 221 proteins. “The extent of these modifications suggests that they are as important in *M. pneumoniae* as they are in eukaryotes,” says Anne-Claude.

Both phosphorylation and lysine acetylation depend on specific enzymes that attach and remove the relevant chemical group. Each of those enzymes is, of course, encoded by a gene, and when Anne-Claude and colleagues knocked out the three *M. pneumoniae* genes required for adding and removing phosphate groups, they saw a surprising effect. Preventing phosphate groups from being added or removed from proteins not only affected phosphorylation, as expected, but also lysine acetylation. However, the two enzymes that add phosphate tags affect acetylation differently: knocking out one led to increased lysine acetylation, whereas the loss of the other reduced it. Similarly, knocking out the enzymes involved in lysine acetylation affected phosphorylation levels, pointing to cross-talk between these two protein-modification systems. “We don’t know exactly how this cross-talk is achieved, but we think that these chemical modifications affect the ability of enzymes to add further modifications,” says Anne-Claude. “Adding a phosphate group to a certain protein might make it easier – or more difficult – for another enzyme to then add an acetyl group.”

Even without a precise picture of the reciprocal links between phosphorylation and acetylation, it’s clear that *M. pneumoniae* makes extensive use of the protein-modification code, just like eukaryotes. This suggests that the code has ancient origins in the common ancestor of both prokaryotes and eukaryotes more than two billion years ago. “Creating diversity in the functions of proteins by attaching chemical tags has been conserved from prokaryotes through to the eukaryotic cells of animals and humans,” says Vera van Noort, one of the scientists in Peer’s group involved in the study.

The systems-level perspective on *M. pneumoniae* afforded by these recent studies vividly illustrates the power of natural selection to squeeze as many functions as possible out of a limited set of ingredients. It’s a skill based on a few core principles, such as adding chemical tags to change the function of proteins, that emerged early in the history of life on earth, and has been preserved in organisms as diverse and distant as prokaryotes and eukaryotes ever since. Apparently, from mice to men, from bacteria to blue whales, these principles — the basic logic of life — are the same everywhere.



Disrupting one protein (red) can affect some of its immediate partners (pink), but the problems may not propagate throughout the whole network.



How enzymes are made

Enzymes are crucial to life, and understanding how they have evolved to perform a diverse range of reactions in all kingdoms of life – from breaking down food to generating movement – is fundamental to many biological studies, especially those leading to new therapeutics. To study this evolution, researchers in Janet Thornton's group at EMBL-EBI teamed up with colleagues at University College London to pinpoint how enzymes change their function over evolutionary time. The protocol they have devel-

oped makes it easier to predict the function of an enzyme, and to design new ones for industrial or medical purposes.

"Our idea was to develop a resource and knowledge base for exploring the evolution of function of enzymes by integrating sequence, structure and chemistry information," explains EMBL-EBI's Nicholas Furnham, who led the study in Janet's group. "This way, we could gain a clearer picture of what enzymes are doing."

The team combined information about protein structure, sequence, phylogeny – or ‘relatedness’ – and the interactions of small molecules to study 276 enzyme superfamilies that are groups of diverse but related enzymes. They drew on their new software, FunTree, which uses tools based on GoogleMaps to visualise the evolutionary relationships between enzymes and their functions. Using this, they were able to piece together an overview of how new enzyme functions evolve.

“The new protocol allowed us to look quite deeply into evolutionary history to understand mechanisms that have allowed enzymes to change their function. We applied it to a large number of enzyme-containing protein superfamilies, which are made up of diverse, distantly related sequences, and were able to identify the evolutionary route taken within each superfamily to change an enzyme function from one reaction to another,” says Nicholas.

The researchers also showed that some types of changes in function are more common than others, which makes it easier to predict what an enzyme might do. They also found that most of the functionality observed in nature is confined to relatively few enzyme families, which implies that our basic chemistry was established very early on and the changes that have occurred since then are simply variations on a theme.

Pinpointing the mechanism by which enzymes evolve now makes it easier for scientists to predict the function of the millions of enzymes that haven’t yet been tested. Importantly, it also helps in the design of new enzymes for industrial or medical purposes.

“This is a starting point for fundamental work in enzyme evolution,” says Nicholas. “If you can look at all the times a certain change has taken place during evolution and identify commonalities, you will be much closer to understanding what it takes to make an enzyme change its function. That is crucial knowledge for enzyme design.”

EXPLORING ENZYMES

Why spend time digging through a bunch of databases to find out about an enzyme? EMBL-EBI’s new Enzyme Portal mines data from many sources and dishes it up in a single website. You can search for an enzyme you already know something about, or browse through what’s on offer – and perhaps discover something new.

The Enzyme Portal brings together data about proteins with enzymatic activity from 10 major public repositories via a single search, delivering information about disease, biochemical reactions, biological pathways, small-molecule chemistry and protein structures as well as the most relevant scientific articles.

It offers information about many species, including the key organisms used in biological research, and makes it simple to compare enzyme activities between them. “The Enzyme Portal is very useful for researchers working in drug discovery or chemical biology,” explains Christoph Steinbeck, Head of Cheminformatics and Metabolism at EMBL-EBI. “It seamlessly bridges the gaps between information in many areas of biology, from small molecules to 3D structures.”

“The Enzyme Portal was designed by users, for users,” says Cheminformatics Coordinator Paula de Matos. “Now, we have a central place where you can access and explore an enormous collection of data on enzymes – including information about how they may be involved in disease.”

“The Enzyme Portal takes the researcher’s perspective,” adds User Experience Analyst Jenny Cham. “It is the first EMBL-EBI resource to have a fully user-centred design from scratch. This approach is cost-effective, and makes decision making and communication about design and technology choices much easier.”

ONLINE EXTRA:

The Enzyme Portal features a short video showing what it can do: <http://youtu.be/Kldp0WXcxUM>

Furnham N, Sillitoe I, Holliday GL, Cuff AL, Laskowski RA, Orengo CA, Thornton JM (2012) Exploring the evolution of novel enzyme functions within structurally defined protein superfamilies. *PLoS Comput Biol.* **8**: e1002403

Furnham N, Sillitoe I, Holliday GL, Cuff AL, Rahman SA, Laskowski RA, Orengo CA, Thornton JM (2012) FunTree: a resource for exploring the functional evolution of structurally defined enzyme superfamilies. *Nucleic Acids Res.* **40**: D776-82

Click'n'glow

“We basically had to find a method to expand the genetic code.”

In many an espionage thriller, the protagonist has to keep tabs on the movement of some precious or dangerous cargo — a secret document or a biological weapon — by tagging it with a hidden locating device. Biologists use a similar approach to track the movements of proteins within cells, to see where they accumulate or how they move about to

carry out their functions. Now EMBL scientists have devised a new way to attach a fluorescent tag to any protein of interest in living cells.

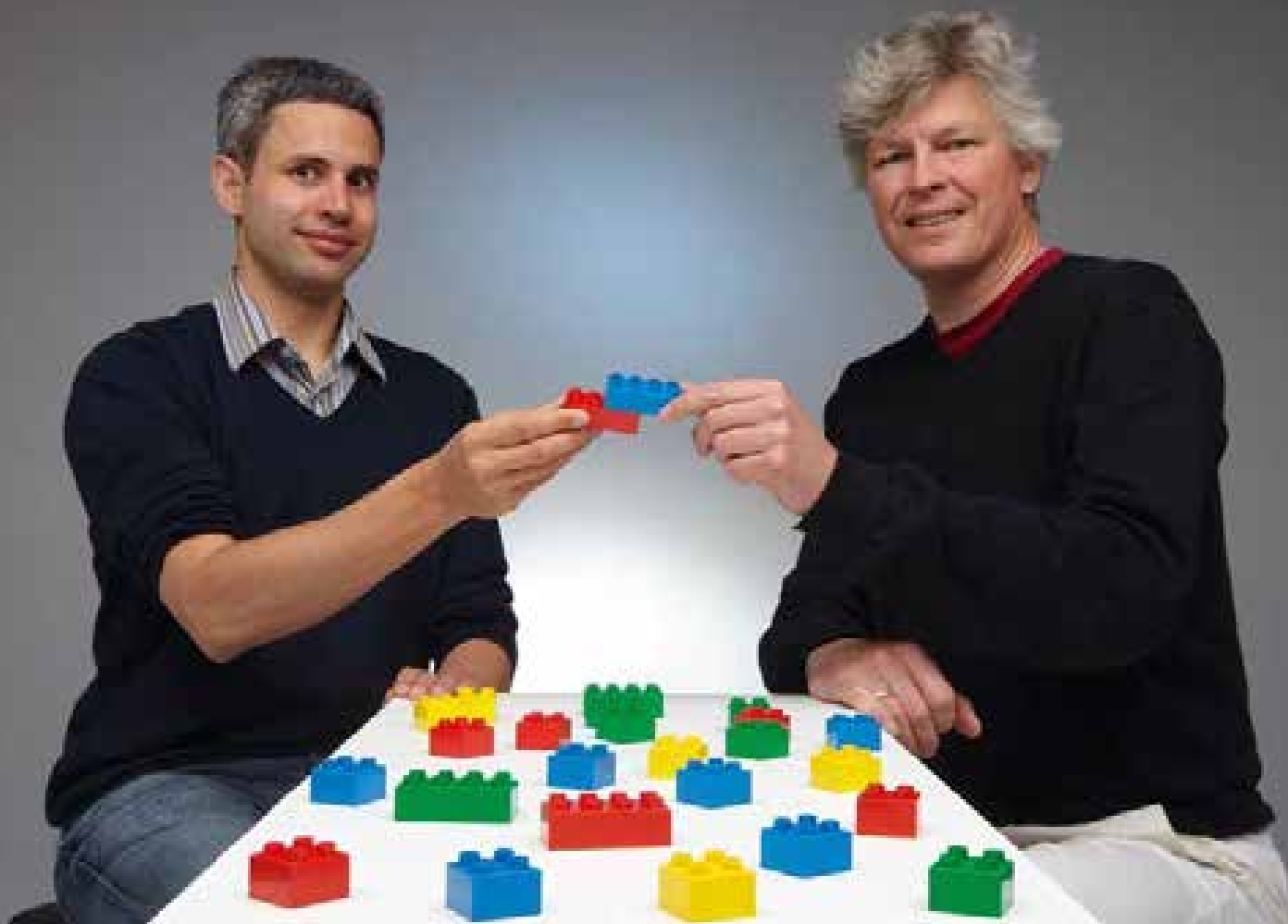
The new method — developed by Carsten Schultz, Edward Lemke and colleagues at EMBL

Heidelberg — gets around the problems associated with existing ways to fluorescently label proteins. One widely used technique involves grafting green fluorescent protein (GFP) onto to a protein of interest, which then glows green and provides a visual tag of the protein's location in the cell. GFP, however, is a relatively large protein, and this bulky addition

can interfere with the function of proteins in their natural environment. Fluorescent proteins also have limited photostability as they can be damaged and bleached by the microscope's light.

So the EMBL scientists set out to create a way to label proteins with small-molecule dyes, which are less susceptible to photobleaching and less likely to affect the function of the proteins they label. The first step was to create a new, artificial amino acid — the building blocks of proteins — that contains a binding site into which dyes can be loaded. “This synthetic amino acid is membrane permeable, so when we add it to the surrounding medium, cells can take it up and use it,” says Edward.

Next, they genetically modified the bacterium *Escherichia coli* so that its protein-synthesising machinery would incorporate the unnatural amino acid when a specific protein was being made. “This was a major challenge,” says Carsten. “We basically had to find a method to expand the genetic code.”



Edward Lemke and
Carsten Schultz

Genes ordinarily encode proteins made out of 20 naturally occurring amino acids. The ‘letters’ in the genetic code are read in triplets called codons, with each codon specifying which amino acid should be added at that position. Not all codons specify amino acids, however, and some are almost never present in genes. The scientists took advantage of this, and genetically modified the machinery that translates genes into proteins so that one of these rarely used codons would denote the addition of the unnatural amino acid. This single codon was then added to the gene encoding the protein to be tagged, so that the protein would be produced containing the unnatural amino acid at one specific site — which could then be loaded with fluorescent dye.

Crucially, this new amino acid is able to bind small-molecule dyes through ‘click chemistry’. “These are reactions that are so easy and so specific that the reacting molecules essentially click together like Lego blocks in a biological environment,” says Edward. This means that they do not need a catalyst, which can often

be toxic to cells, and so this approach represents a major advance over the more common catalysis-dependent approaches.

“For the first time, we’ve synthesised an amino acid that we can do this click chemistry with inside living cells,” says Carsten. And while these experiments focused on adding dyes to proteins so that they can be visualised within cells, the basic approach could be used to attach many kinds of molecules, such as linkers to other proteins and ‘spin labels’ for use in magnetic resonance imaging. “It’s a very widely applicable method,” says Carsten.

Plass T, Milles S, Koehler C, Schultz C, Lemke EA (2011) Genetically Encoded Copper-Free Click Chemistry. *Angew Chem Int Ed Engl* **50**: 3878-81



Neat nets

Competitors in synchronised swimming often use elastic hairnets to keep their locks safely tucked away while they leap, dive and swirl through the pool. Insights from another organism that spends most of its life underwater – the starfish – have now shown that egg cells use a similar net not to keep things in place, but to move them around.

You are here today because at some point in the past an egg cell was fertilised by a sperm cell. However, before that momentous event could take place, an oocyte had to mature into an egg cell, by getting rid of half of its genetic material. When an oocyte is first formed, it has two copies of each chromosome, just like the other cells in the body. But, if an egg cell with two copies of each chromosome fused with a sperm cell that has its own set of chromosomes, the resulting embryo would have a chromosome overload, which could cause serious problems. To avoid this, before it is fertilised the oocyte expels half of its genetic material, keeping only one copy of each chromosome. This eviction isn't as easy as it sounds. Because it holds the nutrients and building materials that the embryo will need in order to grow, the oocyte is an inordinately large cell, with a huge nucleus. To ensure it keeps one copy of each chromosome, the oocyte must gather all the chromosomes, pair them up, and then separate the pairs (see p. 70). The sheer size of its nucleus makes the first step problematic: chromosomes have to be gathered from across this vast sphere, and brought together in one place.

Péter Lénárt and his group at EMBL Heidelberg have now discovered how the oocyte reels in the chromosomes from the farthest reaches of its nucleus. "It's almost embarrassingly simple," Péter says. "It's like fishing with an elastic net. Imagine the net is stretched out; it's under tension. Then if you cut the edges, it all snaps in, trapping the chromosomes."

If a swimmer unpinned all the edges of her hairnet at the same time, it would spring to the middle. But if she left one point attached, and released the rest, the elastic mesh would 'shrink' toward that point – in all likelihood, painfully snagging a few hairs in the process. In essence, this is what the oocyte does. Strands, or filaments, of a protein called actin form a mesh throughout the nucleus. When the time comes to gather up chromosomes, the actin net springs together, dragging the chromosomes with it. Masashi Mori, a postdoctoral fellow in Péter's group, discovered that, like a hairnet kept in place at one point, the actin mesh is anchored to the cortex – a layer of actin filaments just inside the cell's membrane.

Masashi made his discovery by moving the nucleus to different points in starfish oocytes, using a centrifuge. When he moved the nucleus to the centre of the cell, the chromosomes dragged by the actin net all sprung to the middle of the nucleus. When Masashi placed the nucleus close to the cortex, the chromosomes moved to the area of the nucleus closest to the cortex – regardless of whereabouts in the cortex that was. This implies that no other molecules or factors are needed to get the chromosomes to where they have to go. The actin meshwork simply tethers



Starfish show how egg cells use nets to move things around.

itself to the cortex, and this anchor point ensures that when the mesh contracts, the chromosomes are dragged to that point, leaving them in the perfect location for half of them to be expelled in a small bud from the cell's membrane.

The EMBL scientists teamed up with Mark Bathe, an engineer at the Massachusetts Institute of Technology (MIT).

Nilah Monnier from Mark's lab managed to track the movements of chromosomes and of actin fibres, and saw that the farther away they were from their destination, the faster they moved – like the loose end of a bungee cord moves faster than the middle. This proved that all the fibres in the actin net are contracting at the same time and rate, so that ultimately all the chromosomes arrive near the cortex at the same time. By injecting beads of different sizes into the cell and tracing their movement, watching which got dragged by the net, Nilah was also able to show that the mesh is the perfect size to trap chromosomes.

In the meantime, Masashi has discovered that the actin mesh isn't actually released at the edges. "It turns out it's more complicated than that," Péter says. Masashi devised a technique that allows him to sit at the microscope observing and filming the oocyte, and then stop actin production by adding a drug at just the right moment. When he stopped the oocyte from making actin, the mesh contracted much faster than it normally would. Along with other microscopy data, this led the scientists to conclude that as the actin network contracts, new material is constantly being fed onto its fibres, like a climbing assistant gradually paying out rope. This controls the speed at which chromosomes move.

"All in all, it's a very elegant system: you don't need any gradients of signalling molecules, or anything like that," Péter concludes.

This study is also interesting because scientists knew that, in the cortex, actin acts as a scaffold – maintaining the cell's shape and granting it flexibility to move – but so far no-one had a clear idea of actin's role inside cells. The prevailing thought was that actin could transport molecules, but only over short distances. This work by Péter's group, however, lays that idea to rest, as it proves that actin can carry chromosomes over long distances. And supporting evidence is now arising from other sources. EMBL alumna Melina Schuh, who now heads a lab at the Medical Research Council Laboratory of Molecular Biology in the UK recently showed that actin transports vesicles across large distances inside mouse cells.

Next, Péter is looking to get to grips with how the actin mesh works at the molecular level: how it is produced, and how it is anchored to the cortex, for instance. Human oocytes also have an actin net, and probably use it in a similar way, so understanding this process could have wider implications in fields like human fertility and *in vitro* fertilisation. Thus, the starfish's version of the hairnet could prove helpful not only to swimmers, but to other humans too.

ONLINE EXTRA:

Watch a starfish egg cell expel half its chromosomes on the EMBL YouTube Channel:
<http://youtu.be/05r55I96r1w>.

Mori M, Monnier N, Daigle N, Bathe M, Ellenberg J, Lénárt P (2011) Intracellular transport by an anchored homogeneously contracting F-actin meshwork. *Curr Biol*. **21**: 606-611

Safeguarding genes

Just as banks store only the most valuable treasures in their safest of safes, cells prioritise which genes they guard most closely. Researchers in Nicholas Luscombe's group at EMBL-EBI have shown that bacteria have evolved a mechanism to protect their most important genes from random mutation, effectively reducing the risk of self-destruction.

In addition to answering a question that has been hotly debated for decades, the new findings provide insights into how disease-causing mutations arise and how pathogens evolve.

"We discovered that there must be a molecular mechanism that preferentially protects certain areas of the genome over others," says Nicholas. "If we can identify the proteins involved and uncover how this works, we will be even closer to understanding how mutations that lead to diseases like cancer can be prevented."

Mutations make each of us unique: changes in genetic material give rise to variation between individuals, and between cells within individuals. But they do have a darker side. For example, a mutation could render a tumour-suppressing gene useless – with disastrous consequences. However, just as holding all bank deposits in maximum-security safes would be prohibitively expensive, a cell simply cannot expend the energy necessary to protect all of its genes from mutation.

Iñigo Martincorena, a PhD student in Nicholas' group, found that cells have evolved a 'risk-management' strategy to protect their most prized genes. Looking at 120 000 tiny genetic mutations called single nucleotide polymorphisms (SNPs) in 34 strains of the bacterium *Escherichia coli*, Iñigo and other members of the team were able to quantify the mutation rate in different areas of the bacterial genomes. The results showed that key genes – for example, the ones needed for respiration – mutate at a much lower rate than the others. This means that their chances of suffering a detrimental mutation are much lower.

"We were struck by how variable the mutation rate appears to be along the genome," says



Iñigo. "Our observations suggest these bacteria have evolved a clever mechanism to control the rate of evolution in crucial areas of the genome."

Mutation rates reflect how 'valuable' *E. coli*'s genes are to the bacterium.

Scientists have long thought that mutations occur randomly – regardless of whether or not they are beneficial – and that once a mutation occurs, natural selection determines whether it spreads through the population or is eliminated. The new findings challenge this assumption. Using population genetics techniques, Nicholas' group were able to disentangle the effects of natural selection and mutation rate by analysing data from very large populations of bacteria.

"For many years in evolution there has been an assumption that mutations occur randomly, and that selection 'cleans them up,'" explains Iñigo. "But what we see here suggests that genomes have developed mechanisms to avoid mutations in regions that are more valuable than others."

Observations from cancer genome studies suggest that similar mechanisms are involved in the development of cancer. The next step for Nicholas' group will be to investigate exactly how gene-protection 'risk management' works, and what role it may play in tumour cells.

Martincorena I, Seshasayee ASN, Luscombe N (2012) Evidence of non-random mutation rates suggests an evolutionary risk management strategy. *Nature* doi: 10.1038/nature10995

A year in the life of EMBL

April

Girl power at EMBL

It was all about girl (and boy) power in Heidelberg on 14 April, as the main Laboratory welcomed 17 young visitors, aged between 10 and 16, as part of the Girls' Day initiative. Celebrated in many European countries, Girls' Day aims to give schoolgirls a taste of what are thought to be traditionally 'male' careers, such as science, engineering, maths and computing. But, as EMBL is rarely one to follow the crowd, the invitation to experience life in the lab was open to girls and boys. Most of the visitors were family or friends of members of staff, and they shadowed people working in labs, the Core Facilities, the photolab, and even the ISG Hotel. The pupils were able to choose the career that interested them most, and could be found on the day getting to grips with everything from preparing protein gels to artistic direction.



2011

Collaboration matters

EMBL further strengthened links with scientific organisations in Russia on 26 April, with the signing of a Memorandum of Understanding with the Skolkovo Foundation to develop collaborations in key areas of research. Iain Mattaj, EMBL Director General, and Igor Goryanin, Director of the Biomedical Cluster, Skolkovo, formalised the framework agreement at a meeting of the Board of Trustees of the Skolkovo Foundation. The Foundation is a non-profit organisation that, on behalf of the Russian Federation, is dedicated to the development and commercialisation of new innovations and the creation of a new 'science and technology city' in the Moscow suburb of Skolkovo. As envisaged by the collaboration, EMBL and the Skolkovo Foundation will work together on mutual goals, such as the development of scientific exchanges, collaborations, and joint projects between EMBL and laboratories and research organisations in Russia.

May

Rethinking the balance of nature

Urgent challenges facing our natural world were put under the spotlight as experts and participants convened in Cambridge for the fifth annual EMBL-EBI Science & Society symposium on 6 May. Issues discussed at 'Biodiversity and Endangered Species: Rethinking the Balance of Nature' included economics, education and politics in the context of the latest science. As in previous years, the conference attracted a diverse range of speakers and participants, leading to lively debates, particularly during the panel discussion. The topic will also be taken up by this year's EMBL|EMBO Science & Society Conference in Heidelberg on 9-10 November, 'Biodiversity in the Balance: Causes and Consequences'.



Interrogating an insect society

May saw one of the highlights in this year's EMBL Science & Society Programme when Raghavendra Gadagkar from the Indian Institute of Science, who has spent over four decades studying a primitive paper wasp, delivered an EMBL Forum lecture. "There is so much to learn from this organism," he said. "Every time you learn something new, the next question that occurs is even more interesting." In his talk, Raghavendra explained how research on *Ropalidia marginata* continues to shine new light on our understanding of how insects live. A trained molecular biologist, he develops ideas from the experimental methods he learned and also draws inspiration from the ecosystems just outside his window.

The code of life

On 8 June, Sidney Brenner gave the final presentation in the Vision 2020 lecture series. During his talk 'Reading the Human Genome', Sidney laid out his priorities for the future of research to the 400-strong crowd that filled the EMBL Advanced Training Centre auditorium. "A big challenge is to work out the code that underlies different cell types, especially in the brain," he explained. "This is certainly achievable, but big challenges can only be met by engaging young scientists in the research. Today you can outsource a lot, but no one can do the synthesis except the scientist." Such outlines characterised the Vision 2020 series, which involved eight leading scientists from around the world sharing their thoughts and hopes relating to the future of their fields to celebrate the inaugural year of the EMBL Advanced Training Centre.



Lennart Philipson dies

Lennart Philipson, who served as EMBL's second Director General, passed away on 26 June. Lennart headed EMBL for more than a decade between 1982 and 1993, a crucial time for molecular biology when different scientific disciplines in the life sciences were becoming increasingly interlinked. He reorganised the Laboratory into new scientific and instrumentation Units, with a profound impact on both scientific success and the development of innovative technologies in areas such as microscopy. Most influentially, he realised the power of bioinformatics approaches and ensured that EMBL became a stronghold of research and service activities in this area. He also insisted that turnover was key to the long-term success of the Laboratory and ensured that this principle was reflected throughout EMBL.



Science, posters, party – Lab Day!

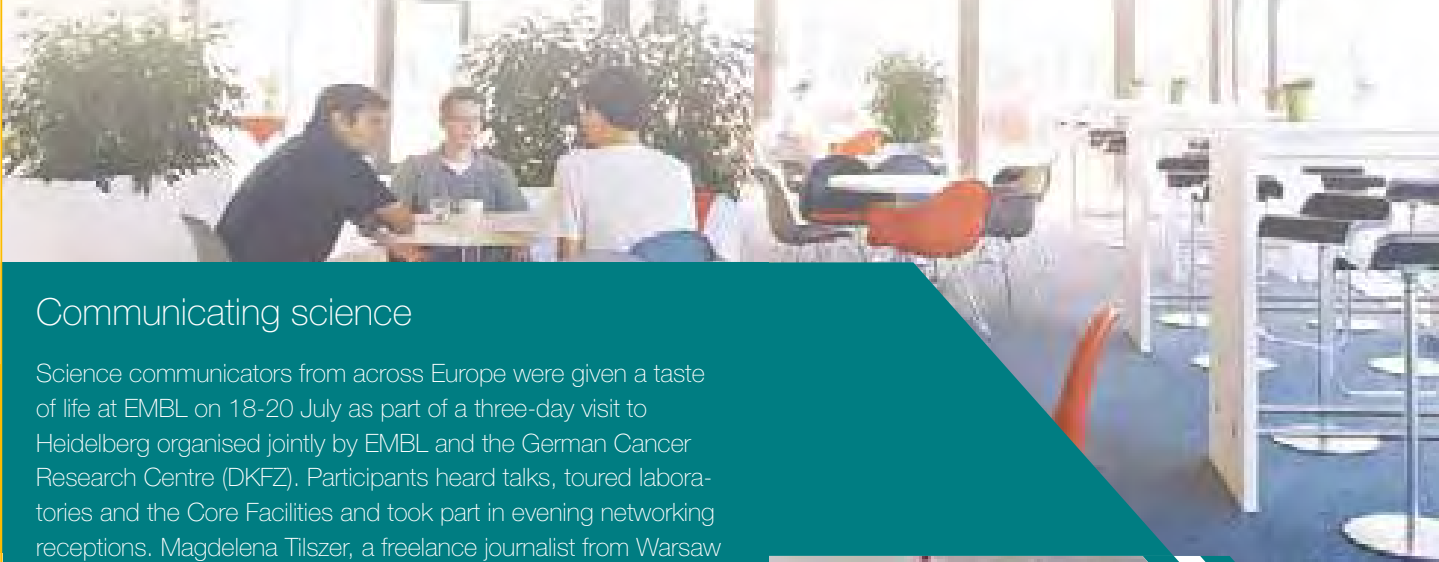
More than 200 staff from across all the EMBL sites attended the events at the main Laboratory on 9 June, which was the highest attendance to date. This year's programme included expert talks, poster displays, the graduation ceremony and the presentation of the prestigious John Kendrew Award to former Monterotondo postdoc Amaicha Depino for her communication of science through workshops and children's books in Argentina. New this year was a scientific programme, organised by EMBL pre- and postdocs, that included presentations from predocs, postdocs, group leaders and other scientific staff on research such as computational models of biological processes, genotypic diversity, and developing the use of new (and old) model organisms in scientific research. Lab Day was preceded by Career Day, during which scientists from all EMBL sites and the local scientific community learned about alternative, non-academic career opportunities from a variety of professionals.



July

Bigger, better, brighter

9 July saw the opening of the new cafeteria at EMBL Heidelberg. In place of the old canteen there is now a contemporary new area divided between the cafeteria and a staff lounge that was opened on 14 September. A wide range of specialities are on offer at the cafeteria including new 'Dishes of the Day', hot sandwiches and delicious smoothies. The new staff lounge provides a variety of games that include a darts board, a Playstation/Wii and a quieter area for those who either want to relax and read a book or enjoy a game of chess.



Communicating science

Science communicators from across Europe were given a taste of life at EMBL on 18-20 July as part of a three-day visit to Heidelberg organised jointly by EMBL and the German Cancer Research Centre (DKFZ). Participants heard talks, toured laboratories and the Core Facilities and took part in evening networking receptions. Magdalena Tilszer, a freelance journalist from Warsaw was impressed with the wide-ranging scientific activities at both institutions. "Learning about the different types of research, from genome and computational biology through to advanced imaging techniques has been fantastic," she said. "The atmosphere is great and it is wonderful to see the opportunities for pre- and postdocs." A total of 22 media professionals attended from 11 countries, and they left with fresh ideas and inspiration. Gorm Palmgren, a Danish journalist who contributes to Science Illustrated magazine, expects to follow up a number of stories. "We heard Mani Arumugam talking about gut flora, and I expect to write a big article on this subject," he said.



International get-together

Throughout the summer, EMBL Heidelberg hosted visits from several international groups, including 40 of the most talented students in Spain as part of the Becas Europa initiative – a three-week tour of some of Europe's most important universities and institutions. Other visitors included students from the US, Canada and the UK, who came as part of the Research Internships in Science and Engineering (RISE) programme that provides opportunities for undergraduate students to work with leading research groups across Germany. In addition, the two winners of the EMBL prize from the European Union Contest for Young Scientists, Kristina Aare (2009) and Raghd Rostom (2010), both completed internships at EMBL Heidelberg.

August

A guided tour of the brain

For the fourth year running the local Heidelberg newspaper 'Rhein-Neckar-Zeitung' organised an excursion to the EMBL main Laboratory. New on the agenda this year was a visit to the teaching laboratory where participants could watch different stages of development in zebrafish under the microscope and Timm Schlegelmilch, a postdoc in Francesca Peri's group, explained the role of microglia in the developing brain.



2011

The human animal

What is humanness? Is the public being misinformed about genetic screening? How are technologies impacting on how we see ourselves? These were just some of the questions considered by 20 participants at the beginning of August at the EMBL|EMBO Science & Society summer school 'The Human Animal: Scientific, Social and Moral Perspectives' held at EMBL Heidelberg. The six-day programme included lectures from EMBL scientists and experts from around the world. Tutors, including Agnar Helgason (deCODE Genetics, Iceland), Tecumseh Fitch (University of Vienna, Austria) and Eric Parens (The Hastings Centre, USA), discussed a broad range of social and scientific issues relating to genetics, genomics, patenting, human enhancement, neuroscience, bioethics, and more. Organised by faculty from EMBL, the European School of Molecular Medicine and the Harvard Kennedy School, the summer school involved pre- and postdocs from both natural and social sciences from countries as far-flung as the US, Mexico, Finland, Israel and Australia.



September

Understanding the personal genome

Staff at EMBL-EBI took part in lively discussions about personal genomics through a series of talks, debates, games and other initiatives jointly organised by EMBL-EBI and the Wellcome Trust Sanger Institute. The campus-wide programme aimed to stimulate discussion about the ethical implications of personal genome sequencing and its impact on society. A seminar series featured engaging topics such as direct-to-consumer genomic testing and the falling cost of genome sequencing, which could have a profound influence

on healthcare. The Sanger Institute also offered a number of staff on campus the chance to have a small portion of their own genome screened anonymously. "As discussed in Nature recently, personal genomics is increasingly being applied in a clinical setting," says EMBL-EBI group leader John Marioni, who helped coordinate the seminar series. "Developing a deeper understanding of the scientific and ethical challenges that this entails is vital. This is especially true for research groups whose work is directly related to this area."

Rowing for charity

Scientists from Edward Lemke's group at EMBL Heidelberg exchanged their pipettes for rowing oars as they took part in a charity regatta in the September sunshine. Although few in the group had any rowing experience, they all took part in gruelling training sessions. And for predoc Swati Tyagi, the challenge was even greater: "In the rules it says that you have to be able to swim, but I could not – so I had to learn quickly!" she says. Research technician Christine Köhler points out that success, in part, came down to the two teams' specially designed EMBL T-shirts. "They are very cool, we like the colours a lot," she says. The teams raised more than 400 Euros for charity.



Nothing beats a retreat

Almost 100 predocs enjoyed two days of warm temperatures, productive meetings and reunions during this year's EMBL PhD retreat held in Mallorca on 23-25 September. The three days provided ample opportunity to discuss science and issues of interest and the programme included a set of research talks on a broad range of subjects, including structural biology, genome analysis and cancer. Interactive activities, organised by a team of predocs, focused on the effective communication of biological results, with many in the audience both amused and surprised when a piece of original research was contrasted with how it had been presented. The meeting provided an opportunity to refresh relationships with EMBL colleagues from all EMBL sites and to discuss ideas relating to PhD projects and more.



October

Showcasing EMBL at the Moscow Science Festival

Young and old went 'bananas' for science as EMBL scientists Vladimir Rybin and Alexey Kikhney helped visitors at Russia's biggest popular science event on 7-9 October to extract banana DNA using household chemicals. The sixth Russian Science Festival brought together hundreds of institutions and members of the public, with a great deal of interest in EMBL's stand following broadcasts in the Russian media. "Our simple experiments attracted the attention of several journalists, including those from some of Russia's biggest television channels," explains Alexey, a postdoc in Dmitri Svergun's group at EMBL Hamburg. "The next day people were hurrying over to our stand saying 'we saw you on TV!'" The three-day event took place in more than 80 locations across Moscow and other cities in Russia. The diverse programme included a symposium on the human brain and its memory functions, together with literary and drawing competitions and a chance to pitch ideas to business leaders. "People want to see what real researchers are like, and showcasing EMBL here is a valuable way of connecting people with science," adds Alexey.



November

Great ideas of biology



More than 350 interested members of the public crammed into the Print Media Academy in Heidelberg to hear Nobel Laureate Paul Nurse outline his vision of 'The Great Ideas of Biology'. Speaking in a Heidelberg Forum on Biosciences and Society lecture, Paul gave a historical overview of the breakthroughs he believes have defined scientific progress in biology – from theories of the gene, evolution by natural selection, the proposal that the cell is the fundamental unit of all life and the organisation of chemistry in the cell. He also speculated about a fifth great idea: how cells and organisms process information and acquire specific forms. "Complexity is going to move biology from the common sense world we essentially inhabit, to a world that is potentially a little bit stranger, where it is not intuitive what is happening," he says. "This would be comparable to quantum mechanics – you cannot get a common sense view of it, it only makes sense in terms of mathematics." The lectures – a joint initiative by EMBL, the German Cancer Research Centre and Heidelberg University – aim to promote an understanding of science to a variety of audiences.

A night to remember

Visitors to EMBL Hamburg's stand at Hamburg's long night of science in November were wowed with hands-on challenges including making and watching crystals grow under the microscope and fishing for crystals using an oversized fishing 'loop'. The event attracted more than 20 000 people, with 13 600 coming to the DESY site where more than 60 attractions awaited – including EMBL's stand in the PETRA III hall. "The children were queuing up to fish for crystals," says Annabel Parret, a staff scientist in Matthias Wilmanns' group. "Some came back several times and kept trying to beat their own score – they really had a lot of fun!" Other parts of EMBL's exhibition included movies of 3D models of molecules and the chance for visitors to get to grips with modelling software and 3D data. Members of the PETRA III team also offered tours of the EMBL beamlines throughout the day. "We have been really busy," says Thomas Schneider, head of the PETRA III team. "It's not only scientists who have shown an interest in the beamlines. It is great to see so many people here who are interested in what we are doing."



Postdoc retreat in Strasbourg

Careers, collaborations, and *Saccharomyces cerevisiae* were on the agenda as more than 70 postdocs from across EMBL's sites convened in Strasbourg from 2-4 November for the annual postdoc retreat. One of the main aims of the retreat is to develop new interactions and collaborations between postdocs throughout the EMBL Units and outstations and provide a platform to discuss ongoing projects, technological developments and wider aspects such as career development. The get-together, which is organised by the Postdoc Association each year, included 20-minute scientific talks, poster sessions and informal discussions that involved all participants. The programme also included keynote lectures from Bernhard Hauer (Institute of Technical Biochemistry, Stuttgart) and Kevin Verstrepen (VIB Laboratory of Systems Biology, Leuven), who discussed topics as diverse as synthetic biology, alternative career paths and the mechanisms underlying rapid evolution in *S. cerevisiae* (and its role in the beer-brewing process).



2011

December

An occasion full of firsts

Friday 16 December was a day full of firsts when the EMBL Alumni Association board met at the Grenoble outstation. It was the first meeting of the newly elected board, the first visit to the Grenoble outstation in the Association's 13-year history, and the first board dinner to be transformed into a staff-alumni get-together. More than 40 board members attended the event organised by Rokhaya Tounkara and Dominique Lancon from EMBL Grenoble Administration. "It was a nice balance between scientific and administrative staff from all levels together with local alumni," comments Maria Vivanco, EMBL Alumni Association (EAA) Vice-Chair. "We are delighted with the decision to hold one board meeting every year at an EMBL outstation, and with the interest of EMBL Grenoble staff today," adds Matthias Hentze, EMBL Associate Director, who represents EMBL at these meetings. The agenda of the meeting included the selection of two John Kendrew Award winners, the composition of four working groups to deal with Association business throughout the year, as well as updates regarding alumni and EMBL news.



January

2012



Of finches and dragons

On the 13th of January, a mixed audience from around the DESY campus attended the science and society talk by Arthur J. Connor Professor of History Harriet Ritvo from MIT. In her presentation entitled "The (very gradual) emergence of Darwin's Evolutionary Theory" Harriet talked about the context in which Darwin developed his theory of evolution by natural selection. It was only through the diligent work of the ornithological expert who categorized some of the specimens collected on the Beagle expedition that Darwin switched his attention from the more sexy Galapagos dragons (iguanas and other lizards) to the adaptive traits of the finches. Darwin continued collecting evidence for his theory for an additional twenty years, until the likelihood of a scoop by Alfred Russel Wallace inspired him to rush publication of 'On the Origin of Species'. The Science and Society talk at EMBL Hamburg was a lively event and was followed by a social get-together with drinks and snacks.

Celebrating productive partnerships

For the third year running, members of the EMBL Advanced Training Centre Corporate Partnership Programme convened at the EMBL ATC in Heidelberg for their annual gala event on 26 January. Managing Directors, Vice-Presidents and other high-level guests from all 16 companies involved in the programme gathered for a reception, followed by scientific talks from EMBL experts – including a presentation by Nadia Rosenthal on 'the Australia experience' – and dinner in the ATC foyer. The EMBL Corporate Partnership Programme was established in 2008 with Leica Microsystems, GE Healthcare, Life Technologies and Olympus as founding partners to provide support for EMBL's scientific conferences, courses and other events. Funds are primarily used to support events in the new EMBL ATC and to keep registration fees at a reasonable level.



February



The ethics of primate brain research

Neurophysiological studies in awake rhesus monkeys are amongst the animal experiments that receive the most critical public attention. In his EMBL Forum talk on 13 February, Stefan Treue, Professor for Biopsychology and Cognitive Neurosciences at the University of Göttingen and Director of the German Primate Centre, used this type of research as an example to illustrate fundamental points about the importance, ethics, politics and the legal aspects of biomedical research with animals. He demonstrated once more the complex role of scientists in the debate about animal research and the impact that this work is having on society.

March

Graham Cameron retires

On 1 March, EMBL-EBI Associate Director Graham Cameron retired after 30 years at the forefront of bioinformatics. He joined EMBL in 1982 when bioinformatics was still a specialist, niche area: „The day EMBL Council agreed to establish the EBI was a proud moment. However, when I came onto this campus, none of the new buildings had even been started – I was a ‘one-man EBI’.“ Team leaders Rolf Apweiler and Ewan Birney have been appointed joint Associate Directors of the outstation to succeed Graham. Since 2007, Rolf and Ewan have been jointly running a large team that develops public domain protein and nucleotide databases and tools. Graham will be working closely with Ewan and Rolf over the coming months to ensure that the transition of stewardship of the world’s most comprehensive range of freely available molecular databases is as smooth as possible.



EMBL goes east

Just a stone’s throw from historical monuments and architectural marvels such as the Kremlin and Red Square, representatives from EMBL joined leading scientists from Russia and worldwide at one of the country’s top scientific meetings on 21-25 March. Director of International Relations Silke Schumacher delivered a keynote presentation, discussing the role of EMBL as Europe’s centre of excellence for molecular life sciences. The conference, ‘Biotechnology: State of the Art and Prospects of Development’, brought together scientists, industry leaders and decision makers from a broad range of disciplines to network, disseminate ideas and discuss strategies such as innovative knowledge transfer. Some 3300 people attended the conference over five days, with EMBL staff speaking to delegates about opportunities such as scientific collaborations, the PhD Programme and the EMBL Interdisciplinary Postdoctoral Programme (EIPOD), which promotes highly interactive research between EMBL’s research Units.

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