Brakes and gas pedals

Meeting: Spetses 2000, molecular mechanisms of development and disease

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Introduction

In September 2000, FEBS and EMBO organized the meeting 'Molecular mechanisms of development and disease' on the Island of Spetses in Greece. The symposium drew 23 speakers from the fields of transcription, developmental biology, cancer biology and signalling. As became readily apparent during the meeting, the study of improper recapitulation or modification of developmental and regulatory processes has led to an understanding of how their alteration or misregulation can cause disease. Importantly, a few of these processes can now be controlled, with beneficial consequences for the treatment of previously chronic or terminal diseases such as cancer. We selected a small fraction of the many superb talks for inclusion in this review, which gives an overview of the presented research.

Developments in development

The completion of the *Drosophila* and *Homo sapiens* genome projects has enabled the rapid discovery of novel genes. Characterization of these genes in *Drosophila* has permitted rapid establishment of their relevance to human disease. Herbert Jäckle (MPI, Göttingen, Germany) discussed one new gene, which encodes a novel G β -like protein that has homologues in mice and humans. Polymorphisms in this gene encourage the growth of adipose tissue at the expense of rapid movement. Variants of a sequence-related gene have been found in the Inuit, Aborigine and other racial groups prone to obesity, but may have originally helped these people make the most efficient use of limited food supplies. The rapid discovery of this gene, and its unintended possible responsibility for a serious public health problem, highlighted the ability of basic developmental

biology, in combination with functional genomics, to identify causes for human disease.

The discovery that a gene induces a particular phenotype, however, does not always shed light on the mechanism of action of the gene product. A good example of this is *eyeless*, a member of the Pax6 family of transcription factors, which induces ectopic eye formation in nearly every part of the fly (Callaerts *et al.*, 1997). Homozygous mutant mice with a non-functional Pax6 gene do not form optic vesicles or lenses. This prevented elucidation of the role of Pax6 with respect to the specific tissues formed during later eye development. To address this question, Peter Gruss (Max-Planck Institut, Göttingen, Germany) discussed some recent data from his group. These concerned conditional knockouts of mouse Pax6 in which the surface ectoderm of the developing eye was affected (Ashery-Padan *et al.*, 2000). Although lens formation is induced in such mice, crystallin is not produced and the lens does not fully develop. Moreover, several separated retinas form, indicating that the lens is required for development of a single functional retina.

In humans, loss of function of the homologous Pax6 gene leads to aniridia, or absence of the iris. However, this alone does not reduce vision, unless the macula does not fully develop. The latter, frequent complication is usually the most significant cause of vision loss in this disease. The observation that the functional lens is required for proper development of the retina may explain why aniridia is often accompanied by improper macular development.

Finally, Peter Gruss also presented interesting data on the connection between the forkhead genes and mouse behaviour. In the brains of mice lacking the forkhead gene *Fox1b*, thalamic neurons never enter the medial mammillary body, which subsequently vanishes by apoptosis. Although the mice are apparently healthy, they do very poorly in Morris water maze and eight-arm maze behaviour tests. These observations are

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consistent with memory loss. As the mammillary body and parts of the thalamus are missing in alcoholics, these data may provide an explanation for the amnesia that is so common among people with this addiction.

Regulating the nucleus

Methylation of mammalian DNA was initially hypothesized to be involved in the recognition of self and non-self DNA, but the importance of chromatin structure and non-sequence related DNA alterations in transcriptional regulation has clearly been underestimated. Research concerning the latter has now led to the unravelling of several regulatory processes that do not involve the original DNA sequence. A recent discovery, described by Alan Wolffe (Sangamo Biosciences, Richmond, CA), is the characterization of remodelling machines that consume ATP in order to rearrange nucleosomes. Such remodelling is essential for the occurrence of other epigenetic events, such as histone acetylation and DNA methylation (Wolffe and Matzke, 1999). The extensive modification of histones by ubiquitylation, ribosylation, methylation, phosphorylation and acetylation opens new perspectives for the accurate regulation of nucleosome 'solubility', DNA accessibility and, thus, regulation of transcriptional activity. Epigeneticists have been able to demonstrate histone acetylation in transcriptional promoters, and cytosine methylation in silenced regions of DNA.

From a medical point of view, the importance of methylation is encapsulated by the advice given to women who wish to become pregnant to supplement their diet with folate. Folate functions as a methyl-group donor during DNA methylation and has been shown to positively effect neural development, suggesting that DNA modifications can be transmitted from parent to child in a non-sequence specific way.

Studying the synergistic fashion in which transcription factors, insulators, enhancer sequences and epigenetic DNA alterations direct the fine-tuned regulation of gene expression will bring us closer to an understanding of how the cell controls its genetic material, how it directs expression of proteins, and how it compensates for and corrects DNA errors. In tumour cells for example, mutation of genes involved in regulation of histone-acetylation has been found to occur. However, during one of the discussion group sessions, it became clear that different cell types react differently to hypo- and hyper-methylation, which remains confusing in light of the opposing effects of methyltransferase drugs on different cancers.

There are still organisms, such as *Caenorhabditis elegans*, for which methylation has not been demonstrated. Previously, this was also thought to be the case for *Drosophila*. However, Rudolf Jaenisch (MIT, Cambridge, MA) presented evidence for the methylation of CpT sequences in *Drosophila* embryos, albeit at a very low level (0.7–0.1%) (Lyko *et al.*, 2000). This suggests an early evolutionary origin of methylation. In view of this recent discovery, it will be interesting to study organisms that do not methylate DNA, and their gene expression requirements, in order to deduce evolutionary relationships for methylation among different species.

How did DNA methylation evolve? As has been known for a long time, the capping of RNA is essential, involves methylation, and does not only function in protection against degradation but

functions in RNA-processing, nuclear export and, later on, in the assembly of the translational apparatus. It is very well possible that DNA duplication of these RNA-methylation enzymes resulted in modified enzymes that were capable of methylating DNA sequences. In this respect, it will be interesting to compare the different methylases used by viruses to the cellular methylases used for RNA- and DNA-methylation. An early evolutionary origin and ubiquity of methylation may hamper the development of non-toxic anti-methylation drugs. Given the role of methylation in the life cycles of viruses, the characterization of viral methyltransferases may permit the discovery of differences between these and the cellular methylation machinery, which could then be exploited by a novel anti-viral methylation inhibitor.

Communication within and across the border

How do cellular components know where to go and when and where to act? This question still has not been answered and may be incredibly complex. However, part of the problem is explained by the recognition of specific signals that target cellular components to the proper cellular compartments. Of the many cellular trafficking processes, importin and exportin-mediated nucleocytoplasmic transport are particularly interesting because they do not directly require input of energy, yet are extremely selective (Görllich and Kutay, 1999). Dirk Görllich (ZMBH, Heidelberg, Germany) presented additional data indicating that the mass flux through nuclear pores is very high (nearly 100 Mda/s) and incredibly rapid ($\sim 10^3$ translocations/s) (Ribbeck and Görllich, 2001). Any minor defect in the nuclear pore proteins, importins or exportins could severely interfere with the speed of these forms of transport, which appear to be essential to the survival of the cell. Iain Mattaj (EMBL, Heidelberg, Germany) also pointed out that nuclear export of some macromolecules, in particular unspliced mRNAs, is so essential to the survival of viruses that they have evolved their own proteins to mediate this transport.

Now what happens during cell division? Iain Mattaj's group recently demonstrated that proteins involved in nuclear transport (RCC1 and RanGTP) bind to chromosomal DNA, are responsible for the formation of the mitotic spindle (Carazo-Salas *et al.*, 1999), and function in the final step of reassembly of the nuclear envelope (Gruss *et al.*, 2001). Relocalization of the nuclear and cytoplasmic proteins is carried out by an ingenious mechanism that shields the chromosomal DNA, ensures the correct positioning of the proteins that are involved in reassembly of the nuclear membrane, and re-establishes the nuclear-cytoplasmic RanGTP gradient.

Transport processes can also have direct consequences for human diseases. In discussing multidrug resistance proteins, Piet Borst (The Netherlands Cancer Institute, Amsterdam, The Netherlands) pointed out that in normal tissues, the expression of membrane pumps such as P-glycoprotein permits specific export of toxic chemicals (or their modified derivatives) out of the cytoplasm, and is essential for maintenance of biological barriers such as the blood-brain barrier and gut tissue. However, improper expression of these proteins in a variety of cancer cells induces multidrug resistance to a variety of commonly used chemotherapeutics. Studying this phenomenon has led to the

discovery of compounds such as cyclosporins that are poor substrates for the transporters. Delivery of chemotherapeutics in combination with these transporter inhibitors permits the killing of previously drug-resistant cancer cells (Borst *et al.*, 2000).

The transport of information, however, does not always require physical transport of a molecule. Conformational changes in proteins that span the membrane, and subsequent modification of these proteins on their intracellular surfaces, can also convey information to the cell. Among the many cell-surface receptors, epidermal growth factor receptor (EGFR) has been particularly well studied. Binding of a ligand induces receptor dimerization, cross-phosphorylation of the cytoplasmic domain, and the subsequent propagation of signals into the cell. Approximately 25 years ago, the similarity of the normal EGFR to a viral oncogene led to the conclusion that constitutive activation of this protein could cause cancer. Consequently, EGFR was selected as a target for anti-cancer drugs.

Now the results of this discovery are starting to yield clinical dividends. Axel Ullrich (Max-Planck-Institut of Biochemistry, Martinsried, Germany) presented results from mouse studies of several novel EGFR family inhibitors, discovered by the company SUGEN. These drugs target vascular endothelial growth factor receptor-2 (VEGFR-2), which is found only on the vascular endothelium. One of these drugs is extremely specific, and inhibits VEGFR-2 without altering the function of closely related receptors, such as the platelet-derived growth factor receptor. It inhibits blood vessel growth in mice. Another candidate drug is slightly less specific, but is capable of completely curing human tumours grafted into mice. Moreover, the results from preliminary clinical tests of these compounds are very promising, and demonstrate the potential role of basic research in identifying and characterizing targets for novel therapies.

Communication between cells is essential for effective oncogenesis. Recently, Robert Weinberg (Whitehead Institute for Biomedical Research, MIT, Cambridge, MA) and colleagues described the creation of human tumour cells by specific introduction of four components: the catalytic subunit from human telomerase (hTERT), the SV40 large and small T antigens, and an activated *ras* protein (Hahn *et al.*, 1999). Recent data that the Weinberg group obtained from further studies on these cells demonstrated essential roles of non-tumorous stromal cell types in the speed and success of tumour formation. Clearly, tumour formation is a complex process requiring angiogenesis and stromal cell recruitment in addition to simple proliferation of transformed cells. The nature of the interactions between cancer cells and normal tissue is however still poorly understood.

Who's afraid of insects or cattle?

Much of this year's course focussed on misregulation of normal biological processes, and its consequence in cancer and other diseases. The agents of infectious diseases, however, also use ingenious molecular mechanisms, genetic and otherwise, to ensure their rapid spread and survival. In accordance, several lectures dealt with infectious diseases, in particular the prion diseases and two arthropod-borne maladies, malaria and sleeping sickness.

Toll/Toll-like receptors & related factors

Fig. 1. Similarities between Toll and NF-κB signalling in *Drosophila* and mammals.

Fotis Kafatos (European Molecular Biology Laboratory, Heidelberg, Germany) presented research on the defense systems that the malaria mosquito, *Anopheles gambiae*, uses to fight the parasite *Plasmodium falciparum*. A recently discovered thioester-containing protein that is involved in the anti-parasite response in the mosquito shows similarities to both serine protease inhibitors and the complement system that is used by *Drosophila* and mammals (illustrated in Figure 1). The *Anopheles* genome project, which is in its early stages, will also try to identify the genetic differences that allow some mosquitos to sequester and kill *Plasmodium*.

Piet Borst discussed some of his research on another parasite, *Trypanosoma brucei*, which causes sleeping sickness. This parasite uses a very specific insect host, the tsetse fly, which bites a broad range of mammals and can thus infect many hosts with the parasite. This broad host range requires the parasite to adapt to the different genetic make-up of the host, and to develop mechanisms for evading the immune system. *Trypanosoma*

brucei has tackled these problems by varying its surface glycoprotein and transferrin receptor, which can produce more than 1000 unique coats and permit uptake of iron via transferrins that can have <70% homology between hosts, respectively. This high variability is not achieved by gene rearrangement but probably by epigenetically regulated gene translocation, resulting in only one transcriptionally active site without changes in promoter sequences.

The talk containing the most worrisome data this year was presented by Charles Weissman (MRC Prion Unit, Imperial College School of Medicine, London, UK). Prion proteins are responsible for spongiform encephalopathies, which have been increasing in incidence over the past few years. In particular, a new variant of Creutzfeldt-Jakob Disease (nvCJD) appears to be caused by a prion protein that is identical to the Bovine Spongiform Encephalopathy (BSE) prion protein, suggesting that this prion has crossed the species barrier and 'adapted' to humans. Insidiously, misfolded-prion protein binds to metal surfaces (from simple steel wire to complex brain electrodes) and, in this state, is actually more effective at causing spongiform encephalopathies. This was exemplified by the fact that three patients in Britain were infected with, and died of CJD after treatment with an electrode used for an electro-encephalogram. The first patient (at that time not diagnosed as CJD positive) carried the disease, which was subsequently transmitted by the electrode (although sterilized) to the other two patients. Currently, incited by the idea that metal is involved in stabilizing the scrapies form, the involvement of copper (Cu) is being studied. Its putative stabilizing role might explain the dominance of the scrapies form over the normal form of the protein. Hopefully, the discovery of the biological role of prion proteins will help in finding a way to fight against this disease, which currently inevitably results in death.

Gene therapy, a dream come true?

In the fight against disease, and more specifically cancer, new therapies and anti-cancer drugs are in the process of development. Major obstacles that have been encountered so far include metastasis, multidrug resistance and efficient targeting of the diseased tissues. Up to this point, most of the therapies that have been discussed in this review have focussed on inhibition of novel targets that have been discovered by molecular biologists. These therapies can cure the disease, but they do not correct the genetic alterations that are its source. Gene therapy mediated by viral vectors has been heavily promoted but has not come into widespread clinical use. Moreover, in one case of adenovirus gene therapy at the stage of a Phase I trial, the patient died two days after treatment (Somia and Verma, 2000), which has resulted in the development of stricter guidelines for the use of gene therapy.

So far, the best results have been obtained with adenovirus associated virus (AAV) vectors and lentivirus vectors. The big advantage of the former is the targeted integration into the genome (although at very low efficiency) by homologous recombination. The new generation of lentiviral vectors, however, has several advantages: the ability to infect both dividing and non-dividing cells, the presence of inducible

promoters, and the ability to induce a mild immune response, so that reuse of the vector in the same patient has become possible.

The first real success of gene therapy was obtained last year with three babies who suffered from the fatal X-linked form of severe combined immunodeficiency syndrome (SCID-XL). Haematopoietic stem cells from the patients were transduced *ex vivo* with a recombinant mouse leukaemia viral vector containing the γ C-receptor gene and infused back into the young patients. After 10 months, T-, B- and NK-cell counts and function were comparable to those of age-matched controls (Cavazzana-Calvo *et al.*, 2000). Nevertheless, it will take a lot more research to eventually render this technique efficient in the fight against genetic and acquired diseases. In the future, it will hopefully be possible to specifically diagnose and analyse every cancer type. This, combined with the possibility of modifying the viral vectors for targeting to specific cell types, might result in a very powerful therapy against cancer.

Other approaches to the development of anti-cancer drugs were presented by Axel Ullrich and Patrick Baeuerle, who discussed antibody therapies against cancer and the mimicking of signal peptides. The few products that are already on the market are extremely effective, but are also very specific and cannot be used against multiple cancer types. The first generation of antibody therapies was also very immunogenic and could not be used more than once. Modifications to these novel drugs, however, might allow the body to fight back against the diseases that it has inflicted on itself.

Conclusion

All in all, the summer school gave a very good overview of the many difficulties in the fight against disease. The combination of all the information that has been gathered from different fields of research is leading to the development of novel anti-disease strategies. Sometimes, a simple understanding of the biology of the system is all that is needed, as in the case of tsetse fly traps. These \$1 traps are used to catch the flies, which effectively limits the spread of sleeping sickness.

In many cases, though, the solution is not as evident. Even after more than 80 years of progress in molecular biology, no one knows how a single eukaryotic gene is regulated. With evolution as the only common theme linking all biology from atomic structure to organismal function, molecular biology will probably remain an empirical science for some time. Nevertheless, the meeting's talks indicated that even in its current state, molecular biology is capable of making significant contributions to the fight against disease. In individual cases, as for e.g. EGFR inhibitors, this may take some time, but scientists will be able to make progress, given sufficient effort and ingenuity (and funding). The recent completion of the genome sequences from several multicellular eukaryotes, including man, should aid in the search for new targets that can then be exploited to regulate cellular processes that lead to disease.

The excitement generated by the topic of this meeting was enhanced by the perfect location on the island of Spetses (Greece). The relaxed setting inspired informal, stimulating discussions between students, post-docs and lecturers. There were over 120 posters concerning an enormous range of different topics, which forced the organization to include an

additional poster session. After two weeks of 'Molecular mechanisms of development and disease' everybody was either reluctant to go home and leave this inspiring meeting, or could not wait to get home to start new experiments in the laboratory.

Finally, we can only wish for researchers to stay critical and to continue to ask the right questions. An influx of new ideas, approaches and solutions will come from the next generation of researchers who will be full of energy, willpower, a bit of idealism, and respect for the complexity of the systems that they investigate.

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