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Meeting report

Molecular analysis of brain development and function

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Recent progress in the field of vertebrate developmental neurobiology has been made not only in areas which have become classical (pattern formation, neural crest) but also in relatively new subjects such as the behavioural analysis of mutations affecting CNS development, and the properties of stem cells and how to exploit them to treat disease. Participants in the meeting ('Molecular Analysis of Brain Development and Function', 14–19 June, Tourtour, France) organized by Peter Gruss and Nicole Le Douarin and funded by the Fondation Les Treilles use approaches to developmental biology that reflect this variety of subjects.

Marnie Halpern (Carnegie Institution, Baltimore) discussed some of the early events of patterning of the neural tube that are already initiated during gastrulation. Previous work has shown that the notochord induces the formation of the floor plate and motor neurons in the ventral neural tube via the signalling molecule sonic hedgehog (*shh*). However, the analysis of zebrafish mutants that affect notochord and/or floor plate development suggests that earlier events also control the formation of floor plate and motor neurons. For example, *shh* and the related *twhh* gene are coexpressed in the shield, the zebrafish equivalent of the 'organizer', that contains precursors for the prechordal mesoderm, notochord and floor plate. Subsequently, the notochord and floor plate express only *shh* and *twhh*, respectively. However, in the nil mutant in which notochord development is blocked, *shh* and *twhh* continue to be coexpressed and there is an expansion of the floor plate, consistent with a switch of notochord precursors to a floor plate fate. The floor plate fails to form and *shh* is not expressed in a mutant of the *cyclops* gene, that encodes a nodal-related signalling molecule expressed in the shield and the prechordal mesoderm. These defects in *cyclops* mutants are rescued by injection of RNA to express *cyclops* in prechordal mesoderm (but not when expressed in notochord), suggesting that this tissue has an important role in induction as it migrates past the presumptive spinal cord. Later in development, *cyclops*, antivin (a TGFβ-related gene), and the pitx2 transcription factor are expressed in an asymmetric manner, on the left side of the presumptive pineal organ in the dorsal diencephalon. These genes form part of a regulatory cascade in which *one-eyed pinhead* (*oep*), required for nodal signalling, is essential. By injecting *oep* RNA into *oep* mutants, early gastrulation defects are rescued, but not the later role in left-right asymmetric gene expression. Intriguingly, this leads to a mispositioning of the normally asymmetric location of the pineal organ, indicating a role in the generation of anatomical asymmetry in the forebrain.

One major theme of the meeting was the role of transcription factors in pattern formation along the anteroposterior and dorsoventral axes in the mouse midbrain and forebrain. **Antonio Simeone** (IIGB, Milan, and Guys Hospital, London) discussed the relative roles of Otx1 and Otx2 in the specification of the anterior brain. The forebrain, midbrain and anterior hindbrain are missing in Otx2 −/− mutants, whereas Otx1 −/− mutants only have later defects in the telencephalon and eye, and in semicircular canals in the inner ear. Otx2 function is required at early stages in anterior visceral endoderm (AVE) that induces anterior neural tissue, and is subsequently expressed in, and required for the maintenance of, forebrain and midbrain territory. Furthermore, the interface of Otx2 and Gbx2 expression at the presumptive midbrain/hindbrain interface is required for formation of the isthmic organizer, a signalling centre expressing FGF8 that induces the midbrain and anterior hindbrain. Whereas the isthmus forms normally in Otx2 +/− mutants, in Otx1 −/−, Otx2 +/− mutants FGF8 expression shifts anteriorly into the posterior forebrain and fails to become restricted to its normal narrow domain. However, forebrain tissue is not transformed into midbrain, suggesting that Otx gene function is required for the competence to respond to FGF8. These results suggest that Otx1 and Otx2 have at least partly overlapping roles, and that a minimum Otx gene dosage is required for anterior brain development. This raises the question of whether the different phenotypes of Otx1 and Otx2 mutants reflect divergent biochemical properties of these transcription factors, or

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differences in their regulation. A 'knock-in' of Otx2 to replace Otx1 rescues the late defects in brain development, but have defects in the semicircular canals, suggesting a specialized role of Otx1 in ear development. The converse replacement of Otx2 by Otx1 leads to a failure to maintain the anterior brain. Intriguingly, although the knocked-in Otx1 gene expresses RNA in the AVE and anterior brain, Otx1 protein is only present in the former tissue. Furthermore, a knock-in that inserts exogenous sequences into the 3′ untranslated region of Otx2 RNA inhibits its translation in the anterior brain but not in the AVE. Taken together, these findings reveal that translation is an important level of control of Otx2 gene expression in the brain.

Wolfgang Wurst (Max Planck Institute of Psychiatry, Munich) discussed the interactions and roles of genes expressed in the isthmic organizer. En1 and En2 are expressed on both sides of the isthmus, whereas expression of FGF8 and Pax2, and of Wnt1 becomes restricted to reciprocal domains, posterior (midbrain) and anterior (anterior hindbrain) to the isthmus respectively. Analysis of En1/En2, Pax2 and Wnt1 mutants reveals that these genes are mutually interdependent for maintenance of their expression and formation of the midbrain and anterior hindbrain. When the boundary of Otx2 gene expression was shifted posteriorly by generating a knock-in of Otx2 into En1, there was a corresponding posterior ectopic expression of Wnt1 and down regulation of Gbx2. Concomitant with this, there is a deletion of anterior parts of the cerebellum that are derived from the anteriormost part of the hindbrain. Together with other data, these findings show that Otx2 and Gbx2 mutually repress each other and position the isthmus, and that they initiate a network of signalling and transcriptional regulation that maintains the organizer and regulates regional fate. It has been assumed that Wnt1 may have a similar role to Wnt genes in other tissues and systems, as a signal that regulates the fate of adjacent cell populations. To test this, the boundary of Wnt1 was shifted posteriorly by creating a knock-in into the En1 gene. Unexpectedly, it was found that there was a major increase in the size but not the patterning of midbrain derivatives, whereas anterior hindbrain derivatives were not affected. This larger size was due to an increase in cell proliferation at the time when the endogenous Wnt1 gene would normally be downregulated, but the knocked-in Wnt1 gene expression was maintained. Wnt1 therefore has an important role in the control of cell proliferation in the midbrain that requires correct temporal regulation of its expression.

The cerebral cortex has been regarded by some developmental neurobiologists as a last frontier to describe and explain with the tools of molecular biology. Today it is widely accepted that cells in the cortical primordium have some information about their presumptive fate even before being reached by incoming thalamic axons. The data of **Edoardo Boncinelli** (DIBIT, Milan) reveal that the transcription factor Emx2 could be part of that information. By means of antibody staining it can be demonstrated that Emx2 is expressed in the ventricular layer of the embryonic cortex in a caudo-rostral and latero-medial gradient. This implies an early polarization (and perhaps an early definition of areas) of the cortex before any subcortical axons have reached it. Consistently, in mice deficient in Emx2 the areal specification is altered, to judge by the expression of markers like Id3, cadherin 6 and Lamp. In general, the mutant cortex is shifted caudally and medially, and its connectivity is altered accordingly. An intriguing hint about how Emx2 could exert its effect is given by the fact that this protein can also be found in the nuclei of a peculiar cell population of layer 1 which expresses reelin (Cajal–Retzius cells). The Emx2 null mutant lacks this population of reelin-expressing layer 1 cells from E14.5 on. The subplate of the mutants is also very reduced, especially at caudal levels (even if Emx2 is not expressed in the subplate). In these mutants, cortical cells cannot migrate through the subplate (perhaps because of the absence of reelin-containing Cajal–Retzius cells?).

Also expressed in the ventricular layer of the early cortex is the transcription factor Pax6. **Anastassia Stoykova** (Max Planck Institut, Göttingen) reviewed the evidence that Pax6 has an essential role in the control of the number and differentiation of cortical radial precursors. A novel function for Pax6 is in the delimitation of the telencephalic neuroepithelium at two borders: those between pallium/subpallium and lateral/medial ganglionic eminences. At the second of these, Pax6 appears to limit the ventralizing activity of sonic hedgehog/Nkx2.1. This is similar to what has been reported for the spinal cord, and suggests that Pax6 might be a common modulatory cue for the dorso-ventral patterning and cell specification in the entire neural tube. The defective dorso-ventral patterning in the telencephalon of the natural Pax6 mutant *Small eye* has as a result morphological defects in the cortico-striatal border, dysgenesis of the piriform and lateral cortex and of the amygdala as well as thalamo-cortical and cortico-fugal pathfinding abnormalities. The results suggest also that in some cortical progenitors Pax6 might regulate the differentiation of a subpopulation cortical precursors, acting upstream transcription factor Ngn2.

Expression studies have suggested that different combinations of POU-III transcription factors are involved in the further partitioning of the basal ganglia neuroepithelium. **Oscar Marín** (Nina Ireland Laboratory, UCSF) showed results indicating that an increasing number of other genes (including Mash1, Nkx2. 1, Emx2) can be added to the list of transcription factors that contribute to the subdivision of the telencephalic neuroepithelium into progenitor domains. The origin and specification of striatal interneurons is an ideal model to test this hypothesis. By combining injections of DiI and retroviral markers with detection of Nkx2.1 expression on cultured slices of mouse embryonic telencephalon, it can be shown that many cells migrating tangentially from the medial ganglionic eminence (MGE) differentiate into interneurons destined to the striatum (and probably to the cortex too). Consistently, mice defi-

cient in Mash1, which have a reduced MGE, show a large decrease in cholinergic interneurons in the lateral ganglionic eminence (LGE), although the numbers of striatal interneurons expressing NPY are much less affected. Mice deficient in Dlx1 and Dlx2 show something of the opposite effect: reduced striatal NPY interneurons, but almost normal numbers of the cholinergic variety. The results show how the large variety of transcription factors and their combinations is at the source of the variety of interneurons that can be found in the basal ganglia. If the progenitor domains of the neuroepithelium are the beginning of the story, perhaps we can consider behaviour as the end towards which brain development is finally oriented.

Gonzalo Alvarez-Bolado (Max Planck Institut, Göttingen) is trying to put together a model that starts with transcription factors in the diencephalon and ends with behavioural alterations. Correct expression of winged helix transcription factor Foxb1 (Fkh5) in a specific population (a subdivision of the zona limitans?) of radial precursors in the diencephalic neuroepithelium is essential for the entrance of mammillary axons (hypothalamus) in the dorsal thalamus. Immediately after this navigational defect, the lateral and medial mammillary nuclei, source of these axons, are dramatically reduced in size owing to apoptosis. Degeneration of the mammillary body is the cause of severe memory problems (anterograde amnesia) in humans affected of Korsakoff syndrome. Data obtained in collaboration with Kostik Radyushkin (Anokhin Institut, Moscow) show that adult Foxb1 mutants score well in a series of experimental paradigms that test for learning abilities (among them fear-conditioning, social transmission of food preference). The mutants show, however, a very specific defect in spatial learning that can be detected in the Morris water maze. This mutation can be a model of how the precise information contained in a gene can affect a number of cellular processes which affect a whole brain system and are then reflected in behaviour.

A further part of the diencephalon is the optic system (eye stalk, eyecup and retina), which is partitioned by the action of transcription factors. **Peter Gruss** (Max Planck Institut, Göttingen) discussed recent genetic and molecular evidence that transcription factors Pax2 and Pax6 inhibit each other to subdivide into optic stalk (Pax2 territory) and retina (Pax6 territory) the limited patch of diencephalic neuroepithelium allocated to the optic primordium. The respective mutant phenotypes are in agreement with this hypothesis: deficiency in Pax2 leads to a reduced and altered optic stalk, while mouse mutant embryos lacking Pax6, do not develop a retina. In addition, the promoters of both transcription factors have binding sites for each other. A key experiment involves Shh, an important regulator of forebrain differentiation expressed in the ventral midline of the forebrain. Overexpression of Shh in a transgenic background leads to embryos with a larger Pax2 expression domain (which translates into a longer optic stalk) and a smaller Pax6 expression domain (resulting in a smaller retina). These results suggest that Pax2 and Pax6 subdivide the optic primordium by competing to regionalize the neuroepithelial patch either as stalk or retina; and that Shh participates in the competition by indirectly activating Pax2 and inhibiting Pax6. Consistently, the reported phenotype of mouse embryos deficient in Shh includes complete absence of optic stalk and one patch of Pax6 expression in the middle of the ventral diencephalon. Injection experiments in *Xenopus* oocytes show that Vax1, a transcription factor expressed in the optic stalk, inhibits the expression of Rx, a key retinal differentiation regulator. This suggests that a 'push–pull' contest between transcription factors could be a general mechanism to regionalize the forebrain neuroepithelium.

In addition to the control of regional and cell type identity discussed in the above talks, the establishment and maintenance of patterns of cellular organization and neuronal connections requires the regulation of cell and axon movement. The Eph receptors and ephrins are important regulators of cell and axon movement, and **Mark Henkemeyer** (University of Texas Southwestern) presented data that reveal new aspects of their developmental functions. EphB receptors interact with the transmembrane ephrin-B proteins, and biochemical and functional studies suggested that each of these components can transduce signals leading to a repulsion response. The analysis of EphB1, EphB2 and EphB3 receptor knockouts indicates that there are defects in the crossing of axons across the midline at a number of locations in the CNS. Whereas in some cases the EphB receptor transduces signals required for axonal pathfinding, in others it is acting as a ligand to activate signalling through ephrin-B protein expressed in axons. In a triple knockout of these three EphB receptors, there is also a defect in the fusion of the dorsolateral neural epithelium to form the neural tube. Furthermore, EphB2fEphB3 null mutants have hypospadia, in which there is a problem in the midline fusion of endodermal cells that normally leads to the separation of the urethra and colon. A similar phenotype is observed after a knock-in in which the intracellular domain of ephrin-B2 is replaced with β-galactosidase such that it can act as a ligand but cannot transduce signals. Since EphB2 is expressed in endodermal cells at the site of normal fusion, and ephrin-B2 is throughout the epithelium, their interaction seems to promote epithelial fusion. These findings provide important in vivo evidence for the emerging idea that in some contexts Eph receptors and ephrins can mediate adhesion rather than repulsion. Still further surprises have come from investigation of defects in inner ear function in EphB2fEphB3 mutants. Rather than being due to problems in innervation, it was found that EphB2 is required for the function of the secretory epithelium. Eph receptors contain an interaction motif for PDZ domain containing proteins, and it was shown that this couples Eph receptors to aquaporin proteins that transport water across the plasma membrane.

Other aspects of Eph receptor and ephrin-B function were discussed by **David Wilkinson** (National Institute for Medi-

cal Research, London). Eph receptors and ephrin-B proteins are expressed in complementary segmental domains in the developing hindbrain, and the results of ectopic activation and blocking experiments in zebrafish embryos had suggested a role in preventing mixing between segments. By mosaically expressing Eph receptor or ephrin-B, it was shown that activation of either component leads to cell sorting within the hindbrain, suggesting that bidirectional responses could occur at the interface between segments. Direct evidence for a role of bidirectional signalling was obtained by analysing cell mixing between a zebrafish animal cap expressing exogenous Eph receptor and an animal cap expressing exogenous ephrin-B. Bidirectional signalling prevented mixing between the cell populations, whereas unidirectional activation of Eph receptor or ephrin-B did not. However, unidirectional activation was sufficient to prevent gap junctional communication. By structure–function mapping in the animal cap assay, evidence was obtained that signalling through ephrin-B proteins involves both tyrosine phosphorylation and interactions with PDZ domain proteins, and that these have distinct roles in the restriction of cell intermingling. These findings support a model in which Eph receptor and ephrin-B activation each lead to a repulsion response, such that bidirectional activation at a boundary underlies a mutual repulsion that prevents each cell population invading the other. These molecules may therefore stabilize hindbrain segments and other tissue domains by preventing cell intermingling, such that they form 'compartments', and by restricting cell communication via gap junctions between segments.

Studies in *Drosophila* embryos have shown that the boundaries between compartments often act as signalling centres that control local patterning. **Donna Fekete** (Purdue University) discussed evidence that such a principle could be involved in generation of the highly complex pattern of the inner ear. A number of genes encoding transcription factors, such as Pax2, Soho1 and Otx1, are expressed in specific domains along the anteroposterior (AP), mediolateral (ML), and dorsoventral axes of the otic placode and vesicle. Furthermore, BMP4 is expressed at the boundaries between some of these domains, and the saccule, cochlear and endolymphatic duct arise at specific locations in relation to the boundaries. Cell lineage analyses show that there are stereotyped cell movements and that there is a restriction to mixing across the AP boundary. Taken together with the effects of gene knockouts, these observations support a model in which transcription factors may act as compartment identity genes, and the boundaries control local patterning. An important aspect of the generation of the highly complex three-dimensional pattern is likely to be the closure of the otic placode to form a vesicle such that new interfaces will form between distinct domains.

Another important area of developmental neurobiology concerns the formation, migration and differentiation of neural crest cells. Two talks focused on the question of how the migration of neural crest cells from the dorsolateral neural plate is initiated. **Chaya Kalcheim** (University of Jerusalem) presented evidence that, in addition being involved in the formation of neural crest, BMP signals induce the delamination and migration of these cells in the trunk. BMP4 is expressed uniformly along the dorsal neural tube, while its antagonist Noggin is expressed in these cells in a high caudal to low rostral gradient. In the rostral spinal cord (low Noggin) neural crest migration has initiated, whereas in the caudal spinal cord (high Noggin) neural crest is still premigratory. Furthermore, application of Noggin, either in vivo or to neural tube explants, inhibits the initiation of migration, whereas exogenous BMP4 accelerates the onset of migration. It is therefore important to understand how the downregulation of Noggin is controlled, thus allowing BMP4 to induce migration. By carrying out tissue ablation experiments, it was shown that the dorsal somite contains an activity required for the downregulation of Noggin, accounting for the coordination of neural crest migration with the rostral to caudal wave of somitogenesis. Activation of BMP receptors induces the expression of genes, such as rhoB and Cad6B, implicated in the morphogenetic movements of delamination and migration, and it was found that Noggin down-regulates the expression of these genes. Noggin also downregulates Wnt1 expression, and by use of an inhibitor of Wnt function, evidence was obtained that Wnt signalling is required for the delamination of neural crest.

Another key regulator of neural crest delamination and migration was discussed by **Angela Nieto** (Cajal Institute, Madrid). In the chick embryo, the *slug* zinc finger gene is the earliest known marker of premigratory neural crest, and previous work implicated it in the control of delamination and migration. The related Snail gene is expressed subsequently in the migrating neural crest in the chick, consistent with the idea that Slug and Snail may act to initiate and maintain migratory behaviour. Surprisingly, it was found that this temporal order is reversed in the mouse, with Snail expressed in premigratory neural crest, and Slug in migrating neural crest. A survey of expression in different vertebrate species found that the pattern observed in the mouse occurs in fish, and that the swap in timing of expression occurred in the lineage leading to reptiles and birds. This finding suggests that Slug and Snail may have similar targets, and indeed overexpression of either gene in the neural tube led to an increase in the number of migrating neural crest cells. Furthermore, overexpression of Snail in epithelial cell lines induces a mesenchymal phenotype, and expression of the endogenous gene has a striking correlation with the invasiveness of tumours. In epithelial cell lines and tumours, E-cadherin expression is downregulated in the presence of Snail, suggesting that loss of this adhesion molecule is an important step in the epithelial to mesenchymal transition. This regulatory relationship is direct, since Snail is a transcriptional repressor that binds to regulatory sequences of the E-cadherin gene.

A major question concerning the development of neural

crest is how their differentiation into a wide variety of derivatives is controlled. **Nicole Le Douarin** (Institut d'Embryologie Cellulaire et Moleculaire, Nogent-sur-Marne) discussed evidence for plasticity and restrictions in the fate of neural crest. Neural crest from specific axial levels gives rise to parasympathetic ganglia whereas at other levels they give rise to sympathetic ganglia. The results of transplantation experiments show neural crest cell populations are not committed to these fates prior to migration, but rather their fate depends upon anteroposterior location. A related issue is whether at the single cell level there are totipotent stem cells and/or more restricted pluripotent precursors, and clonal analysis provides evidence for both classes of cells. Insights into factors regulating the differentiation of cells into melanocytes has come from the analysis of the roles of endothelin3 (ET3) and endothelin receptor B. A requirement in melanocyte differentiation is demonstrated by the observation that mutations in these genes are responsible for coat colour defects (as well as deficiencies in the enteric nervous system). When neural crest cells in culture are treated with ET3, there is a major increase in the number of melanocytes at late stages, but no change in the number of neurons. Furthermore, in clonal analyses it was found that there is an increase in the number of clones giving rise to melanocytes, glial cells or both, but not in glial/neuronal or glial/neuronal/melanocyte clones. Remarkably, if differentiated pigment cells are cultured in the presence of ET3, both pigment and glial cells are produced. Similarly, Schwann cells transdifferentiate to form some pigment cells in the presence of ET3. Taken together, these findings show that ET3 acts specifically on melanocytes, glial cells, and their intermediate precursor, but not on other intermediate precursors or totipotent neural crest. Furthermore, ET3 can induce differentiated cells to revert to an intermediate glial/melanocyte precursor phenotype.

One of the most exciting and rapidly growing fields in developmental neurobiology is that of neural stem cells in the embryonic and adult brain. Neurons are continuously generated in certain regions of the central nervous system. These neurons derive from multipotent, self-renewing neural stem cells. The first observations about adult neurogenesis were made in the cortical subventricular zone, which produces neurons for the olfactory bulb. This system, together with the dentate gyrus, which is known to produce granular cells in the adult, has become a favourite model to study the properties of adult neural stem cells. **Georg Kuhn** (University of Regensburg) discussed data indicating that production of neural cells by these systems can be modulated. Infusion of growth factors like EGF and FGF2 is known to increase neurogenesis and to affect the proportion of neurons to glia formed by the neural stem cells: EGF produces a large increase in the numbers of both neurons and glia, while FGF2 causes a smaller increase in production, but mostly of neurons. There is evidence that growth factors exert their modulatory effects on neurogenesis through tyrosine kinase receptors; these would in turn affect the transcription of genes involved in cell proliferation. Favourite suspects are transcription factors of the E2F family, known regulators of cell cycle progression and cell division. Of the five members of the family, E2F1 and E2F2 are expressed in the ventricular and subventricular zone of the brain. Accordingly, mice mutants deficient in E2F1 or in E2F2 (and particularly those deficient in both) show decreased adult neuronal proliferation. Selective pharmacological destruction of serotonergic, noradrenergic or cholinergic inputs to adult neurogenetic areas has differential effects on the number of BrdU-labeled cells found in the neuroepithelium. This intriguing results suggest that afferent inputs to the proliferating areas differentially affect the rate of adult neurogenesis.

It has been thought that the differentiation potential of adult stem cells was limited to the cell types characteristic of the organ where they appear. **Jonas Frisén** (Karolinska Institut, Stockholm) has used adult neural stem cells from ROSA26 mice (β-galactosidase-expressing) to demonstrate that adult neural stem cells can generate cells of every germ layer. When adult neural stem cells are cultured together with embryoid bodies, many of them differentiate into myocytes. Adult neural stem cells (from the ventricle-lining ependyma or from the subventricular layer) can be cultured as clonal aggregates or 'neurospheres'. Mouse-derived neurospheres injected in the amniotic cavity of chick embryos can incorporate into the embryo and give rise to perfectly differentiated cells in ectoderm-, mesoderm- and endoderm-derived organs. This is also true if the injected neurospheres are clonally formed from one single ependymal cell. Injection of mouse-derived neurospheres into early mouse blastocysts can give rise to chimaeric mouse embryos where β-galactosidase cells can be found in many organs, always fully differentiated according to the host tissue. Although 'blue' cells are also found in the germ line, it is not known at present if they would be functional. This evidence suggests that adult neural stem cells could have a degree of multipotentiality approaching that of embryonic stem cells.

One step beyond 'natural' neurogenesis is the harvesting of embryonic neural stem cells and their utilization to produce neurons in vitro for transplantation or to test new pharmacological compounds. **Clive Svendsen** (MRC, Cambridge, UK) discussed recent progress in the development of consistent and reliable protocols to generate in vitro human neurons with the desired phenotypes. If grown on a substrate in the presence of FGF2, cells isolated from the neural tube of early human fetuses can generate neurons, astrocytes and oligodendrocytes. Human neural precursor cells expanded in culture for short periods can be grafted into the striatum of adult rats with lesions of the dopaminergic system, where only occasionally some of them express tyrosine hydroxylase and can revert the effects of the lesion. This suggests that it is safer to differentiate the human neural precursors in vitro, before transplanting. One

possibility would involve 'shepherding' the neurons through differentiation pathways leading to the dopaminergic phenotype. Recent data obtained in rodents, however, indicate that rodent neurospheres differentiate more often into those neuronal types characteristic of the region of the neural tube from which precursors were collected. Therefore, the neural precursors are regionally specified in such a way that they have different phenotypic potential. Consequently, in order to obtain large numbers of dopaminergic cells in vitro, we are better off if we start with human neural precursors harvested from the midbrain. This regional specification does not exclude the existence of earlier, non-regionalized stem cells common to the whole neural tube; these could simply divide more slowly and be flooded by more committed precursors. Another important finding is that neural precursor cells from mouse, rat and human show different requirements for growth in vitro, so that we cannot directly apply what we learn from animal models to the culture of human neurons. Work with human cells is essential if knowledge with eventual clinical applications is to be obtained.

Of course there is another way to put stem cells to good use: gene trapping represents a tried and true method to go from embryonic stem sells to the discovery of novel genes. One popular strategy is to electroporate the ES cells, then fish for the trapped genes and classify them by sequence. The opposite strategy consists of generating the mutant mice, analysing the patterns of expression of the reporter and focusing on those gene trap events that show a more interesting distribution. The advantage in this case is that, together with the novel gene, the mutant line is immediately available. **Peter Gruss** (Max Planck Institute, Göttingen) presented a selection of novel genes obtained by this method. According to the corresponding mutant phenotypes, all of these genes have interesting developmental functions. *Apaf1* codes for an essential component of the apoptotic pathway whose deficiency leads to overgrown brain and retina, failure to fuse in the midline in the face, and preserved interdigital membranes. The *Querkopf* mutation defines the gene for a histone acetyltransferase of the MYST family involved in cortical development. In its absence, the cortical plate is very reduced, which translates in a smaller adult cortex with a dramatic reduction in GABAergic interneurons and in layer 5 pyramidal cells. *HSP90beta* is a 90 kDa heat shock protein expressed ubiquitously but the effects of whose deficiency are limited to the placenta. Mutant trophoblast cells fail to differentiate leading to placental failure and death of the conceptus.

Developmental neurobiology is progressing at a breathtaking pace. A cell and molecular description of the events leading to neural crest differentiation and brain regionalization seems within reach. Its practitioners are also looking forward to contributing to fields such as behavioural science and the therapy of neurodegenerative diseases. In this exciting and rapidly changing environment, the meeting in the secluded provencal domain of Les Treilles was a particularly welcome occasion to take stock of some of the many areas of progress.