Brain development is a complex process that involves several sequential steps: regional determination, specification of neuronal cell types, control of cell migration, guidance and formation of neural connective networks, and activity-dependent synaptic plasticity. Recent research has demonstrated that these steps are exquisitely controlled by a variety of molecular and cellular mechanisms, including the expression of specific transcription factors, the activity of morphogens and growth factors, the expression of guidance molecules and extracellular proteins, and synaptic activity. Our research focuses on the identification of new genes involved in these processes, and the characterisation of the intracellular signalling pathways activated in growth cones in response to extracellular signals. Moreover, it is known that the adult brain does not regenerate, either after lesions or disease-associated cell-death processes. Studies on the mechanisms that govern the normal development and growth of the nervous system are essential to explain the lack of spontaneous brain repair in adult tissue and to design new regenerative approaches to repair brain lesions.

Further roles of netrins and semaphorins in neuronal guidance

We have further investigated the roles of several guidance molecules in the formation of complex brain structures, such as the cerebral cortex and the cerebellum. For instance, elucidating the way in which GABAergic interneurons in the cerebellar cortex migrate or finding the guidance cues that steer them are part of our research efforts. Recent data show that the development of interneurons starts at the cerebellar germinal epithelium on top of the fourth ventricle. These interneurons continue to proliferate in the postnatal cerebellar white matter and later migrate to their final position in the cerebellar cortex. We have demonstrated a chemorepulsive action of Netrin1 on postnatal cerebellar interneurons in vitro; we have also reported the expression pattern of Netrin1 and its receptors DCC and Unc5 in the developing cerebellar system. Our expression results corroborate that Netrin1 is involved in the migration of GABAergic interneurons in vivo. Moreover, our data point to Bergmann glial fibers as possible tracks for these cells en route to the molecular layer. Finally, experiments using blocking antibodies have allowed us to conclude that DCC, although expressed by postnatal cerebellar interneurons, is not involved in the repulsive response triggered by Netrin1 in these cells (Guijarro et al, 2006).

We have also studied the distribution and role of a specific variant of semaphorin Y/6C (Sema6C) in mouse forebrain development and plasticity. Growth cone collapse of entorhinal and pyramidal neurons, as well as activation of glycogen synthase kinase-3 (GSK-3) through depletion of the inactive pool, is induced by a diffusible Sema6C1 form, thereby suggesting that this protein participates in development. We found this isoform to be widely expressed during development, remaining in the adult and showing variations in distribution when the perforant pathway was axotomised. These changes were detected in both the hippocampal and entorhinal cortices. In axotomised animals, the ipsilateral hippocampus hemisphere, but not the contralateral, showed that Sema6C-IR had moved into the stratum lacunosum-moleculare, the medial molecular layer of the dentate gyrus (DG) and the fibers, but not the cell bodies, of the entorhinal cortex (EC). These results indicate a specific role for Sema6C variants in the generation and/or stability of circuits and synapses (Burgaya et al, 2006).

The tyrosine kinase ACK1/PYK1 in brain development and plasticity

Cytosolic tyrosine kinases play a critical role both in neural development and in adult brain function and plasticity. We have isolated a cDNA that directs the expression of a 125-kD protein that can be autophosphorylated on tyrosines. This clone corresponds to the mouse homologue of Ack1 (Ack1/
Pyk1) and is a non-receptor protein tyrosine kinase that comprises a tyrosine kinase core, an SH3 domain, a Cdc42-binding region, a Ral homology region, and a proline-rich region. The highest levels of Ack1/Pyk1 expression are detected in the brain, particularly in the hippocampus, neocortex, and cerebellum. Electron microscopy studies show that Ack1/Pyk1 protein is expressed both at dendritic spines and presynaptic axon terminals, thereby indicating that this protein is involved in synaptic function. Furthermore, Ack1/Pyk1 mRNA levels are strongly up-regulated by increased neural activity, which points to a role in plasticity. During development, Ack1/Pyk1 is also expressed in the proliferative ventricular zones and in postmitotic migrating and maturing neurons. These results demonstrate that this kinase is up-regulated during development and that it is expressed in proliferative areas and in migratory pathways in the developing brain. In neuronal cultures, Ack1/Pyk1 is detected in developing dendrites and axons, including dendritic tips and growth cones. Moreover, Ack1/Pyk1 colocalises with Cdc42 GTPase in neuronal cultures and co-immunoprecipitates with Cdc42s (Ureña et al., 2006; De la Torre et al., 2006). Activation of integrins by cell adhesion on fibronectin leads to strong tyrosine phosphorylation and activation of Ack. Upon cell stimulation with EGF or PDGF, Ack is tyrosine-phosphorylated and recruited to activated EGF or PDGF receptors, respectively. Moreover, tyrosine-phosphorylated Ack forms a stable complex with the adapter protein Nck via its SH2 domain (Galisteo et al., 2006). Taken together, our findings indicate that Ack1/Pyk1 tyrosine kinase has a functional role as an early transducer of multiple extracellular stimuli, and that it may be involved in adult synaptic function and plasticity and in brain development.

The axonal growth cone: a sophisticated exploring “apparatus” designed to integrate convergent and divergent signalling pathways

During the development of the nervous system, precisely ordered neuronal connections are formed in a stereotyped, stepwise process. Initially, finely orchestrated expression of
axon guidance molecules and their receptors in the projecting and the target area provide positional and directional information for ingrowing axons, which leads to a coarse connection between distinct groups of neurons. Later, activity-dependent processes, including the formation and elimination of new branches, sharpen the projection, resulting in precise point-to-point connections. Throughout this process, the key apparatus of the growing axons is the neuronal growth cone. This cone can be envisaged as an exploring region at the axonal tips which integrates information from the neighbouring ‘milieu’ to transduce signals that finally may stop or increase axonal growth. In recent years, many signalling pathways that regulate axonal navigation have been identified (eg, netrins, semaphorins, ephrins, etc.), each bearing a full complement of receptors and associated intracellular mediators. However, how these signalling pathways, often with opposite effects, interact with each other, the hierarchy among them (if present), or how ligand/receptor complexes talk to other components of cell machinery, like cytoskeletal proteins and proteins regulating membrane trafficking, are not known.

Our research activities explore these issues by means of simple neuronal culture models. For instance, we have recently discovered a protein-to-protein interaction between the DCC guidance receptor and the SNARE proteins Syntaxin 1 and SNAP-25. Furthermore, these SNARE proteins are required for Netrin1/DCC-induced axonal guidance and migration, both in vitro and after electroporation in the spinal cord. These data point to a link between guidance receptors and the cell machinery controlling exocytosis and membrane addition (Cotrufio et al., in preparation).

Similarly, we explore cross-talk mechanisms between guidance molecule receptor systems. For instance, we have evidence of an interaction between the neurotrophin/trk cascade and the Netrin1/DCC and EphrinA-associated signalling pathways. We have recently shown that activation of EphrinA blocks neurotrophin-induced effects on axonal branching and synapse formation (Marler et al., 2008).

Dissecting novel Reelin functions in development and neurodegenerative diseases

Reelin is a glycoprotein that is essential for the correct cytoarchitectonic organisation of the developing central nervous system (CNS). Reelin binds to very low-density lipoprotein receptor and apolipoprotein E receptor 2, thereby inducing mDab1 phosphorylation and activation of the phosphatidylinositol 3 kinase (PI3K) pathway. We have now demonstrated that Reelin activates the mitogen-activated protein kinase/AKT signalling in an SFK/mDab1- and PI3K-dependent manner and that ERK activation is required for Reelin-dependent transcriptional activation, the detachment of forebrain neurons migrating from the SVZ, and the migration of cerebellar granule cells.

The function of Reelin in the adult brain is not understood, although it has been proposed that this protein is involved in signalling pathways linked to neurodegeneration. We have analysed Reelin expression in brains and cerebrospinal fluid (CSF) from patients with Alzheimer’s disease (AD) and from non-demented controls. We found a 40% increase in the Reelin protein levels in the cortex, but not in the cerebellum, of AD patients compared with controls. Similar increases were detected at the Reelin mRNA transcriptional level. This expression correlates with parallel increases in CSF but not in plasma samples. We also studied the pattern of Reelin glycosylation by using several lectins and the anti-HNK-1 antibody. Glycosylation differed in plasma and CSF. Furthermore, the pattern of Reelin lectin binding differed between the CSF of controls and AD patients. Our results show that Reelin is up-regulated in the brain and CSF in several neurodegenerative diseases and that CSF and plasma Reelin have distinct cellular origins, thereby supporting the notion that Reelin is involved in the pathogenesis of a number of neurodegenerative diseases (Botella et al., 2006). To test this hypothesis, we have generated a conditional transgenic mouse model that overexpresses Reelin in the forebrain. This transgenic mouse line is being crossed with several murine models of AD to ascertain whether the over-activation of the Reelin pathway increases neural degeneration in these mice.

Functions of Nogo-66, MAG and CS in axonal regeneration

Damaged axons do not regenerate after axotomy in the adult mammalian CNS. This may be due to local inhibitory factors at the site of injury, such as the overexpression of chondroitin sulfate (CS) proteoglycans (CSPGs), and the presence of myelin-associated inhibitors. To overcome CSPG- or myelin-induced inhibition, strategies based on extrinsic and intrinsic treatments have been developed. For example, NEP1-40 is a synthetic peptide that promotes axonal regeneration by blocking Nogo-66/NgR interaction, thereby prompting axon regrowth. Myelin-associated glycoprotein (MAG) also contributes to the prevention of axonal regeneration. We have studied the role of MAG, Nogo-66 and CS in the regeneration of cortical connections in vitro. We show that MAG expression is regulated in a distinct manner in the EC and the hippocampus in response to axotomy of the perforant pathway. The participation of MAG in preventing axonal regeneration was tested in vitro: neuraminidase treatment of axotomised entorhino-hippocampal cultures potentiates axonal regen-
ereation (Mingorance et al., 2005). We have also examined whether the combination of complementary strategies facili-
citates axonal regeneration in slice co-cultures. The combi-
nation of CS cleavage with ChABC and NEP1-40 strongly facilitates the regrowth of entorhinal axons after axotomy, permitting the re-establishment of synaptic contacts with target cells. However, combined treatments do not improve the regeneration induced by ChABC alone (Mingorance et al., 2006). These results demonstrate that MAG, CS and Nogo-66 limit axonal regeneration in the cerebral cortex, and pro-
vide insights into the development of new assays and strate-
gies to enhance axon regeneration in injured cortical con-
nexions.

Stem cells, neuronal precursor specification, and brain repair

The nervous system is formed by hundreds of types of neu-
rons. The mechanisms by which the different types of neu-
rons are generated and specified remain unclear. We have shown that in the cerebellum the pancreatic transcription
factor Ptf1a is required for the specific generation of Purkin-
je cells and interneurons. Moreover, we have reported that
granule cell progenitors in the external granule cell layer
appear to be unaffected by deletion of Ptf1a. Cell line-
age analysis in Ptf1acre/Cre mice was used to establish that,
in the absence of Ptf1a expression, E12/E13-proliferating
progenitors—normally fated to produce Purkinje cells and
interneurons—shift to a granule cell phenotype and aber-
rantly migrate to the external granule layer. These findings
indicate that Ptf1a is necessary for the specification and
normal production of Purkinje cells and cerebellar interneu-
rons, two essential GABAergic cell types of the cerebellar
cortex. We have also established that Ptf1a is required for the
suppression of the granule cell specification programme
in cerebellar ventricular zone precursors (Pascual et al., in
preparation). Given the key role of Ptf1a in Purkinje cell
specification, we are now exploring whether the induced ex-
pression of this gene in neuronal stem cells of distinct origin
induces their phenotypic differentiation into a Purkinje cell-
like phenotype. If so, we will have devised a method to pro-
duce Purkinje cells in vitro, thereby facilitating cell therapy
approaches in murine models of cerebellar ataxia.

The production of neurons is a temporally restricted proc-
ress that occurs during embryonic life, except in a few brain
areas (the hippocampus, cerebellum, and the subventricular
zone). In fact, new granule neurons are produced in the DG
of rodents and humans throughout adult life. Understand-
ing the mechanisms that control cell proliferation and neu-
ron production in these areas is crucial to devise therapeutic
strategies aimed at producing neurons from the natural "niches" that contain neural stem cells. Recent studies have
also reported adult neurogenesis in the cerebral cortex of
healthy animals and after brain injury. We have analysed
whether the absence of the synaptic input from the main
hippocampal afferents induces neuronal generation in the
hippocampus outside the DG and/or regulates the prolifera-
tion of DG neuroprogenitors. We have shown that the de-
ervation of the hippocampus does not induce neurogenesis
in hippocampal regions other than the DG. However, neuro-
progenitor proliferation in the DG is reduced after fimbria-
forex lesions but not after entorhinal deafferentation. This
observation supports the view that neuroprogenitor prolifer-
ation and differentiation in the DG are controlled from
basal forebrain/septal neurons. We have also studied cell
proliferation in the hippocampus of rodents and the intrinsic
putative neurogenic potential of EC progenitors. We show
that only the DG generates new neurons in the hippocam-
pus. In addition, neurospheres from the EC have the capac-
ity to differentiate into neurons and glia in vitro and after
transplantation in the adult DG (Fontana et al., 2006). In a
more recent study, we have identified Netrin1 as a key factor
controlling neurogenesis and differentiation of neural stem
cells, specifically in the DG (Barallobre et al., in preparation)
and we are currently focusing our research efforts on eluci-
dating the cellular mechanisms that control symmetrical
versus asymmetrical neuronal cell division.

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Research networks and grants

**Ajuts a grups de recerca consolidats**

**Principal investigator:** Eduardo Soriano

**Funciones de nuevos genes candidatos y proteínas asociadas a mielina durante el desarrollo y regeneración de las conexiones corticales**
Spanish Ministry of Science and Innovation, BFU2006-13651 (2006-2009)

**Principal investigator:** Eduardo Soriano

**Identificació i caracterització d’un nou sistema de senyalització associat a exocitosis i neurotrofines: paper en la generació del dolor**
'La MTV3' Foundation, MTV3-071410 (2008-2010)

**Principal investigator:** Eduardo Soriano

**Identificación y caracterización de nuevos genes y vías de señalización implicados en desarrollo cortical**

**Principal investigator:** Eduardo Soriano

**Implicación de las semaforinas transmembranales y sus receptores en plasticidad sináptica y en enfermedades neurales: Estudio celular y análisis de la transducción de señal**
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**Principal investigator:** Ferran Burgaya

**Papel de la reelina en la formación de conexiones sinápticas in vitro e in vivo y en el desarrollo de enfermedades neurodegenerativas**
Instituto de Salud Carlos III, PI070715 (2008-2010)

**Principal investigator:** Albert Martínez

Collaborations

**Functions of the novel tyrosine kinase Pyk1 in brain development**
Joseph Schlesinger, Yale University (Connecticut, USA)

**Interactions between Ephrin and Trk signalling pathways in axonal navigation**
Uwe Drescher, MRC Developmental Neurobiology (London, UK) and Joan X Comella, University of Lleida (Lleida, Spain)

**Role of Alex-3 in mitochondrial biology**
Antoni Andreu, Vall d’Hebron Hospital (Barcelona, Spain), José Berciano, University of Santander (Santander, Spain), Ramón Trullás, CSIC-IIBB (Barcelona, Spain), Pablo Villoslada, CIMA (Pamplona, Spain), Jaume Bertranpetit, Pompeu Fabra University (Barcelona, Spain), Martin Kerschensteiner, Ludwig Maximilians University (Munich, Germany)

**Role of Alex-3 in Wnt/B-catenin signalling pathway**
Eduard Batlle, IRB Barcelona (Barcelona, Spain)

**Role of CREB family transcription factors in brain development**
Günter Schultz, DKMC (Heidelberg, Germany)

**Role of Netrin1 and NogoR in neural development and regeneration**
Marc Tessier-Lavigne, Genentech (San Francisco, USA)

**Role of Reelin/Dab1 in prionic diseases**
Adriano Aguzzi, University of Zurich (Zurich, Switzerland)