



## Developmental neurobiology and regeneration

*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 work 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 revert brain lesions.*

### **Further roles of netrins and semaphorins in neuronal guidance**

We have further studied 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 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 in 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 Ralt 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 of this protein 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 develop-

ing 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 that 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.

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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 (Cotrufo *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. 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/extracellular signal-regulated kinase (ERK) pathway, which leads to the phosphorylation of Erk1/2 proteins. The inhibition of Src family kinases (SFK) blocks Reelin-dependent Erk1/2 activation. This has also been shown in neuronal cultures from mDab1-deficient mice. Although rat sarcoma viral oncogene was weakly activated upon treatment with Reelin, pharmacological inhibition of the PI3K pathway blocked Reelin-dependent ERK activation, which indicates cross-talk between the ERK and PI3K pathways. We have shown that blockade of the ERK pathway does not prevent the chain migration of neurons from the subventricular zone (SVZ) but does inhibit the Reelin-dependent detachment of migrating neurons. We have also demonstrated that Reelin induces the transcription of the early growth response 1 transcription factor (Simó *et al*, 2006). In addition, we have shown a novel role of Reelin in the migration of cerebellar granule cells, which is highly dependent upon ERK activation (Simó *et al*, 2007). These findings indicate that Reelin triggers ERK 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.

### **Stem cells, neuronal precursor specification, and brain repair**

The nervous system is formed by hundreds of types of neurons. The mechanisms by which the different types of neurons 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 Purkinje 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 lineage analysis in *Ptf1a<sup>Cre/Cre</sup>* 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 aberrantly migrate to the external granule layer. These findings indicate that *Ptf1a* is necessary for the specification and normal production of Purkinje cells and cerebellar interneurons, 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*, 2007). Given the key role of *Ptf1a* in Purkinje cell specification, we are now exploring whether the induced expression of this gene in neuronal stem cells of distinct origin induces their phenotypic differentiation into a Purkinje cell-like phenotype. If this is the case, we will have devised a method to produce Purkinje cells *in vitro*, thereby facilitating cell therapy approaches in murine models of cerebellar ataxia.

The production of neurons is a temporally restricted process 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. Understanding the mechanisms that control cell proliferation and neuron 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 proliferation of DG neuroprogenitors. We have shown that the denervation of the hippocampus does not induce neurogenesis in hippocampal regions other than the DG. However, neuroprogenitor proliferation in the DG is reduced after fimbria-fornix lesions but not after entorhinal deafferentation. This observation sup-

ports the view that neuroprogenitor proliferation 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 hippocampus. In addition, neurospheres from the EC have the capacity 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 elucidating the cellular mechanisms that control symmetrical versus asymmetrical neural cell division.

## Scientific output

### Publications

Aguadó F, Díaz-Ruiz C, Parlato R, Martínez A, Carmona MA, Ureña JM, del Río JA, Schütz G and Soriano E. The CREB/CREM transcription factors negatively regulate early synaptogenesis and spontaneous network activity. *J Neurosci*, **29**(2), 328-33 (2009)

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Gil V, Bichler Z, Lee JK, Seira O, Llorens F, Bribian A, Claverol-Tinture E, Soriano E, Sumoy L, Zheng B and Del Río JA. Developmental expression of the oligodendrocyte myelin glycoprotein in the mouse telencephalon. *Cereb Cortex*, Epub Nov 5 (2009)

Mathew M, Amat-Roldan I, Andrés R, Santos SI, Artigas D and Soriano E. Signalling effect of NIR pulsed lasers on axonal growth. *J Neurosci Methods*, **186**(2), 196-201, Epub Nov 27 (2009)

Montolio M, Messeguer J, Masip I, Guijarro P, Gavin R, Messeguer A and Soriano E. A semaphorin 3A inhibitor blocks axonal chemorepulsion and enhances axon regeneration. *Chem Biol*, **16**(7), 691-701 (2009)

### Research networks and grants

*CIBERNED (Enfermedades Neurodegenerativas)*  
Carlos III Health Institute (ISCIII), RCIBERNED (2007-2010)  
Principal investigator: Eduardo Soriano

*Desarrollo y maduración de la conexión septohipocámpica, implicaciones en la enfermedad de Alzheimer*  
Carlos III Health Institute (ISCIII), PI081891 (2009-2011)  
Researcher: Marta Pascual

*Identificació i caracterització d'un nou sistema de senyalització associat a excitotoxicitat i neurotrofines: paper en la generació del dolor*  
'La Marató TV3' Foundation, MTV3-071410 (2008-2010)  
Principal investigator: Eduardo Soriano

*Identificación y caracterización de nuevos genes y vías de señalización implicados en el desarrollo cortical*  
Spanish Ministry of Science and Innovation, SAF2005-00171 (2009-2013)  
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*  
Carlos III Health Institute (ISCIII), PI070500 (2009-2010)  
Researcher: Ferran Burgaya

*Papel de la reelina en la formación de conexiones sinápticas in vitro e in vivo y en el desarrollo de enfermedades neurodegenerativas*  
Carlos III Health Institute (ISCIII), PI070715 (2009-2010)  
Researcher: Albert Martínez

*Papel de la tirosina quinasa Ack1 en la formación de dendritas y axones en neuronas de neocorteza y de cerebelo. Relación con la enfermedad de Alzheimer y los procesos de 'long-term potentiation'*  
Carlos III Health Institute (ISCIII), PI070942 (2009-2010)  
Researcher: Jesús Ureña

*Paper de la proteïna extracel·lular Reelin en l'estudi cognitiu i la patogènesi de la malaltia de l'Alzheimer*  
Caixa Catalunya Obra Social (2008-2011)  
Principal investigator: Eduardo Soriano

*Potencial del gen Ptf1a/p48 en la regeneración del cerebelo*  
'La Caixa' Foundation, BM06-335-0 (2006-2009)  
Principal investigator: Eduardo Soriano

### Collaborations

*Functions of the novel tyrosine kinase Pyk1 in brain development*  
Joseph Schlessinger, 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*  
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*Role of Alex-3 in Wnt/B-catenin signalling pathway*  
Eduard Batlle, IRB Barcelona (Barcelona, Spain)

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

*Role of Syntaxin1 and Podocalyxins in axonal guidance and brain development*  
Thomas Südhoff and José Rizo-Rey, Southwestern University (Dallas, USA) and Esther Stoeckli, University of Zurich (Zurich, Switzerland)

*Role of the glycogen synthase enzyme in neuronal function and degeneration*  
Joan J Guinovart, IRB Barcelona (Barcelona, Spain)

*Role of the pdf1 gene in cerebellar development and repair*  
Paco X Real, Pompeu Fabra University/IMIM (Barcelona, Spain)

*Transmembrane semaphorins and epilepsy*  
Javier de Felipe, Cajal Institute (Madrid, Spain)

*Ultrasort lasers, axonal guidance and brain repair*  
Pablo Loza, Institute of Photonic Sciences (Barcelona, Spain)