Instituto Juan March
de Estudios e Investigaciones

154 CENTRO DE REUNIONES INTERNACIONALES SOBRE BIOLOGÍA

Workshop on

Developmental Mechanisms in Vertebrate Organogenesis

Organized by

G. Oliver and M. Torres

P. Bovolenta
A. L. Calof
T. Curran
J. Epstein
N. W. Gale
F. Giráldez
F. Guillemot
S. K. McConnell
G. Oliver
O. Pourquie
M. A. Ros
B. Sosa-Pineda
D. Stainier
C. Tabin
I. Thesleff
M. Torres
S. Vainio
D. G. Wilkinson
C. V. E. Wright
K. S. Zaret

Instituto Juan March
de Estudios e Investigaciones

154 CENTRO DE REUNIONES
INTERNACIONALES SOBRE BIOLOGÍA

Workshop on

Developmental Mechanisms in Vertebrate Organogenesis

Organized by

G. Oliver and M. Torres

P. Bovolenta
A. L. Calof
T. Curran
J. Epstein
N. W. Gale
F. Giráldez
F. Guillemot
S. K. McConnell
G. Oliver
O. Pourquie
M. A. Ros
B. Sosa-Pineda
D. Stainier
C. Tabin
I. Thesleff
M. Torres
S. Vainio
D. G. Wilkinson
C. V. E. Wright
K. S. Zaret

The lectures summarized in this publication were presented by their authors at a workshop held on the 9th through the 11th of June, 2003, at the Instituto Juan March.

Depósito legal: M- 33011 / 2003
Impresión: Ediciones Peninsular. Tomelloso, 27. 28026 Madrid

INDEX

PAGE

PAGE

Introduction

Guillermo Oliver and Miguel Torres

Recent advances in cellular and molecular mechanisms underlying organ formation and tissue regeneration in vertebrates have once more positioned developmental biology at the forefront of basic science, and emphasize the relevance of the potential therapeutic application of this knowledge. Advances in stem cell biology and the dissection of molecular networks controlling cell differentiation pathways and pattern formation are the two driving forces behind these new perspectives. In this meeting we have reviewed recent advances in the field by bringing together basic scientists who are contributing significantly to these developments.

Understanding how progenitor cells differentiate and self-organize to build an organ or a tissue requires analysis of the molecular networks that control these processes. Molecular developmental biologists are dedicating considerable effort to the identification and characterization of autonomous and non-autonomous molecules governing differentiation and pattern formation. Molecules that trigger organ formation, specify cell lineages, or control the allocation of different cell phenotypes within organs are the subject of intensive analysis in many leading research groups. The identification of these molecules and mechanisms is crucial to understand normal and pathological aspects of organ development. Research in the last decade provided us with the molecular tools to start dissecting some of these processes and the subsequent functional modifications of many gene products has started to unravel some of the signaling mechanisms and pathways operating in early embryonic development. Further to this, during the last few years, emergence of new powerful technologies has revolutionized this area of research. The use of genomics, microarray analysis, conditional knock-outs and RNA interference, to name some of them, provided the scientific community with extraordinary tools to help dissect and understand vertebrate organ formation. The use of these resources in the most popular vertebrate animal models: e.g., mouse, zebrafish, and chick, produces an ever-increasing body of novel information, often difficult to integrate.

Having the opportunity of bringing together researchers working in different organs in different animal models provides an unique opportunity to compare and exchange approaches and ideas between colleagues working in this exciting field. From the early formation of the three tissue layers, to the formation and pattern of the different organs, extensive discussion over these topics strengthen the idea that although limbs, kidney, pancreas, liver, brain, teeth appear quite different in morphology, many of the genes and mechanisms involved in their development are shared by most of them. Beautiful examples of these exciting studies in normal and pathological conditions are represented here by results obtained in areas such as vascular biology, endodermal organ formation, patterning of ectodermal derivatives, differentiation and proliferation of neural and glial precursors, to name just a few.

The opportunity provided by the Juan March Organization to bring together this workshop was extremely valuable and provided the frame for highly stimulating discussions and exchange of information necessary to integrate knowledge from different fields of vertebrate organ formation.

Guillermo Oliver
Miguel Torres

Session 1: Patterning endodermal derivatives
Chair: Guillermo Oliver

Gut development in zebrafish

Didier Stainier

UCSF

During vertebrate development, many organs adopt asymmetric positions with respect to the midline. Much is known about genes that are expressed exclusively on the left or right side of the embryo to provide positional information during organ formation. Virtually nothing is known, however, about the cellular changes and tissue movements that occur downstream of left-right (L-R) gene expression to produce morphological asymmetry. We have found that the lateral plate mesoderm (LPM) forms a columnar epithelium in the precise region of the embryo where the gut endoderm first loops to the left. Prior to looping, the LPM epithelia occupy symmetrical positions, lateral to the endoderm. Concomitant with looping, however, the LPM undergoes an unexpected asymmetric migration, which appears to push the developing intestine to the left. In *heart and soul* and *nagie oko*, two mutants that show defects in the epithelial structure of the LPM, this asymmetric migration is perturbed and the gut fails to loop. Furthermore, reducing left-specific Nodal activity through the use of a *southpaw* morpholino randomizes the pattern of LPM migration and gut looping. This work provides the first insight into the early cellular movements that underlie morphological asymmetry in the viscera, and implicates the LPM as an active player in the L-R morphogenesis of the digestive tract.

In addition, we have recently completed a screen using a transgenic line that expresses GFP in the entire gut and gut-derived organs for mutations that affect the development of these organs and I will report on the recovery and characterization of these mutants.

Patterning the endoderm into liver and pancreas

Kenneth S. Zaret, Roque Bort, Kimberly Tremblay, and Hideyuki Yoshitomi

Cell and Developmental Biology Program
Fox Chase Cancer Center, Philadelphia, PA, USA

Our laboratory investigates the signaling and morphogenetic mechanisms by which the endoderm germ layer becomes patterned into different tissues, including the liver and pancreas. Tissue patterning initiates when the endoderm is topologically a sheet, prior to the formation of a gut tube, implying that mesodermal signals along both the anterior-posterior and medial-distal axes may be critical. Indeed, direct lineage mapping studies show that, prior to their specification, liver and ventral pancreatic progenitor cells initially occur lateral to the midline and move ventrally to close off the gut. We further show that the homeobox-containing transcription factor Hex is critical to promote the growth of the ventral-lateral endoderm past different mesodermal signaling domains, and consequently is necessary at a morphogenetic level for the specification of the ventral pancreas. Prior studies from our laboratory indicate that the induction of the liver domain is coincident with suppression of the ventral pancreatic program in the endoderm, and this requires simultaneous FGF and BMP signals from cardiogenic mesoderm and septum transversum mesenchyme cells, respectively, followed by interactions with endothelial cells that promote growth. Similarly, other laboratories identified three mesodermal cell types, notochord, endothelium, and mesenchyme, that appear to independently promote early pancreatic development. We have now obtained evidence showing that different mesodermal cell types must signal to each other to promote pancreatic development, in addition to signaling to the endoderm. We have further found that specific mesodermal cell interactions induce certain pancreatic transcription factors in the dorsal endoderm, but have no apparent effect on the same transcription factors in the ventral pancreatic endoderm. Taken together, the findings are providing new insight into the dynamic interactions among mesodermal cells and the ways that different mesodermal cell types provide signals that combinatorially pattern the endoderm into different tissues.

References:

Zaret, K.S. (2002) Regulatory phases of early liver development: Paradigms of organogenesis. Nature Reviews Genetics 3, 499-512.

Transcription factors in pancreatic ß-cell differentiation

Beatriz Sosa-Pineda

Department of Genetics, St. Jude Children's Research Hospital. Memphis, Tennessee, USA

The mammalian pancreas is composed of two types of glandular tissue: exocrine tissue, which secretes digestive enzymes into the gut, and endocrine tissue, which secretes hormones into the bloodstream. Most of the pancreas consists of acini of exocrine cells. The endocrine tissue, on the other hand, is organized into islets that consist of four cell types: α, ß, δ and PP; pancreatic endocrine cells collectively produce hormones necessary to maintain normoglycemia. The ß-cells of the pancreas play a central role in glucose homeostasis due to their ability to secrete insulin in response to increased levels of glucose in the blood; alterations in the integrity or functionality of ß-cells result in the development of diabetes. Genetic studies have provided valuable insights into developmental pathways that lead to the formation of mature β-cells. However, the overall mechanism governing this process has not been fully elucidated, including the specific roles that pancreatic transcription factors play and the identity of mesenchymal signals that regulate this event.

The functional inactivation of the homeobox gene Pax4 has demonstrated that this gene is absolutely required for the formation of mature pancreatic ß-cells. We have recently extended these initial findings by performing a detailed characterization of Pax4 expression during pancreas development. This work, together with results obtained by the characterization of the phenotypic alterations observed in ß-cell precursors deficient in Pax4 suggested that the activity of this gene is necessary to initiate ß-cell differentiation. We have determined that this process is also regulated by two other transcription factors: Nkx2.2 and Pax6.

The homeodomain gene *Prox1* is widely expressed in early embryonic pancreata. We have found that throughout pancreas development, Prox1 expression decreases to undetectable levels in differentiating exocrine cells, but it it maintained in endocrine precursors and differentiating endocrine cells. In mature islets Prox1 is expressed at high levels in α-cells and at lower levels in ß-cells. Detailed characterization of pancreata of available *Prox1*-deficient mouse embryos allowed us to determine that Prox1 is a novel regulator of pancreatic organogenesis. Lack of Prox1 function affects pancreas morphogenesis by reducing the endocrine-cell population while increasing the number of exocrine cells. Our results suggest that in pancreatic precursors Prox1 is a critical regulator of fate choices, and that maintenance of the appropriate levels of Prox1 protein in mature pancreatic endocrine cells may be functionally relevant.

In an attempt to start to decipher the molecular pathway(s) controlled by these different homeobox genes during pancreas development, we have started a systematic comparison of gene expression profiles using microarray analysis and generation of double-knock out mouse mutants to identify possible epistatic interactions.

Pancreas transcription factors and organogenesis

Christopher V. E. Wright

A central focus of our research is to help achieve a complete understanding of the gene networks and cell interaction programs that underlie pancreas organogenesis, as well as those involved in the maintenance and physiological function of this critical tissue. Several key issues can be addressed in the form of a set of questions:

(1) How is the pancreas specified to grow out at specific locations in the foregut endoderm?

(2) Do the early pancreas anlagen, derived from endodermal epithelium, contain multipotential stem cells, or progenitor populations, that are directed by extrinsic and intrinsic signals to differentiate into the acinar, duct and islet fates?

(3) Or, are there independent precursor pools that at early stages become allocated towards the separate fates in the pancreas (duct, acinar and endocrine)?

(4) If there is a transition between the cell populations implied by the points 3 and 4, when and where does it occur, how is it controlled, and can the factors regulating it be described?

(5) What intercellular signals and transcription factors are produced by or in specific cells or tissues that direct the process of cellular differentiation?

(6) How are genetic hierarchies initiated and maintained, and can they be triggered by specific sets of intercellular signals and transcription factors?

(7) Can these programs be elicited in a surrogate cell type, perhaps an endodermal stem/progenitor cell, a MAPC (multipotent adult progenitor cell), embryonic stem cell, hematopoietic stem cell, or other suitable cell type, as a way of converting them reproducibly towards the pancreatic, or pancreatic endocrine cell, fate?

Relevant to these issues, we previously showed that the *pdx1* homeobox gene is essential for outgrowth and differentiation of pancreatic buds (also shown by Helena Edlund's group). Using Cre-loxP conditional gene inactivation, we and Dr. Edlund's group have also shown that *pdx1* is required in insulin-producing cells. Our incorporation of genetically based lineage tracing methods into these conditional gene inactivation experiments allowed us to show that the resulting β cell loss induces a large overgrowth of α cells, but that the α cells were not derived from ex-β cells (which seem, in contrast, to undergo rapid apoptosis). We are now dissecting the *in vivo* role of conserved *cis*-regulatory elements in the endogenous *pdx1* gene, trying to identify upstream regulators, and testing *in vivo* how mutation of DNA recognition motifs for specific transcriptional regulators might cause diabetes by abrogating or interfering with normal levels and timing of *pdx1* function.

We have generated a global deletion of a localized region of evolutionarily conserved cis-regulatory sequences (termed Area I-II-III) in the 5' flanking region of *pdx1*, ~1 kb in size located at –2 kb with respect to the transcription start sites, and conserved at up to 95% nucleotide identity across vertebrate species. Global deletion leads to an apancreatic phenotype in $pdx1^{\Delta[I-II-III]/-}$ mice, while other aspects of organogenesis within the *pdx1* expression domain are unaltered. Importantly, the pylorus, bile duct and rostral duodenum show profound anatomical and cell differentiation abnormalities in the PDX1 protein-deficient situation ($pdx1^{-/-}$ mice), indicating an organ-specific role for this *cis*-regulatory domain. We have produced a floxed allele, $pdx1^{flox[I-II-III]}$ to study the acute/long-term, and cell type-specific effect of removing this enhancer region from *pdx1*. We know that the $pdx1^{flox[I-II-III]}$ allele functions equivalently to a wildtype allele by genetic testing against wild type and heterozygous deficiency backgrounds. Lineage tracing will be used to test for trans-fating and loss of specific cellular functions associated with the loss of the enhancer from the endogenous *pdx1* locus. We will also report data obtained from our studies of Δ[I–II–III] /Δ[I–II–III] mice, which lead to the idea that the timing and level of expression of *pdx1* is critical for the normal program of pancreas organogenesis.

Eventually, we hope to move forward to testing *in vivo* whether the binding sites for known MODY (maturity onset diabetes of the youth; a dominantly inherited monogenic, early onset form of type II diabetes), such as HNF1α, are crucial for normal *pdx1* gene activity. We will test for effects on overall organogenesis, as well as cell differentiation and function. These experiments should also produce spin-off models of human MODY and glucose homeostasis deficiency.

References:

Offield, M., Labosky, P., Ray, M., Jetton, T., Magnuson, M., Hogan, B.L.M. and Wright, C.V.E. (1996) Pdx-1 is required for pancreatic outgrowth and differentiation of the rostral duodenum. *Development* **122**, 983-995

Gannon, M., Gamer, L.W., and Wright, C.V.E. (2001) Regulatory regions driving developmental and tissue-specific expression of the essential pancreatic gene *pdx1*. *Developmental Biology* 238, 185-201

Kawaguchi, Y., Cooper, B., Gannon, M., Ray, M., MacDonald, and Wright, C.V.E. (2002) The role of the transcriptional regulator PTF1a in converting intestinal to pancreatic progenitors. *Nature Genetics* **32**, 128-134

Session 2: Central Nervous System Development
Chair: Kenneth S. Zaret

Making boundaries in the hindbrain

Yi-Chuan Cheng, Marc Amoyel, Marisa Cotrina, Andrea Pasini, Alexei Poliakov and David G. Wilkinson

Division of Developmental Neurobiology, National Institute for Medical Research, London NW7 1AA, UK

The establishment of organised patterns of cell types at appropriate locations in the nervous system is achieved by its initial subdivision into regional domains, each specified to form a distinct set of derivatives. An important question is how these regional domains are formed and maintained as discrete structures, despite the potential for them to become scrambled by cells intermingling during tissue growth and cell proliferation. One mechanism by which sharp interfaces are maintained between adjacent cell populations is to specifically inhibit cells from intermingling across the interface. In some cases, a distinct boundary cell population forms at the interface that acts as a source of signals that organise local pattern. However, little is known regarding the mechanisms of formation and roles of boundaries in the vertebrate hindbrain.

Our previous work has shown that bi-directional activation of Eph receptor tyrosine kinases and transmembrane ephrinB proteins at the interface of complementary domains of expression restricts the intermingling of cells across the interface. We also found that Eph receptors and ephrins act upstream of the formation of distinct hindbrain boundary cells. This talk will discuss progress in dissecting the roles of other receptor-ligand systems in the control of cell movement and formation of hindbrain boundaries.

References:

Xu, Q., Mellitzer, G., Robinson, V. and Wilkinson, D.G. (1999) *In vivo* cell sorting in complementary segmental domains mediated by Eph receptors and ephrins. **Nature** *399*, 267-271.

Mellitzer, G., Xu, Q. and Wilkinson, D.G. (1999) Restriction of cell intermingling and communication by Eph receptors and ephrins. **Nature** *400*, 77-81.

Pasini, A. and Wilkinson, D.G. (2002) Stabilising the regionalisation of the developing vertebrate central nervous system. **BioEssays** *24*, 427-438.

Proneural proteins and cell fate specification in the embryonic and postnatal brain

François Guillemot

National Institute for Medical Research, Mill Hill, London, UK

The brain has a limited capacity to replace neurons lost from injury or disease. Progenitor cells are present throughout the adult brain, but only in restricted regions do they differentiate into a limited set of neuronal types. To improve this neurogenic potential, it is important to understand how the decisison of adult multipotent progenitors to generate neurons or glial cells is normally controlled. Proneural bHLH proteins have been implicated in cell fate decisions in the embryonic nervious system. In particular, Mash1 has an essential role in the generation of multiple neuronal populations during the peak period of neurogenesis in the embryonic telencephalon.

Stem cells located in the periventricular region of the postnatal telencephalon constitutively produce interneurons migrating to the olfactory bulb, as well as glial cells. Mash1 is expressed by transit amplifying progenitors from the olfactory interneuron lineage, and by a fraction of oligodendrocyte precursors. In *Mash1* mutant newborns, we observe a reduced production of neurons and oligodendrocytes in the olfactory bulb. Similarly, neurospheres derived from the periventricular region of *Mash1* mutants produce drastically reduced numbers of neurons and oligodendrocytes. Thus, *Mash1* is involved in the generation of two distinct cell lineages by multipotent postnatal progenitors, and it has a similar proneural function in embryonic and postnatal progenitors.

Together, our data data indicate that proneural genes have a central role in cell fate specification in the nervous system, and that they must cooperate with other neurogenic and gliogenic determinants to activate temporally and spatially appropriate differentiation programmes. The same factors appear to control the specification of multipotent progenitors in the embryonic and adult brain, suggesting that these factors may also be involved in repair mechanisms. Therefore, the regulation of expression and activity of proneural proteins may be a key step in modulating stem cell output and improving their regeneration potential following injury.

Co-ordinating the growth and patterning of the neural tube

Sophie Bel-Vialar, Valérie Lobjois, Fabienne Pituello

During spinal cord elongation, the homeobox containing gene *Pax6*, is progressively upregulated in a head to tail sequence, being present in the neural tube but absent from the caudal neural plate. The timing of *Pax6* activation is controlled by the paraxial mesoderm. Young posterior mesoderm provides a signal (Fibroblast Growth Factor, FGF) which represses *Pax6* in the caudal neural plate while older more anterior mesoderm (in the form of somites) no longer expresses FGF and allows *Pax6* expression in the neural tube. Thus in the embryo, the aging of the mesoderm and neural tube are co-ordinated via FGF signalling. In the same way, the onset of neuronal differentiation which follows *Pax6* activation is controlled by the regressing wavefront of FGF activity. Hence, the progression of *Pax6* along the rostro-caudal axis helps to define two neural progenitor cell domains: a caudal one ($Pax6^{off}$) in which neural progenitor cells divide but do not differentiate and a rostral one ($Pax6^{on}$) in which neural progenitor cells can exit the cell cycle and differentiate. In that context, we are studying the importance of the sequence of *Pax6* activation on cell cycle progression and neuronal differentiation.

Feedback regulation of neurogenesis

Anne L. Calof, Hsiao-Huei Wu, Joon Kim, Sylvia Jaramillo, and Alice Ly

Depts of Anatomy & Neurobiology and Developmental & Cell Biology, and the Developmental Biology Center, University of California, Irvine, California 92697-1275, USA

Studies of developing vertebrates suggest that inhibitory signals have important roles in regulating neurogenesis. In the mouse olfactory epithelium (OE) model system, for example, studies in vitro and in vivo indicate that generation of new neurons by neuronal progenitors is inhibited by a signal from neurons themselves (Calof et al., 1996; Mumm et al., 1996). Thus, feedback inhibition of neurogenesis appears to be an important mechanism by which proper neuron number is maintained in the OE. Identifying the molecules that mediate this feedback regulation is likely to be important not only for understanding nervous system development, but also for devising strategies to deal with brain injury and aging, because in these conditions persistent growth-inhibitory signals could thwart attempts to promote regeneration.

In our studies to identify molecular regulators of feedback inhibition in the OE model system, we have used a candidate approach, focusing on signaling molecules of the transforming growth factor-beta (TGF-beta) superfamily, because of their known actions in inhibiting both cell growth and neural induction (Shou et al., 1999; Shou et al., 2000). Our recent studies have provided evidence that growth and differentiation factor 11 (GDF11) functions as a feedback inhibitory signal in the OE, in vitro and in vivo (Wu et al., 2003). Both GDF11 and its receptors are expressed by neurons and neuronal progenitors in the OE. Recombinant GDF11 inhibits OE neurogenesis in vitro, by acting on a specific stage of transit amplifying progenitor in the olfactory receptor neuron (ORN) lineage, the Immediate Neuronal Precursor (the INP is the ultimate dividing cell type in the ORN lineage, and INP progeny undergo terminal differentiation into ORNs (Calof and Chikaraishi, 1989; DeHamer et al., 1994; Calof et al., 2002)). GDF11 appears to inhibit OE neurogenesis by inducing increased expression of the cyclin-dependent kinase inhibitor, $p27^{Kip1}$, and reversible cell-cycle arrest, in INPs.

Mice lacking a functional *Gdf11* gene show abnormally high levels of cell proliferation and increased numbers of neuronal progenitors, especially INPs, in their OE. There is also an increased number of ORNs in the OE of *Gdf11* null mice. Conversely, mice lacking the gene that encodes follistatin, a secreted GDF11 antagonist that is also expressed in OE, show dramatic decreases in cell proliferation, numbers of neuronal progenitors, and numbers of ORNs in their OE (Wu et al., 2003). Thus, one way in which the mammalian nervous system achieves proper neuron number during development is by negative autoregulation of neurogenesis. In the OE, GDF11 and its antagonist, follistatin, are critical regulators of this process. This action of GDF11 is strikingly similar to that of its close homologue,

GDF8/myostatin, in skeletal muscle, suggesting that similar strategies are used to establish and maintain proper cell number during neural and muscular development.

References:

Calof AL, Chikaraishi DM (1989) Analysis of neurogenesis in a mammalian neuroepithelium: proliferation and differentiation of an olfactory neuron precursor in vitro. Neuron 3:115-127.

Calof AL, HagiwarA N, Holcomb JD, Mumm JS, Shou J (1996) Neurogenesis and cell death in the olfactory epithelium. J Neurobiol 30:67-81.

Calof AL, Bonnin A, Crocker C, Kawauchi S, Murray RC, Shou J, Wu HH (2002) Progenitor cells of the olfactory receptor neuron lineage. Microsc Res Tech 58:176-188.

DeHamer MK, Guevara JL, Hannon K, Olwin BB, Calof AL (1994) Genesis of olfactory receptor neurons in vitro: regulation of progenitor cell divisions by fibroblast growth factors. Neuron 13:1083-1097.

Mumm JS, Shou J, Calof AL (1996) Colony-forming progenitors from mouse olfactory epithelium: Evidence for feedback regulation of neuron production. Proc Natl Acad Sci USA 93:11167-11172.

Shou J, Rim PC, Calof AL (1999) BMPs inhibit neurogenesis by a mechanism involving degradation of a transcription factor [see comments]. Nat Neurosci 2:339-345.

Shou J, Murray RC, Rim PC, Calof AL (2000) Opposing effects of bone morphogenetic proteins on neuron production and survival in the olfactory receptor neuron lineage. Development 127:5403-5413.

Wu HH, Ivkovic S, Murray RC, Jaramillo S, Lyons KM, Johnson JE, Calof AL (2003) Autoregulation of neurogenesis by GDF11. Neuron 37:197-207.

Organizing the forebrain: genetic analysis of telencephalic development

Susan K. McConnell

Department of Biological Sciences, Stanford University, Stanford, California, 94305-5020 USA

During development, the embryonic telencephalon is patterned into different areas that give rise to distinct adult brain structures. Several secreted signaling molecules are expressed at putative signaling centers in the early telencephalon. Multiple *Bmp* genes are expressed at the dorsal midline and have been hypothesized to pattern the medial-lateral axis, whereas *Fgf8* is expressed at the anterior end of the telencephalon and may pattern the anterior-posterior axis. Using a *Cre/loxP* genetic approach to disrupt genes during telencephalic development, we addressed the role of BMP and FGF signaling in vivo by abolishing expression of the receptors *Bmpr1a* and *Fgfr1*. In the absence of *Bmpr1a*, cells of the choroid plexus (the most medial/dorsal telencephalic derivative) fail to be specified or differentiate, and instead remain as proliferative cells. These cells do not adopt the fate of neighboring cells, but do express dorsal midline markers. These results suggest that BMPs induce the formation of the choroid plexus and play an essential role in patterning the medial-lateral axis of the telencephalon. In the *Fgfr1*-deficient telencephalon, striking morphological defects are observed at the anterior end of the telencephalon, where the olfactory bulb fails to form normally. Examination of the proliferation state of anterior telencephalic cells supports a model for initial olfactory bulb formation in which a decrease in proliferation is required for bulb evagination. Together the results demonstrate an essential role for *Fgfr1* and *Bmpr1a* in patterning and morphogenesis of the telencephalon. We are extending these studies to examine the role of other FGF and BMP receptors in brain development, as well to explore the molecular basis of dorsal midline formation during the formation of the two cerebral hemispheres.

References:

Hébert JM, Mishina Y, McConnell SK (2002) BMP signaling is required locally to pattern the dorsal telencephalic midline. *Neuron* 35:1029-1041.

Hébert JM, Lin M, Partanen J, Rossant J, McConnell SK (2003) FGF signaling through FGFR1 is required for olfactory bulb morphogenesis. *Development* 130:1101-1111.

Hayhurst M, McConnell SK (2003) Mouse models of holoprosencephaly. *Curr. Opin. Neurol.* 16:135 – 141.

Hébert JM, Hayhurst M, Marks ME, Kulessa H, Hogan BLM, McConnell SK (2003) BMP ligands act redundantly to pattern the dorsal telencephalic midline. *Genesis*, in press.

Normal and neoplastic brain growth: Tales from mutant mice

Tom Curran

St. Jude Children's Research Hospital, Memphis TN 38105 USA

The formation of the mammalian nervous system requires a choreographed series of cellular interactions that involve dramatic reorganization and cell migration during specific time periods. Insights into these processes have been achieved through the study of several mutant strains of mice. Mice lacking *Reelin*, *Disabled-1* (*Dab1*) and both the very low density lipoprotein receptor (*VLDLR*) and the apolipoprotein receptor 2 (*ApoER2*), exhibit defects in neuronal migration throughout the brain resulting in disorganization of many laminar structures. *Reelin* encodes a novel extracellular protein secreted by pioneer neurons in the developing brain that binds directly to lipoprotein receptors on the cell surface. This activates a signaling cascade leading to tyrosine phosphorylation of Dab1. In the cerebellum, defects in the *Reelin* pathway result in a failure of Purkinje cells to migrate outwards to form the Purkinje cell layer. Purkinje cells in the mutant mice remain in deep clusters within the cerebellum and they do not come close enough to granule cell precursors in the external germinal layer to support their proliferation through secretion of sonic hedgehog (*Shh*). Shh secreted by Purkinje cells binds to the receptor Patched-1 (Ptc1) on granule neurons blocking its ability to inhibit the function of Smoothened (Smo) another multipass transmembrane protein that activates transcription of *Gli1*. This pathway is critical for the control of granule cell proliferation. Indeed, heterozygous loss of *Ptc1* causes medulloblastoma in humans and mice. Mice lacking one copy of *Ptc1* and both copies of the *p53* gene develop medulloblastomas by 12 weeks of age. We are now using genetic and pharmacological approaches to investigate the role of this pathway in tumor growth *in vivo*. Finally, it is likely that medulloblastoma cells escape from growth control by accumulating genetic and epigenetic alterations. Therefore, we used a somatic nuclear transfer to determine whether medulloblastoma nuclei can direct normal differentiation and development after epigenetic reprogramming.

References:

D'Arcangelo G, Homayouni R, Keshvara L, Rice DS, Sheldon M, Curran T. Reelin is a ligand for lipoprotein receptors. Neuron 24:471-479, 1999.

Wetmore C, Eberhart D, Curran T. The normal *patched* allele is expressed in medulloblastomas from mice with heterozygous germline mutation of *patched*. Cancer Res 60:2239-2246, 2000.

Wetmore C, Eberhart DE, Curran T. Loss of *p53* but not *ARF* accelerates medulloblastoma in mice heterozygous for *patched*. Cancer Res 61:513-516, 2001.

Rice DS, Curran T. Role of the reelin signaling pathway in central nervous system development. Ann Rev Neurosci 24:1005-1039, 2001.

Keshvara L, Benhayon D, Magdaleno S, Curran T. Identification of reelin-induced sites of Tyrosyl phosphorylation on disabled-1. J Biol Chem 276:16008-16014, 2001.

Magdaleno SM, Keshvara L, Curran T. Rescue of ataxia and preplate splitting by ectopic expression of Reelin in *reeler* mice. Neuron 33:573-586, 2002.

Keshvara L, Magdaleno S, Benhayon D, Curran T. Cdk5 phosphorylates Dab1 independently of Reelin signaling. J Neurosci 15: 4869–4877 2002.

Benhayon D, Magdaleno S, Curran T. Reelin-induced tyrosine phosphorylation of Disabled-1 is mediated by ApoER2 and VLDLR. Molecular Brain Research (in press) 2003.

Session 3: Sensory Organ Development
Chair: Tom Curran

Vertebrate eye morphogenesis: a view from the retina pigmented epithelium

Paola Bovolenta

Instituto Cajal, CSIC, Av. Doctor Arce 37, 28002 Madrid, Spain. bovolenta@cajal.csic.es

Subsequent to the specification of the eye field, the optic vesicle evaginates from the forebrain. The most proximal cells within the vesicle form the optic stalks (OS) while distal cells form the retina. Within the retinal anlage, those cells that contact the surface ectoderm form the neural retina (NR), while more dorsal cells differentiate as the retina pigment epithelium (RPE). Proper specification of these territories is crucial for the development of the entire eye, but how RPE, NR and OS progenitor cells acquire their identity is still poorly understood.

I will present a genetic and molecular analysis that demonstrate that the transcription factors *Otx1* and *Otx2*, in cooperation with *Mitf* genes are required for the specification and the function of the RPE.

Otx and *Mitf* genes are initially expressed in the entire optic vesicle but their expression becomes restricted to the presumptive RPE during optic cup formation (Hodgkinson et al., 1993; Bovolenta et al, 1997). Mice deficient in *Otx (Otx1-/-; Otx2+/-* and *Otx1+ -; Otx2+/-)* present clear defects in the patterning of the RPE. In fact, the outer layer of the optic cup does not acquire its characteristic of a cuboidal monolayered epithelium but it is replaced by a territory with morphological and molecular characteristic of neural retina. In addition, the expression of *Mitf* and *Tyr*, is largely absent and maintained only in little patches of tissue where residual OTX2 expression is also localised (Martinez-Morales et al., 2002). Conversely, in *Mitf* mutants the expression of *Otx2* is specifically down regulated in those areas where RPE does not differentiate (Nguyen and Arnheiter, 2000), indicating that both *Otx* and *Mitf* are responsible for the development of the RPE. This idea is further supported by the observation that *Otx2*, similarly to *Mitf*, is capable of inducing a pigmented phenotype when is over-expressed in avian neural retina cells. In addition, in transient transfection and EMSA assays, OTX2 binds specifically to a bicoid motif present in the promoter regions of three RPE specific genes, leading to their transactivation (Martinez-Morales et al., 2003). These genes are *QNR71*, a transmembrane glycoprotein specifically targeted to the melanosomes (Turque et al., 1996), and *Tyr* and *TRP-1*, two enzymes involved in melanin biosynthesis. The activity of OTX2 on the promoter region of these genes is synergised by the presence of MITF, with which OTX2 can interact as demonstrated by pull down experiments and their co-localization within the nuclei of RPE cells (Martinez-Morales et al., 2003).

These results demonstrate the importance of *Otx2* in RPE differentiation and show that in vertebrates, *Otx2* and *Mitf* operate at the same hierarchical level to establish the identity of the RPE. Interestingly, pigmented cells of the photo-sensing ocellus in ascidian larvae express tyrosinase family members with structural characteristics highly similar to those of their vertebrate counterparts (Sato et al., 2001). However, the relevant regulatory regions of the ascidian genes do not contain MITF binding sites. Rather they appear under the control of *Hroth*, the single ascidian *Otx* gene (Wada et al., 2002), suggesting that *Otx* genes have an ancestral regulatory function in the determination of the pigmented lineage among chordates.

References:

Bovolenta, P., Mallamaci, A., Briata, P., Corte, G. and Boncinelli, E. (1997) *J. Neurosci.* **17**, 4243-4252.

Hodgkinson, C.A., Moore, K.J., Nakayama, A., Steingrimsson, E., Copeland, N.G., Jenkins, N.A. and Arnheiter, H. (1993) *Cell* **74**, 395-404.

Martinez-Morales, J.R., Signore, M., Acampora, D., Simeone, A. and Bovolenta, P. (2001) *Development* 128, 2019-2030.

Martinez Morales J.R., Dolez, V., Rodrigo I., Zaccharini; R., Leconte, L., Bovolenta, P. and Saule, S. (2003). *J. Biol. Chem.* (in press).

Nguyen, M-T.T. and Arnheiter, H. (2000) *Development* **127**, 3581-3591

Sato, S. and Yamamoto, H. (2001) *Pigmented Cell Res* **14**, 428-436.

Turque, N., Denhez, F., Martin, P., Planque, N., Bailly, M., Begue, A., Stehelin, D. and Saule, S. (1996) *EMBO J.* **15**, 3338-3350.

Wada, S., Toyoda, R., Yamamoto, H. and Saiga, H. (2002) *Dev Dyn.* **225**, 46-53.

Pattern and cell fate in the otic placode: The generation of otic neurons

Fernando Giráldez

DCEXS-Universitat Pompeu Fabra, c/Dr.Aiguader 80, 08003-Barcelona

Otic neurons innervate hair cells located in the auditory and vestibular sensory organs of the inner ear. Local mechanical perturbations are transduced by hair cells into synaptic potentials, which elicit the activation of auditory (cochlear) and vestibular neurons, that project towards the homonymous central nuclei, the first input station of the auditory pathway. Auditory neurons inform the brain of the intensity and spectral properties of sound, and vestibular neurons carry information about position, velocity and acceleration. The inner ear derives from the otic placode, an ectodermal thickening that develops adjacent to the hindbrain (Torres and Giraldez, 1998). The otic placode invaginates and pinches off the ectoderm to form the otic vesicle, which is a transient structure that undergoes multiple developmental changes associated with cell proliferation, differentiation and cell-death. This results in the ear labyrinth: the cochlea, the utricle and saccule, and the semicircular canals, each containing their corresponding sensory organs (Fekete and Wu, 2002). The otic vesicle shows developmental autonomy, meaning that it can be explanted from the embryo and exhibits patterning, morphogenesis and cell diversification, including the genesis of mechano-transducing hair cells and neurons. The whole system is both intricate and simple, i.e. it contains morphological complexity, but based on a limited number of cell types, which are originated from the otic vesicle, and it is believed to arise from the early regionalisation of the otic placode by patterning genes.

The generation of otic neurons is a sequential process, first otic neurons are specified in the otic epithelium, then neuronal precursors delaminate to form the cochleo-vestibular ganglion, the CVG, where they proliferate, and then differentiate and innervate back the vestibular and cochlear (auditory) sensory organs. The cochleo-vestibular ganglion (CVG) is a transient condensation of cells that house the nascent delaminating neuroblasts. Further in development, it generates separated cochlear and vestibular ganglions.

I shall concentrate this review on the initial steps of development of otic neurons, at the specification of neural fate in the otic epithelium, and follow it until the initiation of neuronal differentiation in the CVG. The first task will be to compile the cellular and molecular information about neural determination and differentiation in the ear, and to define cell states and the transition steps from precursors to neurons. This part will deal with the expression and function of *proneural* genes in the otic placode. The second aspect we will address the roles of diffusible factors in the control of ear neurogenesis, by reviewing the effects of Fibroblast Growth Factors (FGFs), the Nerve Growth Factor (NGF) family of neurotrophins and Insulin-like Growth Factor-I (IGF-I) (Alsina et al., 2003). One central

problem in cell fate determination is the interaction between extrinsic-environmental signals and intrinsic (cell-autonomous) factors. There is growing evidence that these families of inter-cellular signalling molecules participate in the regulation of early stages of neural development by mediating cell-to-cell interactions.

A model for ear neurogenesis will be presented. The generation of otic neurones can be described by at lest four cell states: 1) the multipotent progenitor -self-renewing epithelial cell, that probably gives rise to both sensory and neuronal lineages, 2) the epithelial neuroblast, which is a cell that is fully a committed as a neuron and delaminates from the otic placode to form the CVG, 3) the ganglionar neuroblast, which goes through transit-amplification in the CVG, and 4) the post-mitotic otic neuron that initiates differentiation and neurite projection. Each state exhibits a particular combination of transcriptor factors of proneural and Lim-homeodomain families, and also a specific sensitivity to external signals. The transition from the multipotent progenitor to the epithelial neuroblasts requires FGF-signalling, probably FGF10. Ganglionar neuroblasts require IGF-1 for survival and transit-amplification. And otic neurons are dependent on the NT-Trk system for target-derived survival and differentiation.

References:

Alsina, B. Giraldez, F. and Varela-Nieto, I. (2003) Growth factors and early development of otic neurones: interactions between intrinsic and extrinsic signals. *Current Topics in Developmental Biology*. Development of the Auditory and Vestibular Systems. Molecular Development of the Inner Ear. *Academic Press*. Edited by R. Romand and I. Varela-Nieto.

Fekete,D.M. and Wu,D.K. (2002). Revisiting cell fate specification in the inner ear. *Curr. Opin. Neurobiol.* **12**, 35-42.

Torres,M. and Giraldez,F. (1998). The development of the vertebrate inner ear. *Mech. Dev.* **71**, 5-21.

Control of inner ear development by FGFs

Yolanda Alvarez, Maria Teresa Alonso, Victor Vendrell, Laura Zelarayan, Pablo Chamero, Thomas Theil, Dieter Riethmacher and Thomas Schimmang

Due to its conserved expression pattern in the developing hindbrain and inner ear in mouse, chicken, Xenopus and zebrafish fibroblast growth factor 3 (FGF3) has been proposed as the inducer of the inner ear in vertebrates. However, loss-of-function experiments in avians and mice have provided conflicting results on the roles of FGF3 during inner ear development. Antisense or antibodies blocked formation of the otic vesicle in chicken embryos (Nature 353, 561) whereas mutant mice for FGF3 only showed defects during differentiation of the inner ear (Development 117,13). We have recently demonstrated that FGF3 promotes the formation of ectopic otic vesicles in chicken (Development 127, 2011-2019).

To further clarify the roles of FGFs during inner ear development and to analyse their potential redundant functions in mammalian embryos we have produced transgenic mice expressing FGF3, FGF2 and FGF10 ectopically in the anterior part of the developing hindbrain. Our results suggest that both FGF3 and FGF10 are able to confer otic fate. In contrast, ectopic expression of FGF10 in chicken embryos has no effect on otic development. We have analysed the expression pattern of FGF10 during inner ear development in mice and have started to examine knockout mice lacking different members of the FGF family which are potentially involved in inner ear induction. Analysis of single and double knockout mice will reveal the essential and redundant functions of FGFs during inner ear induction.

Session 4: Mesodermal Patterning
Chair: Cliff Tabin

Urogenital system development

Seppo Vainio

University of Oulu, Biocenter Oulu & Department of Biochemistry, Linnanmaa, 90570 Oulu, Finland

The urogenital system is a good model system to assay key developmental mechanisms. The system contributes to morphogenesis of several organs including the adrenal gland, gonad and the permanent kidney and is a site of various stem cells. Furthermore, organogenesis involves guided migration of the germ cells and evidence is suggesting that these cells may also migrate to the aorta-gonad-mesonephros region (AGM) and may contribute to generation of the hematopoetic stem cells. However, several important questions of how the urogenital system is patterned to specific organ primordia, how the determination of the bipotential gonad into the female or male sex occurs in the primordia and what cells and signals of the system contribute to the somatic cells of the glands and regulate nephrogenesis of the kidney are still not well understood. Wnt-4 appears to be one critical signal in the urogenital system. It plays a role in sex determination of the female and in the lack of its activity females adopt male characteristics suggesting that Wnt-4 regulates the female pathway. The signaling is also essential for survival of the oocyte. In the metanephric kidney Wnt-4 signaling is critical for formation of the nephron and Wnt mediated induction is also sufficient to trigger tubulogenesis experimentally. Wnt-4 is expressed in the presumptive cortex of the adrenal gland and is a functional factor for adrenal gland development. We have performed microarray screens to identify Wnt-4 target genes in the urogenital system and analysis of some of the selected targets is on the way.

The sex reversal of the *Wnt-4* mutant females appears to be critically mediated by testosterone as testosterone biosynthesis can be measured from the mutant but not the wild type ovary and blocking of androgen action with flutamide leads to degeneration of the ectopic Wolffian in the female and partial reversal of the ovarian phenotype in genotypic female *Wnt-4* deficient embryos. Interestingly we observe ectopic adrenal gland derived cells in the mutant ovary which suggest that the normal function of Wnt-4 may be to prevent cell migration from the presumptive adrenal cortical field to the gonad, to regulate contribution of the steroidogenic cells to the gonad.

Besides *Wnt-4* recent knock out studies revealed a critical role for Wnt-11 and *Sprouty* genes in the control of kidney development. Both of these components contribute to ureteric bud branching and in the case of *Wnt-11* changes in its signaling lead to reduced *GDNF* expression in kidney mesenchyme. Furthermore, Wnt-11 expression is reduced in *c-Ret* knock out and in double mutants between *Wnt-11* and heterozygous c-Ret kidney size of the kidney is further reduced. These results suggest that a signalling loop between Wnt-11/GDNF/c-Ret

regulate kidney development. Finally our studies suggest an important role for a frizzled and endostatin domain containing collagen, type XVIII collagen in embryonic kidney. This factor appears to play a role in epithelial branching and kidney tubulogenesis.

References:

Heikkilä M., Peltoketo H., Leppäluoto J., Ilves M., Vuolteenaho O & Vainio S. (2002). Wnt-4 deficiency alters mouse adrenal cortex function, reducing aldosterone production. ***Endocrinology*** *143, 4358-4365.*

Heikkilä, M., Peltoketo, H. & S. Vainio. (2001). Wnts and the female reproductive system. ***J Exp Zool.*** *290, 616-23.*

Kispert, A., Vainio, S. & McMahon, A. P. (1998). Wnt-4 is a mesenchymal signal for epithelial transformation of metanephric mesenchyme in the developing kidney. ***Development*** 125, 4225-4234.

Lin, Y., Shang, S., Rehn, M., Itäranta, P., Tuukkanen, J., Heljäsvaara, R., Peltoketo, H., Pihlajaniemi, T. & S. Vainio. (2001). Induced repatterning of type XVIII collagen expression in ureter bud from kidney to lung type: association with sonic hedgehog and ectopic surfactant protein C. ***Development****128, 1573-1585.*

Majumdar, S., Vainio,S, Kispert,A., McMahon, J & McMahon A. (2003).Wnt-11 and c-Ret/GDNF pathways cooperate in regulating ureteric branching during metanephric kidney development. **Development (in press).**

Vainio, S., Lin, Y. and Pihlajaniemi, T. (2003). Induced Repatterning of Type XVIII Collagen Associates with Ectopic Sonic Hedgehog and Lung Surfactant C Gene Expression and Changes in Epithelial Epigenesis in the Ureteric Bud. ***J Am. Soc. Nephr.*** (in press).

Vainio S & Lin Y. (2002). Coordinating early kidney development: lessons from gene targeting. ***Nat Rev Genet.*** 3, 533-43.

Vainio, S. Heikkilä, M., Kispert, A.K., Chin, N. & McMahon, A.P. (1999). Female development in mammals regulated by Wnt-4 signalling. ***Nature*** 397, 405-409.

Molecular regulation of ectodermal organ development

Irma Thesleff*, Marja L. Mikkola, Johanna Pispa, Tuija Mustonen, Johanna Laurikkala, Aapo Kangas, Maritta Ilmonen and Risto Jaatinen

Developmental Biology Research Program, Institute of Biotechnology, University of Helsinki, Finland

The ectoderm gives rise to many organs such as hairs, teeth and several exocrine glands e.g. the mammary and salivary glands. Interactions between the ectoderm and underlying mesenchyme regulate the development of all ectodermal organs, and sevaral molecules in the TGFβ, hedgehog, FGF and Wnt signaling pathways have been implicated in the mediation of epithelial-mesenchymal interactions. Recently, a novel member of the tumor necrosis factor (TNF) family, ectodysplasin (Eda) has been shown to be necessary for the development of several, perhaps all, organs developing as ectodermal appendages (1). Mutations in Eda, its receptor Edar, or other components of the signaling pathway cause ectodermal dysplasias in humans and mice. We have shown that Eda-Edar signaling mediates interactions between ectodermal cell compartments during tooth and hair follicle morphogenesis and that it is integrated with Wnt and activin signaling (2). *Tabby* mice lacking functional Eda protein lack some teeth as well as the first wave of hair follicles. In contrast, transgenic mice overexpressing Eda in the ectoderm have extra teeth and their hair development is accelerated and the hairs grow longer than normal. In addition, they have supernumerary mammary glands and the development of their sweat glands and sebaceous glands is stimulated (3). In summary, Eda-edar signaling regulates the initiation as well as morphogenesis and differentiation of ectodermal organs and it acts upstream of many genes expressed in the ectodermal placodes..

References:
1. Thesleff, I ., Mikkola, M. *Science STKE* 2002;/131/pe22
2. Laurikkala, J., Pispa, J. *et al. Development* 129:2541, 2002
3. Mustonen, T., Pispa, J. *et al. Dev. Biol.* (in press)

New insights in vertebrate segmentation

Olivier Pourquie

Stowers Institute for Medical Research. 1000 East 50th Street. Kansas City, Missouri, 64110, USA
Phone: 816 926 4442. Fax: 816-926-2095. E-mail: olp@stowers-institute.org

Among the most overt features characteristic of the vertebrate body plan is the segmented organisation of vertebrae and associated muscles. This segmented aspect first arises during early development through the formation of somites, the precursors of vertebrae and skeletal muscles. Somitogenesis consists in the sequential formation of pairs of epithelial spheres from the mesenchymal presomitic mesoderm. The strict temporal precision and periodicity underlying somitogenesis is believed to rely on a molecular oscillator called the Segmentation Clock acting within presomitic mesoderm cells to control the periodic expression of "cyclic genes". The spatial integration of the clock pulsation into segment boundaries involves a gradient of FGF8, which defines the level at which cells become allowed to respond to the segmentation clock, and thus controls the positioning of segment boundaries. Maintenance of the FGF8 gradient during embryonic development is critical for the proper coordination of axis elongation and segment formation. Recent evidence indicated interactions between the segmentation clock and the spatio-temporal activation of Hox genes suggesting coordination between segmentation and antero-posterior patterning during the development of vertebrate embryos.

Session 4: Limb Patterning
Chair: David G. Wilkinson

Analysis of the role of Bmp4 signaling in the establishment of the anteriorposterior patterning in the limb bud

Maria Félix Bastida[1], M. Dolores Delgado [2], Baoling Wang[3] and María A. Ros[1]

[1] Dpto. de Anatomía y Biología Celular and [2] Dpto. de Biología Molecular. University of Cantabria, Santander, Spain. [3] University of Cornell, USA

The recent analysis of the *Gli3*$^{-/-}$ *Shh*$^{-/-}$ double mutant limbs (Litingtung et al., 2002; te Welscher et al., 2002) has showed that the effects of Shh signaling in the limb are necessarily mediated through Gli3. Shh signaling counteracts Gli3-mediated repression of key regulator genes, cell survival, and distal progression of limb bud development.

Other studies, mainly focussing in the neural tube of *Shh*$^{-/-}$; *Gli3*$^{-/-}$ null mouse, indicate that Gli3 could also mediate anteriorposterior signals from other sources. There is also evidence supporting the existence of an anterior *Bmp4* signaling system in the limb that opposes the Shh signaling system, similarly to the neural tube (Tumpel et al., 2002).

Here we have investigated the possibility that Gli3 mediates other effects besides the Shh signaling pathway in the limb. We have also analyzed the possible interactions with the Bmp4 based signaling system in establishing anteriorposterior patterning. For our analysis we have used the anterior limb mesoderm after removal of the zone of polarizing activity (ZPA). Removal of the ZPA gives a phenotype similar to that developed in the absence of Shh signaling such as the *Shh* null mouse (Kraus et al., 2001; Chiang et al., 2001) and the *ozd* mutation in the chick (Ros et al., 2003). Removal of the posterior mesoderm results in upregulation of *Bmp4* expression in the remaining mesoderm concomitantly with the increment in the repressor form of Gli3. We have performed a variety of experiments addressed to dissect the effects of both pathways and discuss our results in the context of current models of limb development.

References:

Chiang, C. et al. (2001). Manifestation of the Limb Prepattern: Limb Development in the Absence of Sonic Hedgehog Function. *Dev Biol* 236, 421-435

Kraus, P. et al. (2001). Some distal limb structures develop in mice lacking Sonic hedgehog signaling. *Mechanisms of Development* 100, 45-58.

Litingtung, Y. et al. (2002). Shh and Gli3 are dispensable for limb skeleton formation but regulate digit number and identity. *Nature*

Ros et al., (2003).The chick oligozeugodactyly (ozd) mutant lacks sonic hedgehog function in the limb. *Development*. 130:527-37.

Tumpel et al., (2002). Regulation of Tbx3 expression by anteroposterior signalling in vertebrate limb development. *Dev Biol*.;250:251-62.

te Welscher et al., (2002). Mutual genetic antagonism involving GLI3 and dHAND prepatterns the vertebrate limb bud mesenchyme prior to SHH signaling. *Genes Dev*. 16:421-6.

The BMP antagonist Gremlin: A key regulator of epithelial-mesenchymal signaling during organogenesis?

Odysse Michos, Hans Goedemans, Kristina Vintersten, Silwia Kuc, Rolf Zeller and Aimée Zuniga

Morphogenesis of vertebrate organs such as lungs, kidney and limbs is regulated by epithelial-mesenchymal interactions involving signals such as BMPs, FGFs and WNTs and is possibly modulated by antagonists. For example, we have previously obtained evidence that during limb bud development the BMP antagonist Gremlin functions in establishment of the epithelial-mesenchymal signaling interactions, which maintain SHH signaling by the limb bud organiser and distal limb bud patterning through the SHH/FGF signaling feedback loop. Using reverse genetics, we have now generated a loss-of-function Gremlin mutation in the mouse. Indeed, inactivation of Gremlin precludes establishment of the SHH/FGF feedback loop, disrupts up-regulation and propagation of SHH signaling by the limb bud organiser and causes distal limb skeletal abnormalities very similar to the limb deformity phenotype. Furthermore, Gremlin deficient mice are born alive, but die within 24hrs postnatal due to a complete lack of kidneys. Development of the definitive metanephric kidney is induced during mid-gestation by reciprocal signaling interactions involving the ureteric bud and the metanephric mesenchyme. In Gremlin deficient embryos, branching of the ureteric bud is initiated, but induction and differentiation of the metanephric mesenchyme is disrupted at an early stage causing failure of metanephric kidney development. Our ongoing molecular analysis of epithelial-mesenchymal signaling and its antagonism during metanephric kidney development indicates that Gremlin may be the earliest known inducer of the definitive kidney. These results will also be discussed in view of a potential role of BMP antagonism in epithelial-mesenchymal signal modulation during morphogenesis of other organs.

A conditional loss-of-function strategy to assess the contributions of Hox genes to limb development

Kmita M., Logan M., Rijli P., Tabin C., and Duboule D.

Limb development has proved to be an efficient model to study mechanisms related to vertebrate patterning. Similar sets of developmental genes are indeed at work during morphogenesis of both limbs and trunk. An example is the Hox family of transcription factors, and in particular a subset of HoxA and HoxD cluster genes, which are sequentially activated during limb bud development according to their relative order along their respective cluster1,2. Interestingly, morphogenesis of the appendicular skeleton is achieved through a directional and progressive process, such that proximal bony elements form before distal ones. This correlation between Hox genes sequential activation, on the one hand, and the progressive formation of the limb, on the second hand, is a requirement for proper limb patterning.

Loss-of-function experiments have further demonstrated that genes at the extremity of the complex (those activated the latest) pattern the extremity of the limb3,4 (autopod), whereas genes located in more 3' positions (hence activated earlier) are involved in patterning more proximal structures5. Although individual Hox loss-of-function have been produced and analysed, it has become apparent that limb morphogenesis involves a complex set of interactions between several Hoxa and Hoxd genes, working both in redundant and complementary ways. Therefore, the assessment of the integrated qualitative and quantitative contributions of these genes requires the study of compound mutants, involving both paralogous and non-paralogous genes. While a number of such genetic analysis have been undertaken, some key combinations turned out to be impossible to obtain, mostly because inactivation of Hoxa13 is detrimental to the embryo6, before limb patterning is achieved. To circumvent the Hoxa13 loss-of-function embryonic lethality, we took advantage of the loxP-Cre recombination system to produce tissue-restricted conditional mutants.

We generated mice carrying both a HoxA complex flanked by loxP sites, and the Prx1-Cre transgene, which produces the recombinase in limb buds from early stages onwards7. Mice of this condition are expected to delete, in vivo, their entire HoxA complex in the limb field. Even though Prx1-Cre expression is not restricted to the limbs, mutants for the conditional deletion of the HoxA complex are viable. Targeted deletion appeared incomplete at very early stages of limb bud development. However, Hoxa transcripts became rapidly undetectable, thereby allowing us to assess for the effect of full loss of function of Hoxa genes on limb patterning, either alone or in combination with HoxD mutants. In particular, we used mice carrying a complete deficiency of HoxD in combination with the conditional floxed HoxA allele.

While this massive inactivation of virtually all Hox genes active in limb patterning confirmed a number of previously identified functional inputs for Hoxa and Hoxd genes, the results obtained on both single and combined mutants allowed us to gain new insights into Hox gene contributions to limb morphogenesis. It came into view that both gene clusters are required for limb bud growth, as in the absence of both clusters, limbs are severely truncated. However, HoxA/D genes do not appear to be primarily implicated in the formation of the most proximal part of the limb skeleton. Instead, they seem to be progressively required, along with limb outgrowth, for the morphogenesis of the middle and distal part of the limbs. In addition, HoxA/D genes act in a synergistic fashion to control not only morphogenesis along the proximal-distal axis, but also along the anterior-posterior axis. The function of both Hoxa and Hoxd genes for growth and patterning of the precursor cells of the limb skeleton will be discussed.

References:

1. Dolle P. et al. Nature 342 (1989), pp. 767–772.
2. Nelson C.E. et al. Development 122 (1996), pp. 1449–1466.
3. Zàkàny J. et al. Proc Natl Acad Sci U S A. (1997) 94(25), pp. 13695-700.
4. Kmita M. et al. Nature 420 (2002), pp. 145-150.
5. Davis A.P. et al. Nature 375 (1995), pp. 791–795.
6. Fromental-Ramain C. et al. Development 122 (1996), pp. 461–472
7. Logan M. et al. Genesis 33 (2002), pp. 77-80.

Meis genes in organogenesis

Miguel Torres, Valeria Azcoitia, Giovanna Giovinazzo, Carlos G. Arques, Nadia Mercader, Juan José Sanz-Ezquerro

Departamento de Inmunología y Oncología, Centro Nacional de Biotecnología, CSIC, Cantoblanco, 28049 Madrid, Spain

The TALE homeobox genes *Meis1* and *Meis2* are important regulators of metazoan embryo development, showing conserved functions in limb and eye development (Bessa et al., 2002; Capdevila et al., 1999; Mercader et al., 1999; Zhang et al., 2002). During limb development, *Meis1* and *Meis2* function is restricted to the proximal limb region up to the stylopodal-zeugopodal (S-Z) boundary. Ectopic overexpression of Meis genes during chicken limb development inhibits limb distalization and distal limb differentiation. Retinoic acid (RA) is an upstream activator of *Meis1* and *Meis2* in the proximal chicken limb, thereby promoting the proximal character of limb cells (Mercader et al., 2000). A proximalizing effect has also been described for RA during limb regeneration in amphibians (Maden, 1982; Niazi and Saxena, 1979), but the molecular basis of this phenomenon remains unknown. We are exploring the function of the RA-Meis pathway during limb regeneration in urodeles, and comparing it to its roles in embryonic limb development. The role of the RA-Meis pathway in patterning limb main axes during embryonic development and regeneration will be discussed.

For a broader analysis of Meis function in animal development, we are also analysing the roles of Meis genes in genetically modified mice. Some of the roles of Meis proteins in animal development can be explained by their biochemical function as cofactors of Hox proteins. In addition, Hox-unrelated functions have been demonstrated, or are inferred from Meis expression patterns. We will present results from the analysis of *Meis1* mutant mice, in which we found defects that correlate with the Meis Hox-cooperative activity and defects apparently unrelated to Hox proteins activity. Our results indicate that *Meis1* is dispensable for limb patterning, suggesting redundancy with Meis2 in this role.

References:

Bessa J, Gebelein B, Pichaud F, Casares F, Mann RS. 2002 Combinatorial control of Drosophila eye development by eyeless, homothorax, and teashirt. *Genes Dev.* 16:2415-27.

Capdevila, J., T. Tsukui, C. Rodriquez Esteban, V. Zappavigna, and J.C. Izpisua Belmonte. 1999. Control of vertebrate limb outgrowth by the proximal factor Meis2 and distal antagonism of BMPs by Gremlin. *Mol Cell.* 4:839-849.

Maden M. 1982 Vitamin A and pattern formation in the regenerating limb. *Nature.* 295:672-5.

Mercader, N., E. Leonardo, N. Azpiazu, A. Serrano, G. Morata, C. Martinez, and M. Torres. 1999. Conserved regulation of proximodistal limb axis development by Meis1/Hth. *Nature.* 402:425-429.

Mercader, N., E. Leonardo, M.E. Piedra, C. Martínez-A, M.A. Ros, and M. Torres. 2000. Opposing RA and FGF signals control proximodistal vertebrate limb development through regulation of Meis genes. *Development*. 127:3961-3970.

Niazi IA, Saxena S. 1978. Abnormal hind limb regeneration in tadpoles of the toad, Bufo andersoni, exposed to excess vitamin A. *Folia Biol (Krakow)*. 1:3-8.

Zhang X, Friedman A, Heaney S, Purcell P, Maas RL. 2002 Meis homeoproteins directly regulate Pax6 during vertebrate lens morphogenesis. *Genes Dev.* 16:2097-107

Genetic studies of the propagation of long-range signaling from the ZPA

Cliff Tabin, Brian Harfe, Paul Scherz, Jill McMahon, Andy McMahon and Vicki Rosen

When it was first cloned and analyzed, one of the most striking aspects of the Sonic hedgehog (Shh) expression pattern was its colocolization with an experimentally defined domain in the posterior of the developing limb buds known as the Zone of polarizing activity or ZPA. Cells of ZPA will direct mirror image duplications of digits when transplanted into the anterior margin of a host limb bud, in a concentration-dependent manner. Shh can mimic this effect and also does so in proportion to the concentration of Shh protein used. However, whether Shh itself is a morphogen, and (if it is) the mechanism by which it acts over a distance, have remained unanswered and contentious issues. In recent experiments we have used genetic approaches in the mouse to obtain additional insight into these questions. Using a recombinase-based mapping strategy we have determined the identity of the cells in the limb bud which are descended from the Shh-expressing cells of the ZPA. This experiment suggests that there is a temporal, as well as, spatial gradient of Shh in the developing limb. It has been proposed that, in analogy to the role of dpp as a morphogen downstream of hh in the Drosophila wing disk, Shh may achieve its patterning effects indirectly, by inducing the secretion of the BMP homologue. According to this view, BMP2 and not Shh would be the true morphogen. We have tested this by conditionally deleting BMP2 expression within the limb bud using an Prx1 promoter to drive expression of cre recombinase throughout the early limb.

Session 5: Cardiac and Vascular Development
Chair: Christopher V. E. Wright

A stepwise model for lymphatic vasculature development

Guillermo Oliver and Natasha Harvey

Dept of Genetics, St. Jude Children's Research Hospital
Memphis, Tennessee 38105 USA

The most widely accepted model for lymphatic vasculature development was proposed in 1902 by F. Sabin who proposed that isolated primitive lymph sacs bud from the endothelium of veins during early development. We have previously described the identification of the homeobox gene Prox1 as the first specific marker of lymphatic endothelial cells. Functional inactivation of Prox1 in mice demonstrated that its activity is required in a subpopulation of vascular endothelial cells for determining the lymphatic fate of those budding lymphatic endothelial cell progenitors that will eventually give rise to the whole lymphatic vasculature. We also demonstrated that in vitro, forced expression of Prox1 in blood endothelial cells is sufficient to confer a lymphatic phenotype. On the basis of our findings we propose a stepwise model of lymphangiogenesis in which lymphatic vasculature development is initiated by the specific expression of Prox1 in a subpopulation of vascular endothelial cells that subsequently and in a time dependent manner adopt a lymphatic vasculature phenotype. In an effort to better understand the mechanism of Prox1 activity as well as to identify other key players participating in this poorly characterized process, we have started to generate a detailed transcriptional profiling of lymphatic endothelial cells during different stages of vascular development.

Hop is an unusual homeodomain protein that modifies embryonic and adult cardiac function

Jonathan Epstein

We have identified a novel homeodomain protein that we have called Hop, homeodomain only protein. Hop is the smallest known homeodomain protein with only 73 amino acids. Structural analysis indicates that it folds with a helix-turn-helix motif, similar to other homeodomains, but Hop lacks residues critical for DNA binding and it does not bind DNA. It is expressed in the developing heart where it functions downstream of Nkx2.5. Hop interacts with serum response factor (SRF) to modulate cardiac-specific gene transcription. Inactivation of Hop results in defective cardiac development and partial embryonic demise. Knockdown of Hop in zebrafish disrupts cardiac function. Overexpression of Hop in adult mice leads to dramatic cardiac hypertrophy. Mechanisms and gene programs accounting for these phenotypes will be discussed which suggest novel activities for this ancient structural motif.

Notch lateral activation promotes epithelial-mesenchymal transitions during heart development and neoplasic transformation

Luika A. Timmerman, Joaquin Grego, Esther Bertrán, José María Pérez-Pomares, Juan Diez, Sergi Aranda, Sergio Palomo, Angel Raya, Frank McCormick, Juan Carlos Izpisúa-Belmonte & José Luis de la Pompa

The Notch pathway regulates developmental cell-fate choices via mechanisms termed lateral inhibition and lateral activation, the latter being poorly understood in vertebrates. Disruption of Notch has severe developmental consequences and its ectopic activation is oncogenic in mammals. Here we demonstrate that Notch ligands and receptors are co-expressed at high levels in cells of the embryonic endocardia, including cells which overly the heart valve primordial (endocardial cushions). Ablation of Notch1 or the effector RBPJK curtails expression of Notch receptors, ligands, and the target gene HRT1, demonstrating the existence of a positive signalling feed-back loop consistent with lateral activation. Endocardial cells undergo a developmentally-regulated TGFb-mediated endothelial-mesenchymal transition (EMT) to cellularize developing cardiac valves. Loss of Notch activity attenuates expression of TGFb2 and its receptors, impedes local expression of the snail transcriptional repressor, and stabilizes expression of the endothelial cellular adhesion molecule VE-cadherin, resulting in concomitant loss of endocardial EMT. Conversely, transient ectopic activation of Notch in the zebrafish embryo results in abnormally enlarged and hypercellular cardiac cushions. Overexpresion of activated Notch in endothelial cells in vitro also induces severe attenuation of VE-Cadherin expression and loss of contact inhibition. These transformed cells undergo an apparent EMT, as evidenced by independent cell migration on plastic, through collagen, and in xenografts. As this appears to be independent of TGF beta signaling, we conclude that Notch plays an unexpected, novel role in the promotion of EMT in both developmental and tumor formation settings, in part via regulation of the cellular adhesion system.

Collaborative roles of VEGF, Angiopoietin family members in vascular development

Nicholas W. Gale

VEGF and Angiopoietin-1 requisitely collaborate during blood vessel development. While Angiopoietin-1 activates its Tie2 receptor, Angiopoietin-2 can activate Tie2 on some cells while blocking it on others. Our analysis of mice lacking Angiopoietin-2 reveals that it is dispensable for embryonic vascular development, but requisite for subsequent angiogenic remodeling. Unexpectedly, mice lacking Angiopoietin-2 also exhibit major lymphatic vessel defects. Genetic rescue with Angiopoietin-1 corrects the lymphatic but not angiogenesis defects, suggesting that Angiopoietin-2 acts as Tie2 agonist in the former setting but as antagonist in the latter. Our studies define a vascular growth factor whose primary role is in postnatal angiogenic remodeling, and also demonstrate that members of the VEGF and Angiopoietin families collaborate during development of the lymphatic vasculature.

References:

NW Gale, G Thurston, S Davis, SJ Wiegand, J Holash, JS Rudge, GD Yancopoulos (2003) Complementary and Coordinated Roles of the VEGFs and Angiopoietins During Normal and Pathological Vascular Formation. Cold Spring Harbor Symposia on Quantitative Biology, The Cardiovascular System.

DM Valenzuela, AJ Murphy*, D Frendewey*, NW Gale*, A Economides*, W Auerbach, W Poueymirou, N Adams, J Rojas, J Yasenchak, R Chernomorski, M Boucher, L Esau, J Zheng, J Griffiths, X Wang, H Su, Y Xue, M Dominguez, R Torres, L MacDonald, TM DeChiara, and GD Yancopoulos (2002) Velocigene: A High-Throughput Approach For Functionizing the Genome via Custom Gene Mutation and High-Resolution Expression Analysis in Mice. *Nature Biotechnology*; 21(6): pg 652-659

NW Gale, G Thurston, SF Hackett, R Renard, Q Wang, J McClain, C Martin, C Witte, MH Witte, D Jackson, C Suri, PA Campochiaro, SJ Wiegand, and GD Yancopoulos (2002). Angiopoietin-2 is required for postnatal angiogenesis and lymphatic patterning, and only the latter role is rescued by Angiopoietin-1.. *Developmental Cell*, 3 (3): pg 411-423.

Davis S, Papadopoulos N, Aldrich TH, Maisonpierre PC, Huang T, Kovac L, Xu A, Leidich R, Radziejewska E, Rafique A, Goldberg J, Jain V, Bailey K, Karow M, Fandl J, Samuelsson SJ, Ioffe E, Rudge JS, Daly TJ, Radziejewski C, Yancopoulos GD. (2003). Angiopoietins have distinct modular domains essential for receptor binding, dimerization and superclustering. Nat Struct Biol;10 (1):38-44

Yancopoulos GD, Davis S, Gale NW, Rudge JS, Wiegand SJ, Holash J. (2000). Vascular-specific growth factors and blood vessel formation. Nature 14;407 (6801): 242-8

Gale NW, Yancopoulos GD. (1999). Growth factors acting via endothelial cell-specific receptor tyrosine kinases: VEGFs, angiopoietins, and ephrins in vascular development. Genes Dev 1;13 (9): 1055-66

Suri C, McClain J, Thurston G, McDonald DM, Zhou H, Oldmixon EH, Sato TN, Yancopoulos GD. (1998). Increased vascularization in mice overexpressing angiopoietin-1. Science 16;282 (5388): 468-71

Suri C, Jones PF, Patan S, Bartunkova S, Maisonpierre PC, Davis 1, Sato TN, Yancopoulos GD. (1996). Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis. Cell 27;87(7): 1171-80

POSTERS

An inducible mouse model to study Snail function

Cristina Alvarez de Frutos and M. Ángela Nieto

The Snail superfamily of transcription factors play a crucial role in the triggering of the epithelial-mesenchymal transition (EMT). This process is fundamental for the formation of different tissues during embryonic development, including the early mesoderm and the neural crest. As Snail-mutant mice die in gastrulation, we have developed a spatiotemporally inducible mouse model to approach the in vivo analysis of later processes such as formation of the neural crest or differentiation of tissues and organs. We have developed a strategy to activate mouse Snail in a conditional manner by employing a fusion protein, mSna-FT-ERT2, consisting of the Snail coding region linked to a mutated ligand-binding domain of the human estrogen receptor, hERT2, which respond to the estrogen agonist 4-hidroxitamoxifen (4 OHT). This strategy would allow us to study the function of Snail in a spatiotemporally regulated manner.

In a preliminary study, we have activated Snail protein by intraperitoneal injection of tamoxifen into pregnant mice at 12,5 dpc and the corresponding embryos have been analysed at 18,5 dpc. At this particular stage, we have observed a decrease in size in transgenic mice versus wild type littermates, accompanied by deffects in limb development and in the curvature of the spine.

Xrx1 controls anterior neurogenesis and multipotency of retinal progenitors

Massimiliano Andreazzoli, Gaia Gestri, Federico Cremisi, Simona Casarosa, Igor B. Dawid, Giuseppina Barsacchi

In *Xenopus* neuroectoderm, posterior cells start differentiating at the end of gastrulation while anterior cells display an extended proliferative period and undergo neurogenesis only at tailbud stage. Recent studies have identified several important components of the molecular pathways controlling posterior neurogenesis, but little is known about those controlling the timing and positioning of anterior neurogenesis. We observed that the homeobox gene *Xrx1* is expressed in the entire proliferative region of the anterior neural plate delimited by cells expressing the neuronal determination gene *X-ngnr-1*, the neurogenic gene *X-Delta-1*, and the cell cycle inhibitor *p27Xic1*. Positive and negative signals position *Xrx1* expression to this region. *Xrx1* is activated by *chordin* and *hedgehog* signaling, which induce anterior and proliferative fate, and is repressed by the differentiation-promoting activity of neurogenin and retinoic acid. *Xrx1* is required for anterior neural plate proliferation and, when overexpressed, induces proliferation, inhibits *X-ngnr-1*, *X-Delta-1* and *N-tubulin* and counteracts *X-ngnr-1*- and retinoic acid-mediated differentiation. We find that *Xrx1* does not act by increasing lateral inhibition but by inducing the antineurogenic transcriptional repressors *Xhairy2* and *Zic2* and by repressing *p27Xic1*. *Xrx1* effects on proliferation, neurogenesis and gene expression are restricted to the most rostral region of the embryo, implicating this gene as an anterior regulator of early neurogenesis. Similarly, during retinogenesis *Xrx1* is expressed in retinal stem cells. in vivo lipofection of single retinal progenitors show that *Xrx1* overexpression increases clonal proliferation while *Xrx1* functional inactivation exerts the opposite effect. Interestingly, *Xrx1* lipofected retinas show no changes in the proportions of the different cell types, thus suggesting a role in supporting multipotency of retinal progenitors.

Meis1 implication in embryonic hematopoiesis

Azcoitia V, Mercader N, Giovinazzo G, Torres M

The correct spatial and temporal regulation of homeobox gene expression is essential to ensure the proper cellular behaviour during embryogenesis and postnatal life. While during embryogenesis the functional alteration of homeobox factors often provokes congenital defects, during the postnatal life, it leads to oncogenic processes. The homeobox protein Meis1, belonging to the TALE (Three Aminoacids Length Extension) family, is involved in determining segmental identity and proximodistal limb axis during embryogenesis, and in acute myeloid leukemogenesis (AML) in mice during postnatal life. To a large extent, Meis1 roles are explained by its molecular function as cofactor of homeobox proteins of the Hox family which, as Meis1, are involved in segmental identity specification during embryogenesis and induce similar oncogenic processes during postnatal life. Expression analysis suggests the existence of Meis1 undescribed functions and cofactors, involved in the morphogenesis of a variety of organs.

We aim to describe Meis1 functions, to identify novel Meis1 cofactors and their roles during embryonic development. For that purpouse, we have generated the conditional knock-in mouse of the Meis1a isoform gene, where Meis protein is fused to a hormone binding receptor. This fusion protein is translocated to the cell nucleus only upon Tamoxifen administration, behaving as a knock out otherwise.

The phenotype we observe reveals a new function of Meis1 gene directly related to embryonic hematopoiesis. The use of tamoxifen enables us to rescue the early dead of the embryos. Histological and haematological studies are currently under analysis.

Endogenous Cerberus activity is required for anterior head specification in *Xenopus*

Ana Cristina Silva, Mario Filipe, Klaus-Michael Kuerner, Herbert Steinbeisser and José António Belo

We analyzed the endogenous requirement for Cerberus in the organogenesis of the *Xenopus* head. "Knock down" of Cerberus function by antisense morpholino oligonucleotides did not impair head formation in the embryo. In contrast, targeted increase of BMP, Nodal and Wnt signaling in the Anterior-Dorsal-Endoderm (ADE) resulted in synergistic loss of anterior head structures, without affecting more posterior axial ones. Remarkably, those head phenotypes were aggravated by simultaneous depletion of Cerberus. These experiments demonstrated for the first time that endogenous Cerberus protein can inhibit BMP, Nodal and Wnt factors *in vivo*. Conjugates of Dorsal Ectoderm (DE) and ADE explants in which Cerberus function was "knocked down" revealed the requirement of Cerberus in the ADE for the proper induction of anterior neural markers and repression of more posterior ones. This data supports the view that Cerberus function is required in the leading edge of the ADE for correct induction and patterning of the neuroectoderm.

The E3 ubiquitin ligase Arkadia is regulating Nodal signalling

Vasso Episkopou, Rebecca Andrew, James Dixon, Chariclia Petropoulou, Puala Timmons, Kian-Leong Lee

In mice Nodal is essential for gastrulation, including mesoderm endoderm and node/ mesendoderm induction as well as patterning including both the antero-posterior (A-P) and left-right (L-R) axes. Arkadia is a novel putative E3 Ubiquitin ligase shown to be essential for node and mesendoderm formation in the mouse (1,2). Here using compound mutant embryos between Nodal and arkadia, and tetraploid chimeras we show that Arkadia is in the Nodal signalling pathway and that the two genes function together in a range of tissue induction and patterning events required to establish both the A-P and L-R axes. In addition, our data provide evidence that contributes to a body of work in mouse that correlates the level of Nodal signalling with the tissue induced. Finally, we have developed an ES cell assay to show that exogenous Arkadia expression can super-activate gene expression from Nodal signalling target reporters such as FoxH1-dependent enhancers, and that this activity requires an intact RING-H2 domain Arkadia, suggesting a ubiquitination dependent mechanism of action. Moreover, we present evidence that Arkadia interferes with the activity of the Nodal signal-transduction molecule Smad2.

References:

1. Episkopou, V., et al., Induction of the mammalian node requires Arkadia function in the extraembryonic lineages. Nature, 2001. 410(6830): p. 825-30.
2. Niederlander, C., et al., Arkadia enhances nodal-related signalling to induce mesendoderm. Nature, 2001. 410(6830): p. 830-4.

Identification and characterization of mesenchymal target genes of FGF signaling during facial development

Nicole Firnberg, Andreas Leibbrandt, Johannes Werzowa, and Annette Neubüser

Fgf8, a member of the fibroblast growth factor family of signalling molecules, is expressed in the ectoderm covering the midfacial area at early stages of facial development. Mouse embryos in which the Fgf8 gene has been inactivated in the facial region show severe craniofacial defects caused by altered signaling between the mutant ectoderm and the underlying mesenchyme.

In order to understand how FGF8 controls development of the facial mesenchyme we systematically screened for FGF8 inducible genes. For this purpose we have generated a DNA microarray consisting of cDNA clones from subtractive cDNA libraries from facial mesenchyme cultured in the presence or absence of FGF8. This microarray, consisting of more than 10000 clones was probed with cDNA derived from mesenchyme cultured with or without FGF8. The expression patterns of about 400 clones with the strongest differential hybridization signal have been analyzed by whole mount in situ hybridization and inducibility by FGF8 was confirmed by using an in vitro explant culture system.

Through this screen we have identified more than 80 genes that are induced in the facial mesenchyme in response to FGF signaling and we have begun to obtain full length sequences by RACE – PCR and library screening. Functional analysis of known genes like hyaluronan synthase 2 (Has2), or MAPK phosphatase 3 (Mkp3) or of unknown genes like a very long chain fatty acid Co-Acyl synthetase identified by this screen will ultimately help to understand the so far unknown function of FGF signaling during facial development at the molecular level.

Control of pancreas development by the Onecut transcription factors HNF-6 and OC-2

Patrick Jacquemin, Frédéric P. Lemaigre, and Guy G. Rousseau

Hormone and Metabolic Research Unit, Université catholique de Louvain and Institute of Cellular Pathology, Avenue Hippocrate 75, B-1200 Brussels, Belgium

The Onecut (OC) proteins, whose prototype is HNF-6, are evolutionarily conserved, tissue-restricted transcription factors which contain a bipartite DNA-binding domain composed of a single cut domain associated with a divergent homeodomain (Lemaigre et al, 1996; Lannoy et al, 1998). By analyzing the phenotype of Hnf6-/- mice we found that HNF-6 regulates pancreatic endocrine cell differentiation (Jacquemin et al, 2000). Here we show that HNF-6 is expressed in the endoderm where it controls an earlier step in pancreas development. Indeed, the pancreas of Hnf6-/- mice is hypoplastic. The onset of Pdx-1 expression is delayed in the ventral and dorsal endoderm, leading to a reduction in the number of endodermal cells expressing Pdx-1 at the time of pancreatic budding. Our data indicate that HNF-6 controls the timing of pancreas specification by acting upstream of Pdx-1 (Jacquemin et al, 2003). We further show that Oc2, a paralogue of Hnf6, is also expressed in the endoderm and developing pancreas (Jacquemin et al, submitted). As many target genes are recognized by both HNF-6 and OC-2 (Jacquemin et al, 1999), the similar expression pattern of these two factors suggests a functional redundancy. Indeed, the pancreas of Oc2-/- mice appears to be normal. However, Hnf6-/-Oc2-/- embryos have a more severe pancreatic phenotype than the Hnf6-/- embryos. Their dorsal pancreas is more hypoplastic and they have no ventral pancreas. These results suggest several models that account for the partial redundancy of HNF-6 and OC-2 in pancreas development. They also confirm that the programs for dorsal and ventral pancreas organogenesis are distinct.

References:

Jacquemin et al, J. Biol. Chem. 274, 2665-2671(1999).
Jacquemin et al, Mol. Cell. Biol. 20, 4445-4454 (2000).
Jacquemin et al, Dev. Biol., in press (2003).
Lannoy et al, J. Biol. Chem. 273, 13552-13562 (1998).
Lemaigre et al, Proc. Natl. Acad. Sci. USA 93, 9460-9464 (1996).

Snail superfamily genes in brain development. Angiogenesis and neurogenesis processes

Faustino Marin, Francisca Silva and M. Angela Nieto

The Snail-superfamily of transcription factors is subdivided into two families: Snail and Scratch. The Snail family, represented in vertebrates by Snail and Slug, is involved in the triggering of epithelial-mesenchymal transitions (EMT) both during embryonic development (gastrulation, neural crest migration) and tumor progression (1, 2). The first known member of the Scratch family in vertebrates has been proposed to participate in neuronal differentiation. A second gene, Scratch2, has been identified in our lab (1, 2).

Our current project consists of studying these genes in relation to vertebrate brain development at mid and late embryonic stages. We have compared their expression in Xenopus, chicken and mouse embryos. In these model systems, our results show separate respective roles for Snail and Scratch families:
a) Snail is specifically expressed in the choroid plexus, while Slug appears in meninges and brain periendothelial cells (pericytes/ vascular smooth muscle cells). Thus, Snail family members appear to be involved in brain vascularization at late stages (in contrast with their specific role in neural crest development at early neural tube stages). It is particularly interesting the Slug expression in pericytes, since these cells enter the brain migrating from the adjacent mesenchyme.
b) concerning the Scratch family, expression of Scratch1 and Scratch2 (at late embryonic and postnatal stages) appears related to neuronal differentiation.

On the whole, our results suggest that in vertebrates Snail family members are mainly involved in the development of mesenchymal brain annexes (choroidal plexus, meninges, pericytes) while the major function of Scratch genes would be related to proper neuronal development.

References:

1. Manzanares, Locascio and Nieto (2001) Trends Genet 17: 178
2. Nieto (2002) Nature Rev Mol Cell Biol 3: 155

A unique combination of homeodomain proteins marks deep nuclei cells during early cerebellar development

Daniver Morales*, Emili Cid and Mary E. Hatten

Laboratory of Developmental Neurobiology, The Rockefeller University, New York, NY10021, USA

The cerebellum is a structure of the vertebrate brain implicated in balance, control of motor coordination and motor memory formation. Underlying these functions there is a complex circuitry composed of distinct neuronal cell types including granule cells, Purkinje cells, stellate/basket cells, Golgi cells and deep nuclei cells. To better understand the molecular mechanisms that shape cell fate specification of deep nuclei cells, we analyzed the topographic expression patterns of various transcription factor family members including LIM homeodomain (Lhx), basic helix-loop-helix protein (bHLH), Iroquois homeodomain (Irx) and Even-Skipped (Evx), at the time when neurogenesis begins in the cerebellar anlage. Our results reveal that a unique combination of these homeodomain proteins demarcate the territory where deep nuclei cells differentiate in the avian and mammalian cerebellum. We establish, by evaluating different criteria classically used for assigning neuronal identity, that Lhx2,9, Irx3 and Evx1 expression define prospective deep nuclei cells. One feature of this molecular code is that LIM, Even-Skipped and Iroquois gene families exhibit distinct expression patterns in the progenitor and postmitotic cell domains. The LIM and Even-Skipped homeodomain proteins mark postmitotic cell zones. Instead Iroquois transcription factors mark cells both in the proliferative and differentiating zones of the cerebellar anlage. This complements previous work showing that Math1, Pax6 and Zipro1 mark embryonic granule cells. Taken together, the existence of this complex genetic regulatory network defining deep nuclei cells and granule cells supports the idea that unique combinations of transcription factors are crucial for generating cell diversity during development of the central nervous system.

Supported by grants: NS 30532-10 and NS 045433-01

Expression and function of Mash1 proneural gene in postnatal neurogenesis and oligodendrogenesis

Carlos Parras, Olivier Britz, Rossella Galli, Christophe Galichet, Jane Johnson, Angelo Vescovi and François Guillemot

Neural stem cells (NSCs) have been found in the adult brain, in particular in the periventricular region of the telencephalon. NSCs produce transit amplifying cells that generate proliferating neuroblasts migrating to the olfactory bulb where they differentiate into interneurons. In the embryo, the bHLH gene Mash1 functions as a proneural gene for progenitors of the ventral telencephalon. We have found that Mash1 is also expressed in the adult telencephalon, suggesting that it may play a role in postnatal and adult neurogenesis. Here we demonstrate that Mash1 is transiently expressed in a population of proliferating cells distinct from both NSCs and migratory neuroblasts. In addition, some Mash1-positive precursors express oligodendrocytic markers (NG2, PDGFR), suggesting that Mash1 has also a role of in oligodendrogenesis. Using the LacZ reporter expressed from Mash1 regulatory sequences as a short term lineage tracer, we also show that Mash1+ progenitors give rise essentially to migrating PSA-NCAM+ neuroblasts. Thus Mash1+ progenitors have characteristics of transit amplifying or C cells (Doetsch et al., 1997). To further characterize these cells, Mash1::LacZ+ cells were FACSorted from adult brains. Interestingly, when these cells were cultivated in presence of growth factors, they had the capacity to generate neurospheres which could be propagated or differentiated into neurons, oligodendrocytes and astrocytes after mitogen withdrawal. Thus, the in vitro culture environment can drastically alter the properties of Mash1+ progenitors, from transiently proliferating, neuronal-restricted progenitors to self renewing, multipotent progenitors. To directly address Mash1 function in postnatal neurogenesis, we have isolated, propagated and differentiated neurospheres from Mash1 mutant neonates. When compared with wild-type cultures, cultures from Mash1-mutant neurospheres produced severely reduced numbers of neurons, but also of oligodendrocytes. In vivo analysis on Mash1 mutant neonates showed a reduction of NG2+ precursors in the olfactory bulb indicating a partial requirement for Mash1 function in the generation of oligodendrocytes. These results indicate that Mash1 function is required for the production of both neuronal and oligodendrocytic cell types, and suggest that proneural genes must interact with other determinants to specify different cell fates in different cellular contexts.

Identification of genes responsible for thyroid bud migration

Postiglione M. (1), Parlato R. (1), Dogaru M. (1), Sanges R. (2), De Luca P. (2), Bonner R. (3), De Felice M. (1), Di Lauro R. (1)

(1) Stazione Zoologica Anton Dohrn, Villa Comunale 1, 80121, Naples,Italy.
(2) BioGeM, Gene Expression Core Lab, Via Pietro Castellino 111, 80121 Naples, Italy
(3) Section on Medical Biophysics, NIH/NICHD/LIMB, 13 South Drive, Building 13, Room 3W16, Bethesda MD 20892-5772, USA

The thyroid follicular cells (TFCs) are responsible for thyroid hormone biosynthesis and represent the most abundant cell type in the thyroid gland.

TFCs originate in the floor of the primitive pharynx. Their first appearance in mouse embryos can be detected at E 8.5 p.c by the expression of a specific set of transcription factors (TTF-1, TTF-2, Pax8 and Hex). Subsequently these cells entail dorso-caudal migration that brings them in front of the trachea, where they will merge with other cell types derived from the branchial pouches. Functional differentiation, as detected by thyroid hormone biosynthesis, only occurs at the end of the migration process.

In mice deprived of the transcription factor TTF-2, by the knock out of the corresponding *titf2* gene, thyroid cells do not migrate and remain attached at the pharyngeal floor. These cells express all remaining markers (TTF-1, Pax8 and Hex) and will eventually differentiate. Thus, it appears that TTF-2 is specifically involved in controlling migration of the TFCs.

In order to find genes responsible for migration, the thyroid bud from E10.5 wt and *titf2* KO embryo has been captured by laser capture microdissection. RNA has been isolated and used to construct cDNA libraries. Transcripts of the libraries have been hybridized to Affymetrix GeneChip MG U74 set.

In order to find biologically important genes we used stringent criteria, chiefly high level of expression and high fold change. We are focusing our attention on 600 genes, half of which are overexpressed in the wt thyroid while the other half shows a prevalent expression in the cells of the knock-out mice. From these 600 genes half has no known function, while the functional profile of the other half is essentially the same in migrating and non migrating thyroid cells.

The differential expression of these genes is being verified by in situ hybridization.

Neural tube signals are involved in otic vesicle regionalisation in chick

F. Aragón, E. Ulloa, S. Cereghini, B. Alsina, F. Giraldez, C. Pujades

The inner ear is a complex sensory organ responsible for the senses of hearing, balance and detection of acceleration in vertebrates. Development of inner ear starts with the induction of the otic placode within the surface ectoderm adjacent to the prospective hindbrain at the 3-5 somite stage (ss). At this very same stage in the hindbrain region of the vertebrate Central Nervous System (CNS), Antero-Posterior (AP) regionalisation involves a segmentation process leading to the formation of 7-8 morphological bulges called rhombomeres (r). Gene inactivation in mice suggested that genes expressed in the neighbouring hindbrain are involved in the control of otic development. Moreover, in zebrafish it has been shown that the transciption factor vHNF1 could be involved in hindbrain segmentation and otic development.

The main focus of our research is the analysis of the molecular signals from the hindbrain involved in inner ear development in vertebrates. We want to address which are the roles played by hindbrain segmentation genes -like the transcription factor vhnf1- during otic vesicle regionalization.

With that purpose mvhnf1 was expressed ectopically at the level of the hindbrain by the means of the in ovo electroporation in chick. vhnf1 appears to control the expression of MafB, Krox20 and Hoxb1 in the neural tube. Moreover, alterations in the hindbrain segmentation cascade generates a loss of the regionalized expression pattern of otic genes like Lmx1 and Nkx5.1. Our results support a model where the patterning of the otic vesicle is under the adjacent neural tube cues.

Somitogenesis runs differently in the first formed somites

Sofia Rodrigues

Somites are, by definition, reiterated structures that bud off in pairs from the presomitic mesoderm, respecting a specific timing. However, several studies have suggested that the first somites are different from the most posterior ones. For example, several mutations in mice and zebrafish, specifically disrupt the most posterior somites while the first 5 to 8 somites are spared (1). Furthermore, quail-chick chimera fate maps have shown that somites 1 to 4 give rise to the basal part of the skull while somite 5 gives rise to the boundary between the head and the neck, marking the onset of vertebrae formation (2). Also, a study in zebrafish has suggested that the first somites form faster than the most posterior ones (3).

In this work we have analysed the expression pattern of somitogenic related genes in the first formed somites. First, we checked for AP polarity and our results suggest that the first 4 somites do not exhibit AP polarity, and this is in accordance with the fact that these somites do not give rise to segmented structures since the process of resegmentation only starts at somite level 5 (2). Furthermore, our study shows that in the chick, the first somites seem to bud off at a faster rate than more posterior ones, in agreement to what has been suggested for zebrafish. So, could the clock be running faster in the first formed somites?

References:

1- **Pourquié, O.** (2001). Vertebrate segmentation. Annu.Rev.Cell.Dev.Biol.**17**, 311-50.

2- **Huang, R., Zhi, Q., Patel, K., Wilting, J. and Christ, B.** (2000). Contribution of single somites to the skeleton and muscles of the occipital and cervical regions in avian embryos. *Anat. Embryol. (Berl)* **202**. 375-383.

3- **Kimmel, C.B., Ballard, W.W., Kimmel, S.R., Ullmann, B. and Schilling, T.F.** (1995). Stages of embryonic development of the zebrafish. *Dev. Dyn.* **203(3)**, 253-310.

Signalling properties of chick limb bud cells investigated by grafting manipulated, cultured cells

Eva Tiecke1, Juan José Sanz Ezquerro 2, Anne Warner3 and Cheryll Tickle1

Division of Cell and Developmental Biology, Wellcome Trust Biocentre, 1University of Dundee, U.K., 2 Present address Centro Nacional de Biotecnología, Madrid, Spain, 3 University College London, UK, e.tiecke@dundee.ac.uk

We wish to understand how signalling and responsiveness to signals is maintained during limb bud development. We have approached this by culturing limb bud cells under various conditions and then re-grafting them into the limb. We focussed on how signalling in the polarizing region, located at the posterior of the limb bud is maintained. Signalling can be assayed by grafting polarizing region cells anteriorly which induce digit duplications (polarizing activity). FGF signalling from the ridge maintains Shh expression in the polarizing region and Shh is the basis of polarizing activity. FGF signalling is associated with a high density of gap junctions between cells in the polarizing region.

In order to test whether this junctional communication is involved in maintaining signalling activity, we treated cultured cells from posterior mesenchyme with forskolin, which increases gap junctional communication. Forskolin also activates adenyl cyclase, increasing levels of PKA, which interacts with the Shh signal transduction pathway. Cells from anterior mesenchyme were used as controls.

Quail anterior and posterior limb bud cells were cultured with forskolin for 24 h and then grafted back to the anterior margin of chick limb buds. Both treated and control posterior cultures induced digit duplication but, unexpectedly, anterior forskolin treated cultures also induced extra digits while anterior control cultures did not. In order to determine the cellular origin of the extra digits induced by anterior forskolin treated cultures we examined the distribution of grafted quail cells. We found that additional digits contained grafted quail cells but there was also a substantial contribution from the chick host. We could not detect any Shh expression in the anterior treated cultures either at 1 or 24 h after culture (by RT-PCR), and Shh expression was not switched on when the cultures were grafted back into the limb. Forskolin treatment might trigger the intracellular Shh signalling cascade and therefore we examined expression of known Shh target genes. Grafts of anterior forskolin treated cells expressed Ptc and Gremlin and expression of Fgf4 and Fgf8 in the ridge was affected. This suggests that the Shh pathway has been triggered in anterior forskolin treated cells in the absence of the ligand. This result may lead to insights into the mechanisms that control digit formation in the developing limb.

Embryonic olfactory bulb stem cells differentiate in mature neurons. The role of endogenous IGF-I and mechanisms of neuronal specification

María J. Yusta-Boyo, Manuel González, Antonio Bernad, Flora de Pablo and Carlos Vicario-Abejón

Group of Growth Factors in Vertebrate Development, Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas, Madrid, Spain

At early stages of embryonic development, neuroepithelial precursors cells in the vertebrate nervous system have the potential to differentiate into neurons, astrocytes and oligodendrocytes, and the ability to self-renew indicating that they are neural stem cells. We have isolated and characterized stem cells locally born in the embryonic mouse olfactory bulb (OBSC) (E12.5-E14.5). These cells were 99.2% nestin positive and proliferated extensively in culture to at least 150 cells doublings. Clonal analysis demonstrated that neurons ($TuJ1^+$), astrocytes ($GFAP^+$) and oligodendrocytes (O_4^+) could be generated from single-plated cells, indicating that they are multipotent.

Under differentiation conditions, in a serum-free medium, E14.5 olfactory bulb stem cells originated a high number of neurons (52%), followed by astrocytes (26%) and oligodendrocytes (4%). In long term cultures, these cells originated different neuronal subtypes such as Calretinin-, GABA-, GAD- or TH-positive cells. In addition, these neurons expressed the synaptic-related proteins synapsin-I and SV2.

RT-PCR analysis revealed expression of IGF-I in proliferating and differentiating cells. Differentiation and survival of stem cell-generated neurons and glia showed strong dependence on exogenous IGF-I. Furthermore, the percentage of stem cell-derived neurons, astrocytes and oligodendrocytes were markedly lower in the cultures prepared from *Igf-I*$^{-/-}$ mice compared with those of *Igf-I*$^{+/+}$. Concordantly, lack of IGF-I resulted in abnormal formation of the olfactory bulb mitral cell layer and altered radial glia morphology. These results support the existence within the embryonic mouse olfactory bulb of stem cells with specific requirements for insulin-related growth factor for differentiation. In order to analyse molecular mechanism involved in neuronal specification, we used retroviral infection techniques. A retroviral construct expressing GFP infected more than 94% of OBSC, these cells did not change their proliferation and differentiation properties. This technique will allows us to elucidate the role of specific transcription factors during OB neurogenesis.

Identification and functional characterisation of new genes implicated in the control of interdigital programmed cell death

Vanessa Zuzarte Luis

Dpto. de Anatomía y Biología Celular
Fac. de Medicina. Univ. de Cantabria
Cardenal Herrera Oria s/n
39011 Santander

During limb development, cell death by apoptosis has a key role in sculpturing the shape of developing digits, eliminating the interdigital mesoderm. In species with webbed digits, the interdigital mesoderm escape from the death program allowing the presence of webs between the digits.

Previous studies, on the control of interdigital apoptosis in avian embryos, have shown that interdigital cell death constitutes an apoptotic process regulated by the interaction numerous factors; members of the transforming growth factor beta superfamily and their natural antagonists; members of the FGF family; and the retinoic acid signalling pathway. The implication of caspases its regulators in the execution of the death program, which is a characteristic feature of the apoptotic processes, has been also demonstrated. However, the molecular cascade between the apoptotic signal mediated by BMPs and the execution of cell death by caspases remains unknown. Furthermore, it must be taken into account, that the elimination of the interdigital tissue, not only involves mesodermal apoptosis, but also, the activation of phagocytosis, the degradation of the extracellular matrix, the disintegration of the interdigital ectoderm and regression of the blood vessels. In this work we tried to identify and characterise new genes implicated in the control of regression of interdigital tissue, during the development of the chicken embryo. Out of 200 explored genes, about 20 genes implicated, directly or indirectly, in a variety of apoptotic processes were identified. Among these genes some exhibited a regulated pattern of expression in association with interdigital cell death.

LIST OF INVITED SPEAKERS

Paola Bovolenta — Instituto Cajal, CSIC. Av. Doctor Arce 37. 28002 Madrid (Spain). Tel.: 34 91 585 47 17. Fax: 34 91 585 47 54. E-mail: bovolenta@cajal.csic.es

Anne L. Calof — Depts of Anatomy & Neurobiology and Developmental & Cell Biology , and the Developmental Biology Center, University of California, Irvine. 364 Med Surge II. UCI College of Medicine, Irvine, CA. 92697-1275 (USA). Tel.: 1 949 824 4616. Fax: 1 949 824 1104. E-mail: alcalof@uci.edu

Tom Curran — St. Jude Children's Research Hospital. 332 North Lauderdale, Memphis, TN. 38105 (USA). Tel.: 1 901 495 2255. Fax: 1 901 495 2270. E-mail: tom.curran@stjude.org

Jonathan Epstein — Cardiovascular Division. University of Pennsylvania. 954 BRB II. 421 Curie Blvd, Philadelphia, PA. 19104 (USA). Tel.: 1 215 898 8731. Fax: 1 215 573 2094. E-mail: epsteinj@mail.med.upenn.edu

Nicholas W. Gale — Functional Genomics and Angiogenesis. Regeneron Pharmaceuticals, Inc. 777 Old Saw Mill River Rd, Tarrytown, NY. 10591-6707 (USA). Tel.: 1 914 345 7587. Fax: 1 914 347 5045. E-mail: nicholas.gale@regeneron.com

Fernando Giráldez — DCEXS-Universitat Pompeu Fabra. Doctor Aiguader 80. 08003 Barcelona (Spain). Tel.: 34 93 542 2932. Fax: 34 93 542 28 02. E-mail: fernando.giraldez@cexs.upf.es

François Guillemot — National Institute for Medical Research. Mill Hill, London NW7 1AA (UK). Tel.: 44 20 8816 2740. Fax: 44 20 8816 2109. E-mail: fguille@nimr.mrc.ac.uk

Susan K. McConnell — Department of Biological Sciences, Stanford University. 385 Serra Mall, Stanford, CA. 94305-5020 (USA). Tel.: 1 650 725 87 86. Fax: 1 650 725 98 32. E-mail: suemcc@stanford.edu

Guillermo Oliver — Dept of Genetics, St. Jude Children's Research Hospital. 332 North Lauderdale, Memphis, TN. 38105 (USA). Tel.: 1 901 495 2697. Fax: 1 901 526 2907. E-mail: Guillermo.Oliver@stjude.org

Olivier Pourquie — Stowers Institute for Medical Research. 1000 East 50th Street, Kansas City, MO. 64110 (USA). Tel.: 1 816 926 4442. Fax: 1 816 926 2095. E-mail: olp@stowers-institute.org

María A. Ros	Departamento de Anatomía y Biología Celular. University of Cantabria, 39011 Santander (Spain). Tel.: 34 942 20 19 33. Fax: 34 942 20 19 03. E-mail: rosm@unican.es
Beatriz Sosa-Pineda	Department of Genetics, St. Jude Children's Research Hospital. 332 North Lauderdale, Memphis, TN. 38105 (USA). Tel.: 1 901 495 2701. Fax: 1 901 526 2907. E-mail: beatriz.Sosa-Pineda@stjude.org
Didier Stainier	Dept. of Biochemistry & Biophysics. UCSF. 513 Parnassus Avenue, San Francisco, CA. 94143-0448 (USA). Tel.: 1 415 502 5679. Fax: 1 415 476 3892. E-mail: didier_stainier @biochem.ucsf.edu
Cliff Tabin	Genetics Harvard Medical School. 200 Longwood Ave, Boston, MA. 02115 (USA). Tel.: 1 617 432 7618. Fax: 1 617 432 7595. E-mail: tabin@rascal.med.harvard.edu
Irma Thesleff	Developmental Biology Research Program, Institute of Biotechnology, University of Helsinki. Viikinkaari 9, 00710 Helsinki (Finland). Tel.: 358 9 19159 401. Fax: 358 9 19159 366. E-mail: thesleff@Operoni.helsinki.fi
Miguel Torres	Departamento de Inmunología y Oncología, Centro Nacional de Biotecnología, CSIC. Cantoblanco, 28049 Madrid (Spain). Tel.: 34 91 585 48 49. Fax: 34 91 372 04 93. E-mail: mtorres@cnb.uam.es
Seppo Vainio	University of Oulu, Biocenter Oulu & Department of Biochemistry. Linnanmaa, 90570 Oulu (Finland). Tel.: 358 8 553 1190. Fax: 358 8 553 1141. E-mail: svainio@sun3. oulu.fi
David G. Wilkinson	Division of Developmental Neurobiology, National Institute for Medical Research. The Ridgeway, Mill Hill, London NW7 1AA (UK). Tel.: 44 20 8816 2404. Fax: 44 20 8816 2593. E-mail: dwilkin@nimr.mrc.ac.uk
Christopher V. E. Wright	Dept. of Cell and Developmental Biology. Vanderbilt University Medical Center. 465 21st Avenue South, Nashville, TN. 37232-8240 (USA). Tel.: 1 615 343 8256. Fax: 1 615 322 1917. E-mail: chris.wright@vanderbilt.edu
Kenneth S. Zaret	Cell and Developmental Biology Program. Fox Chase Cancer Center. 7701 Burholme Ave., Philadelphia, PA. 19111 (USA). Tel.: 1 215 728 7066. Fax: 1 215 379 4305. E-mail: zaret@fccc.edu

LIST OF PARTICIPANTS

Cristina Alvárez	Instituto Cajal, CSIC. Avda. Dr. Arce 37, 28002 Madrid (Spain). Tel.: 34 91 585 4736. Fax: 34 91 585 4754. E-mail: calvarez@cajal.csic.es
Massimiliano Andreazzoli	Dipartimento di Fisiologia e Biochimica. Universitá di Pisa. Via Carducci 13, 56010 Ghezzano, Pisa (Italy). Tel.: 39 050 87 8356. Fax: 39 05087 8486. E-mail: andream@dfb.unipi.it
Carlos Arques	Centro Nacional de Biotecnología. Campus de Cantoblanco, 28049 Madrid (Spain). Tel.: 34 91 585 46 65. Fax: 34 91 372 04 93. E-mail: cgarcia@cnb.uam.es
Valeria Azcoitia	Dpto. de Inmunología y Oncología. Centro Nacional de Biotecnología, CSIC. Campus de Cantoblanco, 28049 Madrid (Spain). Tel.: 34 91 585 4659. Fax: 34 91 372 0493. E-mail: vazcoitia@cnb.uam.es
José António Belo	Instituto Gulbenkian de Ciência. Rua da Quinta Grande 6. Ap. 14, 2781 901 Oeiras (Portugal). Tel.: 351 21 440 7942. Fax: 351 21 440 7970. E-mail: jbelo@igc.gulbenkian.pt
María José Blanco	Departamento de Ciencias Morfológicas I. Universidad Complutense de Madrid. Avda. Complutense s/n, 28040 Madrid (Spain). Tel.: 34 91 394 13 74. Fax: 34 91 394 13 74. E-mail: mjblanco@cajal.csic.es
José Luis de la Pompa	Institut de Recerca Oncologica (IRO). Hospital Duran i Reynals. Gran Via s/n, km. 2.7, 08907 L'Hospitalet de Llobregat, Barcelona (Spain). Tel.: 34 93 260 7828. Fax: 34 93 260 7426. E-mail: jldelapompa@iro.es
David del Álamo	Centro de Biología Molecular "Severo Ochoa". Campus de Cantoblanco, 28049 Madrid (Spain). Tel.: 34 91 397 8422. Fax: 34 91 397 4799. E-mail: dalamo@cbm.uam.es
Natalie DeWitt	Nature. Nature Publishing Group. 225 Bush Street, San Francisco, CA. 94104 (USA). Tel.: 1 415 403 9027. Fax: 1 415 781 3805. E-mail: n.dewitt@naturesf.com
Vasso Episkopou	MRC Clinical Sciences Centre. Faculty of Medicine. ICSTM. Hammersmith Hospital Campus. Du Cane Road, London W12 0NN (UK). Tel.: 44 20 8383 8277. Fax: 44 20 8383 8303. E-mail: vepiskop@csc.mrc.ac.uk
José L. Ferrán	Dpto. de Anatomía. Facultad de Medicina. Univ. de Murcia. Campus de Espinardo, 30100 Murcia (Spain). Tel.: 34 968 363 954. Fax: 34 968 363 955. E-mail: jlferran@um.es

Nicole Firnberg	Institute of Molecular Pathology. Dr. Bohrgasse 7, 1030 Vienna (Austria). Tel.: 43 1797 30532. Fax: 43 1798 7153. E-mail: firnberg@nt.imp.univie.ac.at
Patrick Jacquemin	Hormone and Metabolic Research Unit. Université catholique de Louvain and Inst. of Cellular Pathology. Avenue Hippocrate 75, 1200 Brussels (Belgium). Tel.: 32 2764 7531. Fax: 32 2764 7507. E-mail: jacquemin@horm.ucl.ac.be
Marie Kmita	Dept. of Zoology. Sciences III. University of Geneva. Quai Ernest Ansermet 30, 1211 Geneva 4 (Switzerland). Tel.: 41 22 702 6780. Fax: 41 22 702 6795. E-mail: marie.kmita@zoo.unige.ch
Miguel Manzanares	Inst. de Investigaciones Biomédicas "Alberto Sols". CSIC-UAM. Arturo Duperier 4, 28029 Madrid (Spain). Tel.: 34 91 585 4493. Fax: 34 91 585 4401. E-mail: mmanzanares@iib.uam.es
Faustino Marin	Dpto. de Neurobiología del Desarrollo. Instituto Cajal. Avda. Dr. Arce 37, 28002 Madrid (Spain). Tel.: 34 91 585 4736. Fax: 34 91 585 4754. E-mail: faustino@cajal.csic.es
Nadia Mercader	Dpto. de Inmunología y Oncología. Centro Nacional de Biotecnología, CSIC. Campus de Cantoblanco, 28049 Madrid (Spain). Tel.: 34 91 585 4665. Fax: 34 91 372 0493. E-mail: nmercader@cnb.uam.es
Thimios Mitsiadis	École Normale Supérieure de Lyon. 46, Allée d'Italie, 69364 Lyon (France). Tel.: 33 4 7272 8196. Fax: 33 4 7272 8080. E-mail: efthimios.mitsiadis@ens-lyon.fr
Aixa V. Morales	Dpto. de Neurobiología del Desarrollo. Instituto Cajal, CSIC. Avda. Dr. Arce 37, 28002 Madrid (Spain). Tel.: 34 91 585 4736. Fax: 34 91 585 4754. E-mail: aixamorales@cajal.csic.es
Daniver Morales	Laboratory of Developmental Neurobiology. The Rockefeller University. 1230 York Ave., New York, NY. 10021 (USA). Tel.: 1 212 327 7211. Fax: 1 212 327 7140. E-mail: moraled@rockefeller.edu
Carlos Parras	Division of Molecular Neurobiology. National Institute for Medical Research. The Ridgeway, Mill Hill, London NW7 1AA (UK). Tel.: 44 20 8816 2741. Fax: 44 20 8816 2109. E-mail: cparras@nimr.mrc.ac.uk
Fabienne Pituello	Centre de Biologie du Développement. UMR 5547-CNRS. Université Paul Sabatier. 118 rte de Narbonne, 31062 Toulouse (France). Tel.: 33 561 55 67 42. Fax: 33 561 55 65 07. E-mail: pituello@cict.fr

Maria Postiglione	Stazione Zoologica Anton Dohrn. Villa Comunale 1, 80121 Naples (Italy). Tel.: 39 0815 833 283. Fax: 39 0815 833 285. E-mail: mppost@szn.it
Cristina Pujades	Grup de Biologia del Desenvolupament. DCEXS. Universitat Pompeu Fabra. Dr. Aiguader 80, 08003 Barcelona (Spain). Tel.: 34 93 542 2944. Fax: 34 93 542 2802. E-mail: cristina.pujades@cexs.upf.es
Isabel Rodrigo	Instituto Cajal, CSIC. Avda. Dr. Arce 37, 28002 Madrid (Spain). Tel.: 34 91 585 47 15. Fax: 34 91 585 47 54. E-mail: irodrigo@cajal.csic.es
Sofia Rodrigues	Instituto Gulbenkian de Ciência. Rua da Quinta Grande 6. Ap. 14, 2781 901 Oeiras (Portugal). Tel.: 351 214 464 632. Fax: 351 214 407 970. E-mail: sofiar@igc.gulbenkian.pt
Thomas Schimmang	Center for Molecular Neurobiology. Univ. of Hamburg. Falkenried 94, 20251 Hamburg (Germany). Tel.: 49 40 42803 6273. Fax: 49 40 42803 6598. E-mail: schimman@zmnh.uni-hamburg.de
Eva Tiecke	Division of Cell and Developmental Biology. Wellcome Trust Biocentre. University of Dundee. Dow Street, Dundee DD1 5EH (UK). Tel.: 44 1382 345 861. Fax: 44 1382 345 386. E-mail: e.tiecke@dundee.ac.uk
María J. Yusta-Boyo	Group of Growth Factors in Vertebrate Development. Centro de Investigaciones Biológicas. CSIC. Velázquez 144, 28006 Madrid (Spain). Tel.: 34 91 564 45 62. Fax: 34 91 562 75 18. E-mail: mjyusta@cib.csic.es
Aimée Zuniga	Dept. of Developmental Biology. University of Utrecht. Padualaan 8, 3584 Utrecht (The Netherlands). Tel.: 31 30 253 3573. Fax: 31 30 253 2837. E-mail: A.Zuniga@bio.uu.nl
Vanessa Zuzarte	Dpto. de Anatomía y Biología Celular. Fac. de Medicina. Universidad de Cantabria. Cardenal Herrera Oria s/n, 39011 Santander (Spain). Tel.: 34 942 201 923. Fax: 34 942 201 903. E-mail: zuzartev@unican.es

Texts published in the
SERIE UNIVERSITARIA
by the
FUNDACIÓN JUAN MARCH
concerning workshops and courses organized within the
Plan for International Meetings on Biology (1989-1991)

---

*: Out of stock.

*246 **Workshop on Tolerance: Mechanisms and Implications.**
Organizers: P. Marrack and C. Martínez-A.

*247 **Workshop on Pathogenesis-related Proteins in Plants.**
Organizers: V. Conejero and L. C. Van Loon.

*248 **Course on DNA - Protein Interaction.**
M. Beato.

*249 **Workshop on Molecular Diagnosis of Cancer.**
Organizers: M. Perucho and P. García Barreno.

*251 **Lecture Course on Approaches to Plant Development.**
Organizers: P. Puigdomènech and T. Nelson.

*252 **Curso Experimental de Electroforesis Bidimensional de Alta Resolución.**
Organizer: Juan F. Santarén.

253 **Workshop on Genome Expression and Pathogenesis of Plant RNA Viruses.**
Organizers: F. García-Arenal and P. Palukaitis.

254 **Advanced Course on Biochemistry and Genetics of Yeast.**
Organizers: C. Gancedo, J. M. Gancedo, M. A. Delgado and I. L. Calderón.

*255 **Workshop on the Reference Points in Evolution.**
Organizers: P. Alberch and G. A. Dover.

*256 **Workshop on Chromatin Structure and Gene Expression.**
Organizers: F. Azorín, M. Beato and A. A. Travers.

257 **Lecture Course on Polyamines as Modulators of Plant Development.**
Organizers: A. W. Galston and A. F. Tiburcio.

*258 **Workshop on Flower Development.**
Organizers: H. Saedler, J. P. Beltrán and J. Paz-Ares.

*259 **Workshop on Transcription and Replication of Negative Strand RNA Viruses.**
Organizers: D. Kolakofsky and J. Ortín.

*260 **Lecture Course on Molecular Biology of the Rhizobium-Legume Symbiosis.**
Organizer: T. Ruiz-Argüeso.

261 **Workshop on Regulation of Translation in Animal Virus-Infected Cells.**
Organizers: N. Sonenberg and L. Carrasco.

*263 **Lecture Course on the Polymerase Chain Reaction.**
Organizers: M. Perucho and E. Martínez-Salas.

*264 **Workshop on Yeast Transport and Energetics.**
Organizers: A. Rodríguez-Navarro and R. Lagunas.

265 **Workshop on Adhesion Receptors in the Immune System.**
Organizers: T. A. Springer and F. Sánchez-Madrid.

*266 **Workshop on Innovations in Proteases and Their Inhibitors: Fundamental and Applied Aspects.**
Organizer: F. X. Avilés.

267 **Workshop on Role of Glycosyl-Phosphatidylinositol in Cell Signalling.**
Organizers: J. M. Mato and J. Larner.

268 **Workshop on Salt Tolerance in Microorganisms and Plants: Physiological and Molecular Aspects.**
Organizers: R. Serrano and J. A. Pintor-Toro.

269 **Workshop on Neural Control of Movement in Vertebrates.**
Organizers: R. Baker and J. M. Delgado-García.

Texts published by the CENTRE FOR INTERNATIONAL MEETINGS ON BIOLOGY

1 **Workshop on What do Nociceptors Tell the Brain?**
Organizers: C. Belmonte and F. Cerveró.

*2 **Workshop on DNA Structure and Protein Recognition.**
Organizers: A. Klug and J. A. Subirana.

*3 **Lecture Course on Palaeobiology: Preparing for the Twenty-First Century.**
Organizers: F. Álvarez and S. Conway Morris.

*4 **Workshop on the Past and the Future of Zea Mays.**
Organizers: B. Burr, L. Herrera-Estrella and P. Puigdomènech.

*5 **Workshop on Structure of the Major Histocompatibility Complex.**
Organizers: A. Arnaiz-Villena and P. Parham.

*6 **Workshop on Behavioural Mechanisms in Evolutionary Perspective.**
Organizers: P. Bateson and M. Gomendio.

*7 **Workshop on Transcription Initiation in Prokaryotes**
Organizers: M. Salas and L. B. Rothman-Denes.

*8 **Workshop on the Diversity of the Immunoglobulin Superfamily.**
Organizers: A. N. Barclay and J. Vives.

9 **Workshop on Control of Gene Expression in Yeast.**
Organizers: C. Gancedo and J. M. Gancedo.

*10 **Workshop on Engineering Plants Against Pests and Pathogens.**
Organizers: G. Bruening, F. García-Olmedo and F. Ponz.

11 **Lecture Course on Conservation and Use of Genetic Resources.**
Organizers: N. Jouve and M. Pérez de la Vega.

12 **Workshop on Reverse Genetics of Negative Stranded RNA Viruses.**
Organizers: G. W. Wertz and J. A. Melero.

*13 **Workshop on Approaches to Plant Hormone Action**
Organizers: J. Carbonell and R. L. Jones.

*14 **Workshop on Frontiers of Alzheimer Disease.**
Organizers: B. Frangione and J. Ávila.

*15 **Workshop on Signal Transduction by Growth Factor Receptors with Tyrosine Kinase Activity.**
Organizers: J. M. Mato and A. Ullrich.

16 **Workshop on Intra- and Extra-Cellular Signalling in Hematopoiesis.**
Organizers: E. Donnall Thomas and A. Grañena.

*17 **Workshop on Cell Recognition During Neuronal Development.**
Organizers: C. S. Goodman and F. Jiménez.

18 **Workshop on Molecular Mechanisms of Macrophage Activation.**
Organizers: C. Nathan and A. Celada.

*19 **Workshop on Viral Evasion of Host Defense Mechanisms.**
Organizers: M. B. Mathews and M. Esteban.

*20 **Workshop on Genomic Fingerprinting.**
Organizers: M. McClelland and X. Estivill.

21 **Workshop on DNA-Drug Interactions.**
Organizers: K. R. Fox and J. Portugal.

*22 **Workshop on Molecular Bases of Ion Channel Function.**
Organizers: R. W. Aldrich and J. López-Barneo.

*23 **Workshop on Molecular Biology and Ecology of Gene Transfer and Propagation Promoted by Plasmids.**
Organizers: C. M. Thomas, E. M. H. Willington, M. Espinosa and R. Díaz Orejas.

*24 **Workshop on Deterioration, Stability and Regeneration of the Brain During Normal Aging.**
Organizers: P. D. Coleman, F. Mora and M. Nieto-Sampedro.

25 **Workshop on Genetic Recombination and Defective Interfering Particles in RNA Viruses.**
Organizers: J. J. Bujarski, S. Schlesinger and J. Romero.

26 **Workshop on Cellular Interactions in the Early Development of the Nervous System of Drosophila.**
Organizers: J. Modolell and P. Simpson.

*27 **Workshop on Ras, Differentiation and Development.**
Organizers: J. Downward, E. Santos and D. Martín-Zanca.

*28 **Workshop on Human and Experimental Skin Carcinogenesis.**
Organizers: A. J. P. Klein-Szanto and M. Quintanilla.

*29 **Workshop on the Biochemistry and Regulation of Programmed Cell Death.**
Organizers: J. A. Cidlowski, R. H. Horvitz, A. López-Rivas and C. Martínez-A.

*30 **Workshop on Resistance to Viral Infection.**
Organizers: L. Enjuanes and M. M. C. Lai.

31 **Workshop on Roles of Growth and Cell Survival Factors in Vertebrate Development.**
Organizers: M. C. Raff and F. de Pablo.

32 **Workshop on Chromatin Structure and Gene Expression.**
Organizers: F. Azorín, M. Beato and A. P. Wolffe.

*33 **Workshop on Molecular Mechanisms of Synaptic Function.**
Organizers: J. Lerma and P. H. Seeburg.

*34 **Workshop on Computational Approaches in the Analysis and Engineering of Proteins.**
Organizers: F. S. Avilés, M. Billeter and E. Querol.

35 **Workshop on Signal Transduction Pathways Essential for Yeast Morphogenesis and Cell Integrity.**
Organizers: M. Snyder and C. Nombela.

36 **Workshop on Flower Development.**
Organizers: E. Coen, Zs. Schwarz-Sommer and J. P. Beltrán.

*37 **Workshop on Cellular and Molecular Mechanism in Behaviour.**
Organizers: M. Heisenberg and A. Ferrús.

38 **Workshop on Immunodeficiencies of Genetic Origin.**
Organizers: A. Fischer and A. Arnaiz-Villena.

39 **Workshop on Molecular Basis for Biodegradation of Pollutants.**
Organizers: K. N. Timmis and J. L. Ramos.

*40 **Workshop on Nuclear Oncogenes and Transcription Factors in Hematopoietic Cells.**
Organizers: J. León and R. Eisenman.

*41 **Workshop on Three-Dimensional Structure of Biological Macromolecules.**
Organizers: T. L Blundell, M. Martínez-Ripoll, M. Rico and J. M. Mato.

42 **Workshop on Structure, Function and Controls in Microbial Division.**
Organizers: M. Vicente, L. Rothfield and J. A. Ayala.

*43 **Workshop on Molecular Biology and Pathophysiology of Nitric Oxide.**
Organizers: S. Lamas and T. Michel.

*44 **Workshop on Selective Gene Activation by Cell Type Specific Transcription Factors.**
Organizers: M. Karin, R. Di Lauro, P. Santisteban and J. L. Castrillo.

45 **Workshop on NK Cell Receptors and Recognition of the Major Histocompatibility Complex Antigens.**
Organizers: J. Strominger, L. Moretta and M. López-Botet.

46 **Workshop on Molecular Mechanisms Involved in Epithelial Cell Differentiation.**
Organizers: H. Beug, A. Zweibaum and F. X. Real.

47 **Workshop on Switching Transcription in Development.**
Organizers: B. Lewin, M. Beato and J. Modolell.

48 **Workshop on G-Proteins: Structural Features and Their Involvement in the Regulation of Cell Growth.**
Organizers: B. F. C. Clark and J. C. Lacal.

*49 **Workshop on Transcriptional Regulation at a Distance.**
Organizers: W. Schaffner, V. de Lorenzo and J. Pérez-Martín.

50 **Workshop on From Transcript to Protein: mRNA Processing, Transport and Translation.**
Organizers: I. W. Mattaj, J. Ortín and J. Valcárcel.

51 **Workshop on Mechanisms of Expression and Function of MHC Class II Molecules.**
Organizers: B. Mach and A. Celada.

52 **Workshop on Enzymology of DNA-Strand Transfer Mechanisms.**
Organizers: E. Lanka and F. de la Cruz.

53 **Workshop on Vascular Endothelium and Regulation of Leukocyte Traffic.**
Organizers: T. A. Springer and M. O. de Landázuri.

54 **Workshop on Cytokines in Infectious Diseases.**
Organizers: A. Sher, M. Fresno and L. Rivas.

55 **Workshop on Molecular Biology of Skin and Skin Diseases.**
Organizers: D. R. Roop and J. L. Jorcano.

56 **Workshop on Programmed Cell Death in the Developing Nervous System.**
Organizers: R. W. Oppenheim, E. M. Johnson and J. X. Comella.

57 **Workshop on NF-κB/IκB Proteins. Their Role in Cell Growth, Differentiation and Development.**
Organizers: R. Bravo and P. S. Lazo.

58 **Workshop on Chromosome Behaviour: The Structure and Function of Telomeres and Centromeres.**
Organizers: B. J. Trask, C. Tyler-Smith, F. Azorín and A. Villasante.

59 **Workshop on RNA Viral Quasispecies.**
Organizers: S. Wain-Hobson, E. Domingo and C. López Galíndez.

60 **Workshop on Abscisic Acid Signal Transduction in Plants.**
Organizers: R. S. Quatrano and M. Pagès.

61 **Workshop on Oxygen Regulation of Ion Channels and Gene Expression.**
Organizers: E. K. Weir and J. López-Barneo.

62 **1996 Annual Report**

63 **Workshop on TGF-β Signalling in Development and Cell Cycle Control.**
Organizers: J. Massagué and C. Bernabéu.

64 **Workshop on Novel Biocatalysts.**
Organizers: S. J. Benkovic and A. Ballesteros.

65 **Workshop on Signal Transduction in Neuronal Development and Recognition.**
Organizers: M. Barbacid and D. Pulido.

66 **Workshop on 100th Meeting: Biology at the Edge of the Next Century.**
Organizer: Centre for International Meetings on Biology, Madrid.

67 **Workshop on Membrane Fusion.**
Organizers: V. Malhotra and A. Velasco.

68 **Workshop on DNA Repair and Genome Instability.**
Organizers: T. Lindahl and C. Pueyo.

69 **Advanced course on Biochemistry and Molecular Biology of Non-Conventional Yeasts.**
Organizers: C. Gancedo, J. M. Siverio and J. M. Cregg.

70 **Workshop on Principles of Neural Integration.**
Organizers: C. D. Gilbert, G. Gasic and C. Acuña.

71 **Workshop on Programmed Gene Rearrangement: Site-Specific Recombination.**
Organizers: J. C. Alonso and N. D. F. Grindley.

72 **Workshop on Plant Morphogenesis.**
Organizers: M. Van Montagu and J. L. Micol.

73 **Workshop on Development and Evolution.**
Organizers: G. Morata and W. J. Gehring.

*74 **Workshop on Plant Viroids and Viroid-Like Satellite RNAs from Plants, Animals and Fungi.**
Organizers: R. Flores and H. L. Sänger.

75 **1997 Annual Report.**

76 **Workshop on Initiation of Replication in Prokaryotic Extrachromosomal Elements.**
Organizers: M. Espinosa, R. Díaz-Orejas, D. K. Chattoraj and E. G. H. Wagner.

77 **Workshop on Mechanisms Involved in Visual Perception.**
Organizers: J. Cudeiro and A. M. Sillito.

78 **Workshop on Notch/Lin-12 Signalling.**
Organizers: A. Martínez Arias, J. Modolell and S. Campuzano.

79 **Workshop on Membrane Protein Insertion, Folding and Dynamics.**
Organizers: J. L. R. Arrondo, F. M. Goñi, B. De Kruijff and B. A. Wallace.

80 **Workshop on Plasmodesmata and Transport of Plant Viruses and Plant Macromolecules.**
Organizers: F. García-Arenal, K. J. Oparka and P.Palukaitis.

81 **Workshop on Cellular Regulatory Mechanisms: Choices, Time and Space.**
Organizers: P. Nurse and S. Moreno.

82 **Workshop on Wiring the Brain: Mechanisms that Control the Generation of Neural Specificity.**
Organizers: C. S. Goodman and R. Gallego.

83 **Workshop on Bacterial Transcription Factors Involved in Global Regulation.**
Organizers: A. Ishihama, R. Kolter and M. Vicente.

84 **Workshop on Nitric Oxide: From Discovery to the Clinic.**
Organizers: S. Moncada and S. Lamas.

85 **Workshop on Chromatin and DNA Modification: Plant Gene Expression and Silencing.**
Organizers: T. C. Hall, A. P. Wolffe, R. J. Ferl and M. A. Vega-Palas.

86 **Workshop on Transcription Factors in Lymphocyte Development and Function.**
Organizers: J. M. Redondo, P. Matthias and S. Pettersson.

87 **Workshop on Novel Approaches to Study Plant Growth Factors.**
Organizers: J. Schell and A. F. Tiburcio.

88 **Workshop on Structure and Mechanisms of Ion Channels.**
Organizers: J. Lerma, N. Unwin and R. MacKinnon.

89 **Workshop on Protein Folding.**
Organizers: A. R. Fersht, M. Rico and L. Serrano.

90 **1998 Annual Report.**

91 **Workshop on Eukaryotic Antibiotic Peptides.**
Organizers: J. A. Hoffmann, F. García-Olmedo and L. Rivas.

92 **Workshop on Regulation of Protein Synthesis in Eukaryotes.**
Organizers: M. W. Hentze, N. Sonenberg and C. de Haro.

93 **Workshop on Cell Cycle Regulation and Cytoskeleton in Plants.**
Organizers: N.-H. Chua and C. Gutiérrez.

94 **Workshop on Mechanisms of Homologous Recombination and Genetic Rearrangements.**
Organizers: J. C. Alonso, J. Casadesús, S. Kowalczykowski and S. C. West.

95 **Workshop on Neutrophil Development and Function.**
Organizers: F. Mollinedo and L. A. Boxer.

96 **Workshop on Molecular Clocks.**
Organizers: P. Sassone-Corsi and J. R. Naranjo.

97 **Workshop on Molecular Nature of the Gastrula Organizing Center: 75 years after Spemann and Mangold.**
Organizers: E. M. De Robertis and J. Aréchaga.

98 **Workshop on Telomeres and Telomerase: Cancer, Aging and Genetic Instability.**
Organizer: M. A. Blasco.

99 **Workshop on Specificity in Ras and Rho-Mediated Signalling Events.**
Organizers: J. L. Bos, J. C. Lacal and A. Hall.

100 **Workshop on the Interface Between Transcription and DNA Repair, Recombination and Chromatin Remodelling.**
Organizers: A. Aguilera and J. H. J. Hoeijmakers.

101 **Workshop on Dynamics of the Plant Extracellular Matrix.**
Organizers: K. Roberts and P. Vera.

102 **Workshop on Helicases as Molecular Motors in Nucleic Acid Strand Separation.**
Organizers: E. Lanka and J. M. Carazo.

103 **Workshop on the Neural Mechanisms of Addiction.**
Organizers: R. C. Malenka, E. J. Nestler and F. Rodríguez de Fonseca.

104 **1999 Annual Report.**

105 **Workshop on the Molecules of Pain: Molecular Approaches to Pain Research.**
Organizers: F. Cervero and S. P. Hunt.

106 **Workshop on Control of Signalling by Protein Phosphorylation.**
Organizers: J. Schlessinger, G. Thomas, F. de Pablo and J. Moscat.

107 **Workshop on Biochemistry and Molecular Biology of Gibberellins.**
Organizers: P. Hedden and J. L. García-Martínez.

108 **Workshop on Integration of Transcriptional Regulation and Chromatin Structure.**
Organizers: J. T. Kadonaga, J. Ausió and E. Palacián.

109 **Workshop on Tumor Suppressor Networks.**
Organizers: J. Massagué and M. Serrano.

110 **Workshop on Regulated Exocytosis and the Vesicle Cycle.**
Organizers: R. D. Burgoyne and G. Álvarez de Toledo.

111 **Workshop on Dendrites.**
Organizers: R. Yuste and S. A. Siegelbaum.

112 **Workshop on the Myc Network: Regulation of Cell Proliferation, Differentiation and Death.**
Organizers: R. N. Eisenman and J. León.

113 **Workshop on Regulation of Messenger RNA Processing.**
Organizers: W. Keller, J. Ortín and J. Valcárcel.

114 **Workshop on Genetic Factors that Control Cell Birth, Cell Allocation and Migration in the Developing Forebrain.**
Organizers: P. Rakic, E. Soriano and A. Álvarez-Buylla.

115 **Workshop on Chaperonins: Structure and Function.**
Organizers: W. Baumeister, J. L. Carrascosa and J. M. Valpuesta.

116 **Workshop on Mechanisms of Cellular Vesicle and Viral Membrane Fusion.**
Organizers: J. J. Skehel and J. A. Melero.

117 **Workshop on Molecular Approaches to Tuberculosis.**
Organizers: B. Gicquel and C. Martín.

118 **2000 Annual Report.**

119 **Workshop on Pumps, Channels and Transporters: Structure and Function.**
Organizers: D. R. Madden, W. Kühlbrandt and R. Serrano.

120 **Workshop on Common Molecules in Development and Carcinogenesis.**
Organizers: M. Takeichi and M. A. Nieto.

121 **Workshop on Structural Genomics and Bioinformatics.**
Organizers: B. Honig, B. Rost and A. Valencia.

122 **Workshop on Mechanisms of DNA-Bound Proteins in Prokaryotes.**
Organizers: R. Schleif, M. Coll and G. del Solar.

123 **Workshop on Regulation of Protein Function by Nitric Oxide.**
Organizers: J. S. Stamler, J. M. Mato and S. Lamas.

124 **Workshop on the Regulation of Chromatin Function.**
Organizers: F. Azorín, V. G. Corces, T. Kouzarides and C. L. Peterson.

125 **Workshop on Left-Right Asymmetry.**
Organizers: C. J. Tabin and J. C. Izpisúa Belmonte.

126 **Workshop on Neural Prepatterning and Specification.**
Organizers: K. G. Storey and J. Modolell.

127 **Workshop on Signalling at the Growth Cone.**
Organizers: E. R. Macagno, P. Bovolenta and A. Ferrús.

128 **Workshop on Molecular Basis of Ionic Homeostasis and Salt Tolerance in Plants.**
Organizers: E. Blumwald and A. Rodríguez-Navarro.

129 **Workshop on Cross Talk Between Cell Division Cycle and Development in Plants.**
Organizers: V. Sundaresan and C. Gutiérrez.

130 **Workshop on Molecular Basis of Human Congenital Lymphocyte Disorders.**
Organizers: H. D. Ochs and J. R. Regueiro.

131 **Workshop on Genomic vs Non-Genomic Steroid Actions: Encountered or Unified Views.**
Organizers: M. G. Parker and M. A. Valverde.

132 **2001 Annual Report.**

133 **Workshop on Stress in Yeast Cell Biology... and Beyond.**
Organizer: J. Ariño.

134 **Workshop on Leaf Development.**
Organizers: S. Hake and J. L. Micol.

135 **Workshop on Molecular Mechanisms of Immune Modulation: Lessons from Viruses.**
Organizers: A. Alcami, U. H. Koszinowski and M. Del Val.

136 **Workshop on Channelopathies.**
Organizers: T. J. Jentsch, A. Ferrer-Montiel and J. Lerma.

137 **Workshop on Limb Development.**
Organizers: D. Duboule and M. A. Ros.

138 **Workshop on Regulation of Eukaryotic Genes in their Natural Chromatin Context.**
Organizers: K. S. Zaret and M. Beato.

139 **Workshop on Lipid Signalling: Cellular Events and their Biophysical Mechanisms.**
Organizers: E. A. Dennis, A. Alonso and I. Varela-Nieto.

140 **Workshop on Regulation and Functional Insights in Cellular Polarity.**
Organizers: A. R. Horwitz and F. Sánchez-Madrid.

141 **Workshop on The Structure of the Cortical Microcircuit.**
Organizers: R. Yuste, E. M. Callaway and H. Markram.

142 **Workshop on Control of NF-kB Signal Transduction in Inflammation and Innate Immunity.**
Organizers: M. Karin, I. M. Verma and J. Moscat.

143 **Workshop on Engineering RNA Virus Genomes as Biosafe Vectors.**
Organizers: C. M. Rice, W. J. M. Spaan and L. Enjuanes.

144 **Workshop on Exchange Factors.**
Organizers: X. R. Bustelo, J. S. Gutkind and P. Crespo.

145 **Workshop on the Ubiquitin-Proteasome System.**
Organizers: A. Ciechanover, D. Finley, T. Sommer and C. Mezquita.

146 **Workshop on Manufacturing Bacteria: Design, Production and Assembly of Cell Division Components.**
Organizers: P. de Boer, J. Errington and M. Vicente.

147 **2002. Annual Report.**

148 **Workshop on Membranes, Trafficking and Signalling during Animal Development.**
Organizers: K. Simons, M. Zerial and M. González-Gaitán.

149 **Workshop on Synaptic Dysfunction and Schizophrenia.**
Organizers: P. Levitt, D. A. Lewis and J. DeFelipe.

150 **Workshop on Plasticity in Plant Morphogenesis.**
Organizers: G. Coupland, C. Fankhauser and M. A. Blázquez.

151 **Workshop on Wnt Genes and Wnt Signalling.**
Organizers: J. F. de Celis, J. C. Izpizúa Belmonte and R. Nusse.

152 **Workshop on Molecular and Genetic Basic of Autoimmune Diseases: SLE and RA.**
Organizers: A. Coutinho, W. Haas and C. Martínez-A.

153 **Workshop on The Dynamics of Morphogenesis: Regulation of Cell and Tissue Movements in Development.**
Organizers: C. D. Stern and M. A. Nieto.

---

*: Out of Stock.

The Centre for International Meetings on Biology
was created within the
Instituto Juan March de Estudios e Investigaciones,
a private foundation specialized in scientific activities
which complements the cultural work
of the *Fundación Juan March.*

The Centre endeavours to actively and
sistematically promote cooperation among Spanish
and foreign scientists working in the field of Biology,
through the organization of Workshops, Lecture
Courses, Seminars and Symposia.

From 1989 through 2002,
a total of 176 meetings,
all dealing with a wide range of
subjects of biological interest,
were organized within the
scope of the Centre.

Instituto Juan March de Estudios e Investigaciones
Castelló, 77 • 28006 Madrid (España)
Tel. 34 91 435 42 40 • Fax 34 91 576 34 20
http://www.march.es/biology

The lectures summarized in this publication were presented by their authors at a workshop held on the 9th through the 11th of June, 2003, at the Instituto Juan March.

All published articles are exact reproduction of author's text.

There is a limited edition of 400 copies of this volume, available free of charge.