Instituto Juan March
de Estudios e Investigaciones

127 CENTRO DE REUNIONES
INTERNACIONALES SOBRE BIOLOGÍA

Workshop on

Signalling at the Growth Cone

Organized by

E. R. Macagno, P. Bovolenta and A. Ferrús

P. W. Baas
P. Bovolenta
J. Culotti
B. J. Dickson
C. G. Dotti
U. Drescher
A. Ferrús
M. A. González-Gaitán
W. A. Harris
C. E. Holt
K. Kalil
C. Klämbt
P. C. Letourneau
L. Luo
E. R. Macagno
C. A. Mason
P. C. Salinas
N. C. Spitzer
D. L. Van Vactor
K. Zinn

Instituto Juan March
de Estudios e Investigaciones

127 CENTRO DE REUNIONES
INTERNACIONALES SOBRE BIOLOGÍA

Workshop on

Signalling at the Growth Cone

Organized by

E. R. Macagno, P. Bovolenta and A. Ferrús

P. W. Baas
P. Bovolenta
J. Culotti
B. J. Dickson
C. G. Dotti
U. Drescher
A. Ferrús
M. A. González-Gaitán
W. A. Harris
C. E. Holt
K. Kalil
C. Klämbt
P. C. Letourneau
L. Luo
E. R. Macagno
C. A. Mason
P. C. Salinas
N. C. Spitzer
D. L. Van Vactor
K. Zinn

The lectures summarized in this publication were presented by their authors at a workshop held on the 8th through the 10th of October, 2001, at the Instituto Juan March.

Depósito legal: M-48.265/2001
Impresión: Ediciones Peninsular. Tomelloso, 27. 28026 Madrid.

INDEX

Introduction

P. Bovolenta and A. Ferrús

The growth cone was first described by Santiago Ramón y Cajal in 1890, who coined the term to suggest a key role for this unique structure in establishing neural connectivity. The growth cone, we now know, is responsible for guiding neuronal projections to their proper targets. The growth cone achieves this goal through its capacity to integrate a large number of extra- and intracellular signals that result, through specific modifications of its cytoskeleton, in its progress through complex environments to its target. For over a century, the growth cone has fascinated many researchers, who have focused their efforts either identifying substrate derived cues and signals or on understanding the internal machinery that controls growth cone behaviour. In the last ten years, however, a number of mechanisms have been described that link substrate interactions with internal processes. In particular, surface receptors and protein complexes that integrate convergent signalling pathways have been identified, beginning to shed light on the network of mechanisms that generate the behaviour of this structure. Also, novel techniques are being applied to study the dynamics of the cytoskeletal components and of the plasma membrane. Finally, structural and functional similarities are being revealed between proteins and processes that occur in the growth cone and in other cell motility events, including mitosis. In other words, the "black box" between signal and response is now opening and revealing a fascinating content. The meeting on growth cone physiology was therefore very timely.

The first two sessions were devoted to external signalling. The optic system was thoroughly used in several organisms to review current data on molecular systems involving Ephrins, Neurotrophins, F-spondin and Sonic hedgehog. The growth cone dynamics was analyzed under novel visualization techniques that served to emphasize the role of microtubuli and their interaction with peripheral actin as molecular events subserving shape changes. Internal mechanisms were also reviewed including membrane traffic, small GTPases and Ca^{2+}. Finally, a renewed interest on the role of regulatory mechanisms of gene transcription, and mRNA translation at the growth cone became evident through the presentations in the last session.

Future studies on the growth cone demand a more quantitative approach of the implicated mechanisms. In addition, present techniques to visualize specific molecules or structures *in situ* would have to incorporate the capacity to detect and track several components in real time. However, these experimental advances require the previous framing of the key questions to be answered. At the meeting, we set up discussions focused on the identification of the major issues to be addressed experimentally. One discussion, for example, assessed whether simpler models would need to be developed to reproduce multi-protein interactions. Developing ways to approach the study of the properties of the plasma membrane *in situ* is another possible challenge. Another possibility is whether we should emulate the comprehensive approach used to elucidate the synaptic terminal, and should aim to identify the full repertoire of proteins localized at the growth cone.

Paola Bovolenta and Alberto Ferrús

Session 1: External signals (I)
Chair: Carol A. Mason

Attempts to elucidate axon guidance signaling pathways in *C. elegans*

J. Culotti, D. Merz, A. Colavita, M. Killeen, A. Krizus, J-F. Tong, and Tony Pawson

S.L. Research Institute of Mt. Sinai Hospital, Toronto, M5G 1X5 Canada

We have used several genetic approaches to obtain mutations in genes that identify novel components of netrin signaling in *C. elegans*. One approach was to misexpress UNC-5, the *C. elegans* UNC-6/netrin receptor, thereby creating an axon guidance phenotype that we could show depended on UNC-6/netrin signaling. Suppressors of this phenotype identified UNC-44 (ankyrin), UNC-34 (enabled), and UNC-129 (a novel TGF-beta), plus 3 new genes that have no phenotype on their own and may have redundant functions in axon guidance. A second approach has been to identify enhancers of weak alleles of the *unc-5* gene and the *unc-40* gene. This approach has identified known cytoskeletal regulators and at least 10 new genes we are trying to clone. With some rather large assumptions about the hierarchy of gene function, we have created the outline of a molecular signaling pathway involved in guided growth cone and cell migrations.

In other studies, we have focused on the cytoplasmic domain of UNC-5 and have found that it is tyrosine phosphorylated. We have also shown a correlation between UNC-5 function and its ability to be tyrosine phosphorylated *in vivo*. Finally, we have identified a large region of the UNC-5 cytodomain that is required for its UNC-40 dependent functions and another region required for its UNC-40 independent functions in repulsion. Interestingly, the UNC-40 dependent region does not include the region of vertebrate UNC-5 (RCM) required for interaction with the UNC-40 homolog DCC.

Molecular mechanisms of photoreceptor axon guidance in the *Drosophila* visual system

Barry J. Dickson[1], Takashi Suzuki[1], Satoko Suzuki[1], Georg Dietzl[1], Timothy Newsome[1], Yan Sun[1], Julian Ng[2], Timothy Nardine[2], Matthew Harms[2], Julia Tzu[2], Liqun Luo[2], Corinne Maurel-Zaffran[3], Jessica E. Treisman[3]

[1]Institute of Molecular Pathology, Dr. Bohr-Gasse 7, A-1030 Vienna, Austria
[2]Department of Biological Sciences, Stanford University, Stanford, CA 94305, U.S.A.
[3]Skirball Institute, New York University School of Medicine, New York, NY 10016, U.S.A.

During development of the *Drosophila* adult visual system, photoreceptors (R cells) in the eye imaginal disc project axons into the optic lobe. The eye consists of some 750 ommatidia, each containing 8 R cells (R1-R8). R1-R6 axons connect to targets in a single layer of the first optic ganglion, the lamina. R7 and R8 axons terminate in distinct layers of the second ganglion, the medulla. The termini of R axons form smooth topographic arrays in both the lamina and medulla.

We have taken a genetic approach to investigate the molecular mechanisms that establish this pattern of connections. We screened over 32,000 mutant lines, examining them histologically for abnormal connectivity patterns. 210 mutants we recovered, representing some 60 different genes. 22 of these genes have now been identified. One of these encodes the LAR receptor tyrosine phosphatase. LAR is required for the correct targeting of R7 axons to the medulla. LAR can specify correct R7 targeting by either of two signalling modes. In the "forward" mode it acts autonomously in the R7 cell, and strictly requires its cytoplamic domain. In the "reverse" mode, LAR can act non-autonomously from the R8 cell. In this mode, the cytoplasmic domain is dispensible. These data suggest that LAR may be able to mediate bidirectional signalling in a manner similar to Eph receptor tyrosine kinases.

Two other genes identified in our screen are *enabled* and *trio*. The mutant phenotypes of these two genes suggest that they have more general functions in R axon guidance and targeting. However, dosage-sensitive genetic interactions also hint at a specific role for Enabled and Trio in the LAR signalling pathway. Trio is a guanine nucleotide exchange factor. *In vitro*, it activates Rac GTPases. To determine whether Trio acts via Rac GTPases *in vivo*, we generated loss-of-function mutations in all three *Drosophila Rac* genes: *Rac1*, *Rac2* and *Mtl*. *Rac* triple mutants show a phenotype similar to *trio* mutants. Loss of *Rac1* or *Rac2* function also suppresses a *trio* gain of function phenotype. These data suggest that the Rac GTPases are indeed targets for Trio , and that Rac1 and Rac2 are its preferred substrates.

Signaling through the *Drosophila* type II BMP receptor wishful thinking is required for proper synaptic maturation and function

Guillermo Marqués

The formation and maintenance of synapses require closely coordinated pre- and post-synaptic interactions, generally thought to involve intercellular signaling mechanisms. However, the molecular identity of these pathways remains largely unknown. Here we report a novel signaling pathway involving the type II receptor for BMPs wishful thinking (wit). wit is expressed in the embryonic and larval nervous system,as well as in the gut and imaginal disks. wit mutants die as pharate adults, with weak and partially penetrant patterning defects and no obvious morphological abnormalities. Closer examination reveals that embryonic motoneurons and neurosecretory cells of the larval nervous system are affected, as indicated by the loss of expression of specific markers. Morphological characterization of the third instar larval neuromuscular junction indicates a substantial reduction in synapse size. Electrophysiological studies show that evoked synaptic transmission is severely compromised, with normal quantal size and a five- fold decrease in the frequency of spontaneous release. The lethality and associated phenotypes can be rescued with neuronal expression of the wit cDNA. These results indicate that Wit acts as a neuronal receptor essential for the development and maturation of normal synaptic function. Immunohistochemical localization of components of the BMP signaling system to the neuromuscular junction synapse strongly suggests that growth factors of this family are involved in retrograde signaling during synapse formation or maturation.

EphrinAs and their receptor function in topographic projections

Uwe Drescher[1], Bernd Knoell[1], Konstantinos Zarbalis[1] and Wolfgang Wurst[1]

[1]MRC Centre for Developmental Neurobiology, King's College London, New Hunts House, SE1 1UL, London, U.K. and [2]Clinical Neurogenetics, Max-Planck-Institute of Psychiatry, Kraepelinstr. 2, 80804 Munich

The eph family of Eph receptor tyrosine kinases and their 'ligands', the ephrins, has been implicated in a large number of developmental processes such as boundary formation, axon guidance, cell migration and vasculogenesis (Wilkinson, 2001). A characteristic of this family is their capacity for bi-directional signalling. This means that both Ephs and ephrins can function as receptors and ligands. A receptor function has been shown for transmembrane-anchored ephrinBs (Brückner et al., 1997; Holland et al., 1996) as well as GPI-anchored ephrinAs (Davy et al., 1999; Davy and Robbins, 2000; Huai and Drescher, 2001).

We will present data on functional characterisations of the axonal ephrinA expression in the retinotectal and vomeronasal projection which suggest that the eph family is involved in the establishment of neuronal connections by using both repulsive and attractive axon guidance mechanisms (Knöll et al., 2001).

References:

Brückner, K., Pasquale, E. B., and Klein, R. (1997). Tyrosine phosphorylation of transmembrane ligands for Eph receptors, Science *275*, 1640-1643.

Davy, A., Gale, N. W., Murray, E. W., Klinghofer, R. A., Soriano, P., Feuerstein, C., and Robbins, S. M. (1999). Compartmentalized signaling by GPI-anchored ephrinA5 requires the fyn tyrosine kinase to regulate cellular adhesion, Gen Dev *13*, 3125-3135.

Davy, A., and Robbins, S. M. (2000). EphrinA5 modulates cell adhesion and morphology in an integrin-dependent manner, EMBO J *19*, 5396-5405.

Holland, S. J., Gale, N. W., Mbamalu, G., Yancopoulos, G. D., Henkemeyer, M., and Pawson, T. (1996). Bidirectional signalling through the eph-family receptor Nuk and its transmembrane ligands, Nature *383*, 722-725.

Huai, J., and Drescher, U. (2001). An ephrinA-dependent signaling pathway controls integrin function and is linked to the tyrosine phosphorylation of a 120 kDa protein, J Biol Chem *276*, 6689-94.

Knöll, B., Zarbalis, Z., Wurst, W., and Drescher, U. (2001). A role for the EphA family in the topographic targeting of vomeronasal axons, Development *128*, 895-906.

Wilkinson, D. G. (2001). Multiple roles of eph receptors and ephrins in neural development, Nature Neurosci *2*, 155-163.

The role of reverse Ephrin-B signalling in retinotectal topographic map formation

Fanny Mann, Christine Holt, and Bill Harris

Dept. Anatomy, University of Cambridge, Downing St, Cambridge CB2 3DY, UK

There is a complementary graded distribution of EphB receptors and Ephrin-B ligands along the dorsal ventral axis of the *Xenopus* retina. EphB receptors are highest in the ventral retina, while Ephrin-B ligands are highest in the dorsal retina. In the tectum, there is a corresponding gradient, with EphBs highest in the ventral regions (where dorsal axons project) and Ephrin-Bs highest dorsally (where ventral axons project). To test the role of these molecules in axon growth and targeting in this system, we disrupted EphB/Ephrin-B signalling by applying a soluble EphB2 ectodomain (EphB2-Fc) to the optic pathway in an exposed brain preparation. We found that retinal axons were able to navigate correctly to the optic tectum, but projected more dorsally than control axons. Moreover, the ventral tectum was devoid of dorsal retinal axons. This suggests that reverse signalling through Ephrin-B might be important in topographic mapping. To assess reverse signalling directly, we cultured embryonic *Xenopus* retinal explants on membrane substrates from mouse L-cells expressing expressing recombinant EphB4. We compared their axon growth to those cultures on membranes from control L-cells. Axons from the dorsal retinal explants, expressing high levels of Ephrin-B, were significantly shorter when grown on EphB4 membranes than control membranes. Axons from ventral retinal explants were unaffected. To show that the signal was indeed coming through the Ephrin-B ligand, we performed the same experiment with explants overexpressing a truncated Ephrin-B ligand (Ephrin-B2$^{\Delta C}$) that lacks the cytoplasmic domain. This abolished the response of dorsal axons to EphB4. To bring this back to the *in vivo* setting, we lipofected Ephrin-B2$^{\Delta C}$ into the dorsal embryonic retina and traced the axons of the lipofected cells to the tectum. The transfected RGCs projected more dorsally in the tectum than did control axons. These results show that reverse signalling through the Ephrin-B ligand is important for the correct topographic projection from dorsal cells. Similarly normal signalling, in the ligand to receptor direction, is likely to be important in the topographic projection of ventral axons to the dorsal tectum. We are in the process of testing this idea in the context of a model for the dorsoventral mapping in the tectum.

Session 2: External signals (II)
Chair: Peter W. Baas

Signal transduction mechanisms involved in axon guidance and synaptogenesis during neural development in *Drosophila*

Kai Zinn, Rachel Kraut, Kaushiki Menon, Benno Schindelholz, Peter M. Snow, and Bruce Burkemper

Division of Biology, California Institute of Technology, Pasadena, CA 91125 USA

We will describe experiments directed toward an understanding of the roles of receptor tyrosine phosphatases (RPTPs) in axon guidance, and also describe a gain-of-function screen for genes involved in axon guidance and synaptogenesis in the larval neuromuscular system. We have recently identified DPTP52F, the sixth RPTP to be discovered in *Drosophila* (Schindelholz et al., *Development*, in press). Our genomic analysis indicates that there are likely to be no additional RPTPs encoded in the fly genome. DPTP52F is selectively expressed in the CNS of late embryos, as are DPTP10D, DLAR, DPTP69D, and DPTP99A. *Ptp52F* single mutant embryos have axon guidance phenotypes affecting CNS longitudinal tracts. This phenotype is suppressed in *Dlar Ptp52F* double mutants, indicating that DPTP52F and DLAR interact competitively in regulating CNS axon guidance decisions. *Ptp52F* single mutations also cause motor axon phenotypes that selectively affect the SNa nerve. We have also performed experiments to identify proteins associated *in vivo* with a 'substrate-trap' version of the DPTP69D RPTP whose overexpression causes axon guidance phenotypes.

In the gain-of-function screen (Kraut et al., *Current Biology* 11, 417-430, plus an online supplement, pp. S1-S24), we drove high-level expression of genes in motor neurons by crossing 2293 GAL4-driven 'EP' element lines with known insertion site sequences to lines containing a pan-neuronal GAL4 source and UAS-green fluorescent protein elements. This allowed visualization of every synapse in the neuromuscular system in live larvae. We identified 114 EPs that generate axon guidance and/or synaptogenesis phenotypes in F1 EP x driver larvae. Analysis of genomic regions adjacent to these EPs defined 76 genes that exhibit neuromuscular gain-of-function phenotypes. 41 of these ('known genes') have published mutant alleles; the other 35 ('new genes') have not yet been characterized genetically. To assess the roles of the 'known genes', we surveyed published data on their phenotypes and expression patterns. We also examined loss-of-function mutants ourselves, identifying new guidance and synaptogenesis phenotypes for 8 genes. At least 3/4 of the 'known genes' are important for nervous system development and/or function in wild-type flies. The 'known genes', the 'new genes', and a set of previously analyzed genes with phenotypes in the *Adh* region display similar patterns of homology to sequences in other species and have equivalent EST representations. We infer from these results that most 'new genes' will also have nervous system loss-of-function phenotypes. The proteins encoded by the 76 identified genes include GTPase regulators, vesicle trafficking proteins, kinases, and RNA-binding proteins. We will discuss unpublished results on two or three of these genes.

Signals for growth cone passage at the optic chiasm midline

Carol A. Mason, Lynda Erskine, Eloisa Herrera, Riva Marcus, Rivka Rachel, Takeshi Sakurai, and Scott Williams

Department of Pathology, Center for Neurobiology and Behavior, Columbia University, College of Physicians and Surgeons, NY, NY

Our lab has been studying the mechanisms of retinal ganglion cell (RGC) axon guidance during optic chiasm formation (1). Because growth cones of RGC axons make a decision either to cross or avoid the midline, the basis for this binary decision is a model for dissecting how growth cones navigate the midline of the neuraxis. In mouse, the permanent uncrossed projection arises from RGCs located within the ventrotemporal (VT) crescent of the retina, while crossed RGCs are found primarily in other retinal regions. Our previous studies have delineated the paths and dynamic behaviors of RGC growth cones during retinal axon divergence in the chiasm. An ensemble of precocious neurons and radial glia is positioned in and around the site of divergence in the optic chiasm and ventral diencephalon. Morphological and analyses and assays have implicated these neurons and glia in patterning the visual projection during optic chiasm formation. Our recent work focuses on the molecular basis for the crossing decision at the midline of the optic chiasm. Two issues will be discussed:

I. The role of guidance factors expressed in the ventral diencephalon, during retinal axon divergence and chiasm formation

Three families of guidance factors have been analyzed, in experiments based on analysis of pathways, guidance molecule expression, and *in vitro* assays in wildtype and mutant mice:

Ephs/Ephrins - This family of receptor tyrosine kinases has been implicated in axon targeting and in pathfinding. We and others have localized Eph receptors and ephrin ligands in both the retina and in the ventral diencephalon. Experiments on the ephrin A's (2) have shown that they function to prevent invasion of the ventral and caudal regions of the diencephalon, during the organization and growth of the chiasm. Studies on *Xenopus* by the Holt lab (3) have shown that ectopic expression of ephrin-B2 induces a ipsilateral projection prcociously in otherwise crossing axons. Our analyses in the mouse (4) have confirmed mRNA expression of ephrinB2 at the midline just dorsal to the point of divergence, where the midline radial glial cell bodies reside. Expression is coincident with the time course of the formation of the ipsilateral projection. When retinal explants are grown on ephrin-B substrates, ephrin-B2, but not ephrin-B1, is a more potent inhibitor of neurite outgrowth from VT uncrossed axons than from axons from other quadrants of the retina. In a preparation of the retina, optic nerves and ventral diencephalon incubated with EphB4-Fc, which blocks ephrin-B2 function, the ipsilateral projection is reduced. Together, these data suggest that ephrinB2 plays a role in regulating the crossing decision at the midline of the mouse optic chiasm.

Nr-CAM - a member of the L1 family of cell adhesion molecules, has also been investigated in RGC growth through the chiasm. Nr-CAM mRNA is expressed at the midline, and the protein expression pattern matches the disposition of the midline radial glia (5). Based on studies of the floor plate, we hypothesized that this molecule is important for crossing the

midline. In the isolated visual system preparation, application of Nr-CAM-Fc leads to an *increase* in ipsilateral RGC axon projection. This is of interest as Nr-CAM serves as a receptor for other recognition molecules such as TAG-1, contactin, neurofascin, and receptor tyrosine phosphatase. Analysis of mice mutant for NrCAM (6) is underway to analyze whether the absence of NrCAM also leads to a failure of axons to cross the midline. Thus, NrCAM appears to function during crossing of RGC axons at the midline.

Robos/Slits – Slit proteins are evolutionarily conserved secreted factors, controlling axon crossing at the midline. Several Robos, receptors for Slits, have been identified in fly and in vertebrates. In the fly CNS, Slit functions to prevent ipsilaterally projecting fibers from ever crossing the midline and contraterally projecting fibers from recrossing, and helps to determine the distance from the midline that crossed fibers travel. Robos and Slits are expressed in the retina and in the ventral diencephalon (7). Slit2 has been a candidate for regulating divergence in the mouse optic chiasm because of its expression at the midline, though it is localized more rostral to ephrin-B2 and Nr-CAM studies show that Slit1 and Slit2 inhibit the outgrowth of RGC axons from all quadrants of the retina. Mice mutant for either Slit 1 or Slit2 display little aberrant growth or perturbation of the divergence pattern. However, in mice mutant for both Slit1 and 2, a large additional chiasm develops anterior to the true optic chiasm and retinal fibers extend ectopically dorsal and lateral to the chiasm (8). Few effects on divergence per se were seen, in that an ipsilateral component develops. Therefore, during the formation of the mouse optic chiasm, Slit proteins cooperate to establish a corridor through which the RGC axons project and act to define the precise locus in the ventral diencephalon where the optic chiasm forms.

These studies of the function of cues expressed within the ventral diencephalon, have pinpointed candidates for regulating divergence of crossed and uncrossed fibers and for patterning the optic chiasm. They will also be discussed in the context of current models on hierarchies of cues.

II. Regulatory genes in the retina: specification of crossed and uncrossed axon projection phenotype

A number of regulatory genes expressed in the forebrain and telencephalon are expressed in the ventral telencephalon, defining zones where RGC axons do not project and others along whose borders or domains in which RGCs extend (9). The winged helix transcription factors BF-1 and –2 are potential candidates regulating the crossed/uncrossed phenotype as they are expressed in nasal and temporal retina, respectively (10). To test whether BF-2 is involved in the development of the ipsilateral pathway, the retinal axonal projections from BF-2-/- mice were analyzed with dye labeling, and displayed a greatly increased ipsilateral component (11). Expression of markers and other guidance cues expressed by the specialized glia and neurons in the diencephalon populations appears undisturbed. However, in culture assays, VT retina from BF-/- mice show enhanced growth when grown on BF-2 +/+ or -/- chiasm cells, as though the response to inhibition by chiasm cells has been reduced in BF-2 -/- retina. Moreover, ephrin-A5 is expressed both in nasal and temporal retina, suggesting that the altered pathfinding in $BF2^{-/-}$ mice could be attributed to the ectopic expression of this and other Ephs and ephrins. The enlarged ipsilateral projection must result from de-regulation of a number of other genes and guidance factors, as the RGCs giving rise to the ipsilateral projection are randomly distributed across the retina.

Together, these data indicate that BF-2 in the retina influences laterality of axon trajectory, likely upstream of factors specifying crossing, and by affecting both expression of and response to guidance factors. These studies are part of an effort to identify which genes directly contribute to the specification of the uncrossed pathway.

References:

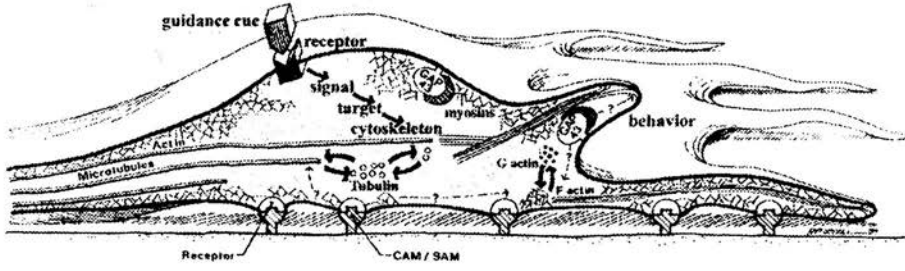
1. Mason CA and Erskine L (2000) 44: 260-270.
2. Marcus RC, Matthews, GA, Gale NW, Yancopoulos GD, Mason CA (2000) Dev. Biol. 221: 132-147.
3. Nakagawa S, Brennan C, Johnson KG, Shewan D, Harris WA, Holt CE (2000) Neuron 25: 599-610.
4. Williams S, Erskine LE, Sakurai T, Yancopoulos G, Gale N, Mason CA (2001) Soc. Neurosci. Abstr.
5. Lustig M, Erskine L, Mason CA, Grumet, and Sakurai T. (2001) J. Comp. Neurol. 433: 113-28.
6. Sakurai, T, Lustig, M, Babiarz J, Furley AJW, Tait S, Brophy PJ, Brown S, Brown L, Mason CA and Grumet M. 2001 J Cell Biol (in press).
7. Erskine L, Williams SE, Brose K, Kidd T, Rachel, RA. Goodman CS, Tessier-Lavigne M, Mason CA (2000) J. Neurosci. 20,: 4975-4982.
8. Plump, A S, Erskine L, Sabatier C, Brose K, Epstein CJ, Goodman CS, Mason CA, Tessier-Lavigne M. (2001) (under revision, Neuron).
9. Marcus RC, Shimamura K, Sretavan D, Lai E, Rubenstein J, Mason CA (1999) J Comp Neurol 403: 346-358.
10. Hatini V, Tao W, Lai E (1996) J. Neurobol. 251: 1293-1309.
11. Herrera E, Marcus R, L S, Lai E, and Mason CA (2001) Soc. Neurosci. Abstr.

Signaling by neurotrophins and other guidance cues interact to regulate growth cone behaviors

Gianluca Gallo, Alan Ernst, William Jurney, Vassil Dontchev, Steven McLoon and Paul Letourneau

Department of Neuroscience, University of Minnesota, Minneapolis MN 55455

The growth cones of extending axons navigate through complex environments in developing tissues and in their targets to establish proper neural circuits. Growth cone migration is driven by cytoskeletal machinery of actin filaments and microtubules to extend the leading cell margin, explore the local terrain and advance the developing axon. Migrating growth cones are guided by extracellular molecules that act as positive cues to promote migration along correct paths and as negative cues that inhibit growth cones from entering incorrect terrain. Extrinsic guidance cues trigger cytoplasmic signals that regulate the dynamics and organization of the growth cone cytoskeleton. While navigating through complex developing tissues, growth cones simultaneously encounter multiple cues, and thus, growth cone behaviors likely reflect the integration of regulatory signaling through several transduction pathways.



Neurotrophins are potent growth factors that regulate gene expression to determine neuronal survival and differentiation. Neurotrophins are present along peripheral pathways and in targets of sensory neurons, where they also act to locally regulate growth cone behaviors. An important role of neurotrophins may be to modulate the effects of negative guidance cues in regulating growth cone behaviors. Semaphorin 3A (Sema3A) is characterized as a negative guidance cue with dynamic spatial and temporal distribution in the developing central and peripheral nervous systems. The axons of NGF-responsive sensory neurons avoid Sema3A-rich regions in the spinal cord and peripheral tissues of developing embryos. Sma3A induces *in vitro* collapse of growth cones of NGF-responsive sensory neurons. However, collapse of sensory neuronal growth cones in response to Sema3A is reduced by neurotrophin signaling in a manner that depends on activity of the PKA kinase.

In the developing visual system, retinal ganglion cell axons make synapses across the optic tectum in a pattern that duplicates their topographic organization in the retina. An important role in patterning retinal axons in the tectum is played by negative guidance cues,

ephrin-A2 and ephrin-A5, which are distributed in increasing rostral-caudal gradients across the tectum. Growth cone receptors for these ephrins are distributed in a gradient across retinal axons, being highest on axons from temporal retina. Temporal retinal axons do not terminate in caudal tectum, where they encounter high levels of ephrins- A2 and A5. Temporal retinal growth cones are collapsed *in vitro* by ephrins- A2 and A5. The neurotrophin BDNF is also made by tectal cells, and similar to the effects of NGF signaling on Sema3A-mediated collapse of sensory neuronal growth cones, BDNF inhibits collapse of temporal retinal growth cones induced by ephrin-A2. Finally, refinement of the patterning of retinal axons across the tectum is dependent on neural activity in a manner that requires nitric oxide (NO) production. NO collapses retinal axons *in vitro*. However, BDNF selectively inhibits NO-induced growth cone collapse of retinal axons in a manner that is dependent on PKA signaling.

These examples indicate how neurotrophins modulate growth cone responses to negative guidance cues and factors that induce growth cone collapse. There is much that remains to be understood about the signaling pathways that are triggered in growth cones by neurotrophins and by other guidance cues. Similarly, the cellular mechanisms of growth cone collapse are not fully understood. These topics will continue to be of interest to students of the "cone de croissance".

This research was supported by grants from the NIH and the NSF.

References:

1. 1. Gallo, G. and Letourneau, P.C. 2000. Neurotrophins and the dynamic regulation of the neuronal cytoskeleton, *J. Neurobiol.* 44:159-173.
2. Ernst, A.F., Gallo, G., Letourneau, P.C. and McLoon, S.C. 2000. Stabilization of growing retinal axons by the combined signaling of nitric oxide and BDNF. *J. Neurosci.* 20:1458-1469.
3. Ernst, A.F., Wu, H.H., El-Fakahany, E.E., and McLoon, S.C. 1999. NMDA receptor-mediated refinement of a transient retinotectal projection requires nitric oxide. *J. Neurosci.* 19:229-235.
4. Gallo, G., Ernst, A.F., McLoon, S.C. and Letourneau, P.C. Transient PKA activity is required for initiation but not for maintenance of BDNF-mediated protection from nitric oxide induced growth cone collapse. *Submitted for publication.*

Plasmin-mediated release of the guidance molecule F-spondin from the extracellular matrix

Vered Tzarfaty-Majar1, Roser López-Alemany2,Yael Feinstein1, Lourdes Gombau2, Orit Goldshmidt3, Eduardo Soriano4, Pura Muñoz-Cánoves2, & Avihu Klar1

1 Dept. of Anatomy and Cell Biology, Hebrew University - Hadassah Medical School, Jerusalem. 2 Institut de Recerca Oncologica (IRO), Centre d'Oncologia Molecular, Barcelona. 3 Department of Oncology, Hadassah-Hebrew University Hospital, Jerusalem 4Department of Animal and Plant Cell Biology, Faculty of Biology, University of Barcelona, Barcelona

Serine proteases are implicated in a variety of processes during neurogenesis, including cell migration, axon outgrowth, and synapse elimination. Tissue-type plasminogen activator and urokinase-type activator are expressed in the floor plate during embryonic development. F-spondin, a gene also expressed in the floor plate (Klar et al., 1992), encodes a secreted, extracellular matrix-attached protein that promotes outgrowth of commissural axons and inhibits outgrowth of motor axons (Burstyn-Cohen et al., 1999; Tzarfati-Majar et al., 2001) F-spondin is processed in-vivo to yield an amino half protein that contains regions of homology to reelin and mindin, and a carboxyl half protein, that contains either six or four thrombospondin type I repeats (TSR). We have tested F-spondin to see whether it is subjected to processing by plasmin and to determine whether the processing modulates its biological activity. Plasmin cleaves F-spondin at its carboxyl terminus. By using nested deletion proteins, and mutating potential plasmin cleavage sites, we have identified two cleavage sites – the first between the 5th and 6th TSR repeats, and the second at the 5th TSR repeat. Analysis of the ECM attachment properties of the TSR repeats, revealed that the 5th and 6th TSR repeats bind to the ECM, whilst repeats 1-4 do not. Structural functional experiments reviled that two basic motives are required to elicit binding of TSR module to the ECM. We further demonstrate that plasmin releases the ECM-bound F-spondin protein (Tzarfaty-Majar et al., 2001).

References:

Burstyn-Cohen, T., Tzarfaty, V., Frumkin, A., Feinstein, Y., Stoeckli, E., and Klar, A. (1999). F-spondin is required for accurate pathfinding of commissural axons at the floor plate, Neuron 23, 233-246.

Klar, A., Baldassare, M., and Jessell, T. M. (1992). F-spondin: A gene expressed at high levels in the floor plate encodes a secreted protein that promotes neural cell adhesion and neurite extention, Cell 69, 95-110.

Tzarfati-Majar, V., Burstyn-Cohen, T., and Klar, A. (2001). F-spondin is a contact-repellent molecule for embryonic motor neurons, Proc Natl Acad Sci USA 98, 4722-4727.

Tzarfaty-Majar, V., López-Alemany, R., Feinstein, Y., Gombau, L., Goldshmidt, O., Soriano, E., Muñoz-Cánoves, P., and Klar, A. (2001). Plasmin-mediated release of the guidance molecule F-spondin from the extracellular matrix, J Biol Chem In press. (published on line).

Control of retinal ganglion cell axon growth: a new role for SHH

Françoise Trousse[1*], Elisa Martí[1*], Peter Gruss[2], Miguel Torres[3] and Paola Bovolenta[1]

[1]Instituto Cajal, CSIC, Av. Doctor Arce 37, 28002 Madrid, Spain
[2] Dept. Molecular and Cell Biology, MPI, 3400 Göttingen, Germany
[3]Centro Nacional de Biotecnologia, CSIC, Canto Blanco, Madrid, Spain

* Equally contributing authors

Retinal ganglion cell (RGC) axons grow towards the diencephalic ventral midline during embryogenesis guided by cues whose nature is largely unknown. *Shh* expression is down-regulated at the ventral hypothalamic region where optic fibres cross the midline (Trousse et al., 2001). In *Pax2* deficient mice the optic chiasm is altered and RGC axons never cross the midline, leaving the projections entirely ipsilateral. Interestingly, in these mice *Shh* expression is ectopically maintained along the entire ventral midline (Torres et al., 1996). Similar results were observed in *noi* zebrafish mutants, which have an alteration in the *pax2/5/8-like* gene (Macdonald et al., 1997). Based on these data we posed the hypothesis that the continuous expression of *Shh* at the axial midline is directly or indirectly responsible for the failure of RGC axons to cross the midline. We have performed a series of experiments , reported in Trousse et al., 2001, that show that SHH directly suppresses the growth of RGC axons. Using collagen gel matrix, we co-cultured chick retina explants with beads soaked in human recombinant N-SHH. N-SHH dampened both the number and the length of neurites emerging from retina but not neural tube explants, as compared to control cultures. As determined by in situ hybridisation of chick retinas the SHH receptor Patched1 localises to of RGC. Direct addition of SHH to neurites grown over a laminin substrate either induced their retraction or prevented their further growth. This effect was counteracted by the use of anti-SHH blocking antibody. We further showed that SHH-induced growth cone's retraction was mediated by decreased levels of cAMP in the growth cone itself, in agreement with previous data indicating that propagation of hedgehog signalling involves the activity of PKA (Ingham, 1998), one of the principal mediators of cAMP effects. Furthermore, *in ovo* experiments using RCAS to target to the chick visual pathway ectopic expression of *Shh*, clearly show that *Shh* expression interfered with the normal growth of RGC axons along the nerve and the hypothalamic region. In conclusion, we provide evidence that in vivo *Shh* expression at the chiasm borders defines a constrained pathway for RGC axons within ventral midline. These data demonstrate a novel role for SHH, the ability of controlling retinal ganglion cell growth cone movements.

References:

Ingham, P.W. (1998) The *patched* gene in development and cancer. *Curr Opin Gen Dev*, 8, 88-94.

Macdonald R., scholes J., Strahle U., Brennan C., Holder N., Brand M., Wilson S.W. (1997). The pax protein noi is required for commissural axon pathway formation in the rostral forebrain. *Development*,124, 2397-2408

Torres M., Gomez-Pardo E., Gruss P. (1996) Pax2 contributes to inner ear patterning and optic nerve trajectory. *Development*, 122, 3409-3418.

Trousse, F., Marti, E., Gruss P., Torres M. and Bovolenta, P. (2001) Control of retinal ganglion cell axon growth: a new role for SHH. *Development*, 128 (in press).

Session 3: Growth cone dynamics
Chair: Nicholas C. Spitzer

Role of motor proteins in the organization of the cytoskeleton during growth cone navigation

Peter W. Baas

Department of Neurobiology and Anatomy
MCP Hahnemann University
2900 Queen Lane
Philadelphia, PA 19129

Axons can extend over exceedingly long distances to reach their target tissues during the differentiation of the nervous system. The development of the axon involves bouts of elongation and retraction, as well as the formation of branches which can also elongate and retract. The parent axon and its branches are tipped by highly motile structures called growth cones which forage through the embryo to find specific targets for innervation. The movement of these growth cones is highly sensitive to environmental factors such as cell-surface molecules and chemotactic signals. Some factors attract axons and promote their elongation while other factors repel axons or cause retraction. Despite substantial work on the visualization of such responses and characterization of attractive and repulsive factors, surprisingly little is known about how these factors actually cause an axon to elongate or retract. Most articles on this topic have noted that axonal elongation and retraction must involve alterations in the cytoskeletal arrays that provide architectural support for the axon. Dense arrays of cytoskeletal polymers are contained within the axon and are necessary for its extension and maintenance. The prevailing view is quite simple; that axonal growth involves the assembly of new cytoskeleton while axonal retraction involves the disassembly of existing cytoskeleton.

This view is mainly based on the results of pharmacologic studies which show that wholesale microtubule depolymerization can cause axons to bead-up along their lengths, become thinner, wither, and retract. Interestingly, however, there is virtually no evidence to support the idea that axons physiologically elongate and retract by building and tearing down cytoskeletal polymers in such a wholesale fashion. Certain repulsive factors have been shown to produce local microfilament disassembly in growth cones, but there is no evidence of widespread microfilament disassembly or any kind of dramatic microtubule disassembly during physiological axonal retraction. In fact, experimental studies have shown that axons can develop and generate branches fairly normally under conditions of suppressed microtubule assembly and disassembly. In recent years a great deal of progress has been made in elucidating the biochemical pathways that transduce environmental signals into molecular changes within the growth cone and the axon, but it remains unclear exactly how these changes alter the cytoskeleton. For example, it has been proposed that changes in the activity of small GTPases and kinases can cause retraction by inducing disassembly of microfilaments, but paradoxically, pharmacologic studies have shown that disassembly of microfilaments actually prevents axons from retracting, rather than causing them to retract. We are exploring another potential mechanism by which environmental factors might alter the cytoskeleton to elicit elongation or retraction of the axon. In this new model, cytoskeletal elements are subjected to forces generated by molecular motor proteins. The modulation of these forces determines whether there is a net forward or backward movement of the

cytoskeletal elements, and these movements, in turn, determine whether the axon elongates or retracts.

In one set of studies, we sought to determine whether or not axonal retraction involves a net depolymerization of cytoskeletal elements. Nitric oxide and semaphorin-3a, known axonal repellent guidance cues, were applied to cultured chick sensory neurons grown under low-adhesion conditions. The majority of axons showed dramatic retraction within 30-60 minutes. Retracting axons were characterized by an enlarged distal region, a thin trailing remnant, and sinusoidal bends along the shaft. Immunofluorescence quantification of microtubule levels showed no detectable loss of microtubule mass during retraction. Instead, microtubules accumulated in a disto-proximal fashion as the axon retracted, suggesting a retrograde movement of intact microtubules. Although quantification of microfilament levels is not as reliable as quantification of microtubule levels, it was also our impression that there was no major loss of microfilaments during retraction. We are currently testing further the conclusion that retrograde movement of microtubules and microfilaments, rather than their wholesale disassembly, underlies axonal retraction in response to physiological cues.

In another set of studies, we sought to determine whether manipulation of motor-driven forces could induce axonal retractions reminiscent of those observed in response to the physiological factors. These studies were also performed on chick sensory neurons cultured under low-adhesion conditions. Microinjection of recombinant dynamitin into the cell bodies of these neurons induced the axons to retract with characteristics remarkably similar to those observed with nitric oxide and semaphorin-3a. Retracting axons were characterized by an enlarged distal region, a thin trailing remnant, and sinusoidal bends along the shaft. There was no loss of microtubules during retraction, and the microtubules accumulated disto-proximally. Retractions were inhibited by depolymerizing microfilaments prior to injection of dynamitin, or by co-injection of NEM-modified S1 with dynamitin. Dynamitin is a component of the dynactin complex that, when introduced into cells at abnormally high levels, causes the complex to dissociate. The dynactin complex is required for all known functions of cytoplasmic dynein, and hence, its dissociation curtails dynein functions. NEM-modified S1 prevents the interaction of endogenous myosin motors with the microfilaments, and thereby curtails myosin functions. These studies suggest that axonal retraction requires actomyosin-driven contractile forces, and that these forces are attenuated by dynein-driven forces. These observations are consistent with the view that cytoplasmic dynein generates forces between the microtubule and microfilament arrays.

It is our proposal that physiological factors that cause axons to retract influence the cytoskeleton not by causing wholesale depolymerization of the cytoskeleton, but rather by regulating the balance of forces generated by motors such as cytoplasmic dynein and myosins. We suspect that additional forces are imposed by kinesin-related motors, and that these forces can also reconfigure the cytoskeleton in response to environmental cues. Ongoing studies are aimed at further testing this model.

Changes in membrane and cytoskeletal dynamics preceding and accompanying axon formation in hippocampal neurons in culture

Carlos G. Dotti

Cavalieri Ottolenghi Scientific Institute. Fondazione Cavalieri Ottolenghi.
Universita degli Studi di Torino

Proper neuronal connectivity, and thus proper neuronal function, requires the proper orientation and the proper navigation of the newly formed axons and dendrites. How do these events normally happen? The numerous growth-guiding factors spread throughout the brain activate receptor/s present on the membrane of the growth cones of the different neuronal populations. The occupied receptors activate signaling events that in turn determine the axon's response. However, how do diverse factors and signaling events modify the cells' intracellular machinery to produce a simple response such as axonal growth or axonal arrest? To address these questions we studied cytoskeleton and membrane changes during axonal and dendritic formation in isolated hippocampal neurons in culture. We observed that in the homogeneous environment of a tissue culture dish membrane organelles and proteins are delivered preferentially to one of the multiple neurites of a newborn neuron, this neurite later becomes the cells' axon (Bradke and Dotti, 1997). Studies on actin dynamics revealed that polarized membrane flow is accompanied by a localized instability of the actin filaments present in the neurite growth cone and by the maturation of the membrane biosythetitc machinery (Bradke and Dotti, 1999; Ledesma et al., 2000). These observations suggest that destabilization of the actin filaments in a pre-condition for axonal formation and that assembly of membrane with a different comosition is a condition for functional maturation. One of the challenge for the future is to determine the molecular mechanisms acting upstream actin dynamics in axonal and dendritic growth cones, how different growth-arrest factors modify actin organization, and how it occurs signaling to the cell body for membrane maturation. Such knowledge will help in our understanding of the molecular basis of neurological diseases characterised by defects in network formation-maintenance.

References:

Bradke, F., and Dotti, C.G. (1997) Polarized membrane trafficking precedes and accompanies axonal formation. Neuron 19, 1175-1186

Bradke, F., and Dotti, C. G. (1999) Localized actin instability specifies axon formation. Science 283: 1931-1934

Ledesma, M.D. , Brugger B., Bunning, C., Wieland, F., and Dotti, C. G. (1999) Maturation of the axonal plasma membrane requires upregulation of sphingomyelin synthesis and formation of protein-lipid complexes. The EMBO J. 18: 1761-1771

WNT signalling regulates axonal microtubule organization

Patricia C. Salinas

Department of Biological Sciences, Imperial College of Science, Technology and Medicine London UK

The conversion of a growth cone into a pre-synaptic terminal requires changes in the axonal cytoskeleton and in the accumulation of the pre-synaptic machinery involved in neurotransmitter release. The signalling molecules that induce pre-synaptic differentiation have remained unknown until recently. Members of the WNT family have recently been implicated in this process. WNTs increase growth cone size, axon branching while decreasing axonal extension. Our studies of WNT-7a in developing axons and in the *Wnt-7a* mutant mouse have shown that WNT-7a regulates the morphological maturation of multisynaptic structures in the cerebellum. Our data is consistent with the view that WNT-7a acts as a retrograde signal that regulates pre-synaptic differentiation. Changes in axonal morphology induced by WNTs are associated with increased microtubule unbundling and the formation of looped microtubules at enlarged growth cones. To understand the mechanism by which WNT signalling regulates microtubules in developing axons, we have examined Dishevelled, a downstream component of the WNT signalling pathway. Dishevelled is associated to axonal microtubules and increases microtubule stability. In the canonical WNT pathway. Dishevelled regulates ß-catenin-TCF transcriptional activation by inhibiting GSK-3ß, a serine/threonine kinase. We have shown that Dishevelled microtubule stabilising function is mediated through the inhibition of GSK-3ß. However, our results demonstrate that Dishevelled stabilises microtubules through a divergent WNT pathway in which ß-catenin and TCF activation are not required. More importantly, Dishevelled stabilises microtubules in a transcriptional-independent manner. Our data support a model in which the localised action of Dishevelled on axonal microtubules regulates microtubule stability by inhibiting a pool of GSK-3ß.

From the growth cone surface to the cytoskeleton: one journey, many paths

David L. Van Vactor

Department of Cell Biology, Program in Neuroscience, Harvard Cancer Center, Harvard Medical School, 240 Longwood Avenue Boston

In recent years, it has become clear that growth cone behaviors are regulated by protein phosphorylation, under control of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). In addition to receptor-class proteins that directly link PTK or PTP catalysis to highly conserved extracellular domains, a number of intracellular enzymes have been implicated in axon guidance decisions as signaling partners of separate receptor proteins. In , the Abelson PTK (Abl) has been implicated in the functions of several transmembrane proteins required for accurate axon pathfinding. *Abl* is required for the formation of many axon pathways; however, the most dramatic phenotypes are seen in mutants that simultaneously lack *Abl* and a small group of intracellular proteins that appear to function in the *Abl* signaling pathway. Functional studies of certain Abl-interactors, such as the substrate protein Enabled, the Actin-binding protein Profilin, and their homologues in other species, suggest that Abl's role in axonogenesis involves the regulation of cytoskeletal dynamics.

It has long been thought that actin structures at the growth cone leading edge are amongst the most important effectors for achieving directional motility. Many observations suggest that the navigational response of the growth cone to both attractive and repellent cues is mediated by rapid remodeling of actin cytoskeleton. Moreover, recent evidence suggests that the same signaling machinery is required for both the attractive and repellent response, depending on the levels of cyclic nucleotides within the growth cone. This has raised the intriguing question of how opposing guidance signals are translated into cytoskeletal dynamics through shared components. Current data in suggest that Abl forms part of this shared signaling machinery, presumably through interactions with partners like Enabled and Profilin. However, our understanding of this mechanism is far from complete.

Here we demonstrate that *capulet* (*capt*), a homologue of the Adenylyl Cyclase-Associated Protein that binds and regulates Actin in yeast, collaborates with Abl during axon guidance at the midline choice point where Slit functions to restrict inappropriate axon crossing. *Capt* displays potent genetic interactions with *Abl* and *slit*, suggesting that Capt is part of the repellent response. Similar genetic interactions are observed with combinations of *roundabout* genes, encoding the receptors of Slit. Capt protein associates with Abl in cells, suggesting a direct functional relationship. Using S2 cells, we show that Capt expression can antagonize Actin structures. These studies identify Capulet as part of an emerging pathway linking guidance signals to regulation of cytoskeletal dynamics.

References:

1) Kaufmann, N., Wills, Z.P., and Van Vactor, D. (1998) Drosophila Rac1 Controls Motor Axon Guidance. Development, 125: 453-461.

2) Wills, Z., Marr, L., Zinn, K., Goodman, C.S. and Van Vactor, D. (1999) Profilin and the Abl Tyrosine Kinase are Required for Motor Axon Outgrowth in the *Drosophila* Embryo. Neuron 22, 291-299.

3) Wills, Z., Bateman, J., Korey, C., Comer, A., and Van Vactor, D. (1999) The Tyrosine Kinase Abl and its Substrate Enabled Collaborate with the Receptor Phosphatase Dlar to Control Motor Axon Guidance. Neuron 22, 301-312.
4) Bateman, J., Shu, H., Van Vactor, D. (2000) The Guanine Nucleotide Exchange Factor Trio Mediates Axonal Development in the Embryo. Neuron 26, 93-106.

Session 4: Intracellular mechanisms
Chair: Carlos G. Dotti

Synaptic vesicle recycling through the endocytic pathway

Marcos A. González-Gaitán

Max-Planck-Institute of Molecular Cell Biology and Genetics, Pfotenhauerstrasse 108, Dresden, D01307, Germany

During synaptic transmission a rapid, repetitive and reliable exocytosis of the neurotransmitter is possible due to the existence of a reservoir of synaptic vesicles at the presynaptic terminal (SV). To ensure a stable SV pool, upon arrival of an action potential, exocytosis is temporally coupled to the reinternalization of vesicle membrane by endocytosis[1-3]

We showed that, after endocytosis, vesicle membrane recycling involves trafficking through an intermediate endosomal compartment[4] mediated by the small GTPase Rab5[5,6]. Vesicles recycled through Rab5-dependent endosomal trafficking show an improved neurotransmitter release probability[4]. This suggests that quality control of the release machinery takes place during recycling via the Rab5 endosome. Our results also indicate that Rab5 levels determine the kinetics of recycling and the release probability of the recycled vesicles. Therefore Rab5-dependent trafficking of SVs through the endosome facilitates synaptic transmission and might thereby contribute to synaptic plasticity.

References:

1. Ceccarelli, B., Hurlbut, W. P. & Mauro, A. Depletion of vesicles from frog neuromuscular junctions by prolonged tetanic stimulation. *Journal of Cell Biology* **54**, 30-8 (1972).
2. Ceccarelli, B., Hurlbut, W. P. & Mauro, A. Turnover of transmitter and synaptic vesicles at the frog neuromuscular junction. *Journal of Cell Biology* **57**, 499-524 (1973).
3. Heuser, J. E. & Reese, T. E. Evidence for recycling of synaptic vesicle membrane during transmitter release at the frog neuromuscular junction. *J. Cell. Biol* **57**, 315-344 (1973).
4. Wucherpfennig, T. & Gonzalez-Gaitan, M. A. Rab5-dependent endosomal trafficking controls synaptic transmission. *Submitted* (2001).
5. Bucci, C. et al. The small GTPase rab5 functions as a regulator factor in the early endocytic pathway. *Cell* **70**, 715-728 (1992).
6. Gorvel, J. P., Chavrier, P., Zerial, M. & Gruenberg, J. rab5 controls early endosome fusion in vitro. *Cell* **64**, 915-25 (1991).

Rho GTPase in growth cone signaling

Liqun Luo

Department of Biological Sciences
Stanford University
Stanford, CA 94305
USA

I will present two recent pieces of work in our continuing effort to study the mechanisms of Rho GTPase signaling in different aspects of neuronal development.

1) Growth, guidance and branching of axons are all essential processes for the precise wiring of the nervous system. Are the cytoskeletal changes that mediate each of these morphogenic processes controlled by distinct molecular mechanisms, or does a common mechanism underlie these processes? Rho family GTPases transduce extracellular signals to regulate the actin cytoskeleton. Studies using dominant mutants have implicated Rac in axon growth and guidance. We report here the loss-of-function phenotypes of three Rac GTPases in the Drosophila mushroom body (MB) neurons. Drosophila Rac1, Rac2 and Mtl work together to specify MB axon development. Progressive loss of combined Rac GTPases activity leads first to defects in axon branching, followed by guidance, and lastly growth. Expression of a Rac1 effector domain mutant that does not bind Pak rescues growth, partially rescues guidance, but does not rescue branching defects of Rac mutant neurons. Mosaic analysis reveals both cell autonomous and non-autonomous functions for Rac GTPases, the latter manifesting itself as a strong community effect in axon guidance and branching. These results demonstrate the central role of Rac GTPases in multiple aspects of axon development in vivo and suggest that axon growth, guidance and branching are controlled by differential activation of Rac signalling pathways.

2) Mechanisms that regulate axon branch stability are largely unknown. Genome-wide analyses of Rho GTPase activating protein (RhoGAP) function in *Drosophila* using RNA interference identified p190 RhoGAP as essential for axon stability in mushroom body neurons, the olfactory learning and memory center. p190 inactivation leads to axon branch retraction, a phenotype mimicked by activation of GTPase RhoA and its effector kinase Drok and modulated by the level and phosphorylation of myosin regulatory light chain. Thus, there exists a retraction pathway from RhoA to myosin in maturing neurons, which is normally repressed by p190. Local regulation of p190 could control the structural plasticity of neurons. Indeed, genetic evidence supports negative regulation of p190 by integrin and Src, both implicated in neural plasticity.

Mechanism and function of mRNA transport to growth cones of developing neurons

Gary J. Bassell

The active transport of specific mRNAs to developing neuronal processes and growth cones may permit local synthesis of cytoskeletal proteins and regulate growth cone organization and function. Using electron microscopy and immunocytochemistry, we have observed polyribosomes and translational components in developing axonal and dendritic growth cones of cultured hippocampal neurons. Fluorescence *in situ* hybridization revealed that β-actin mRNAs were prominent in neuronal processes and growth cones in the form of RNA granules that associate with microtubules. The neurotrophin, NT-3, was shown to rapidly signal the transport of β-actin mRNAs to growth cones. This response was dependent on microtubules and also a cAMP dependent signaling pathway. Recent work has identified a ribonucleoprotein (RNP) complex between a β-actin mRNA localization sequence (zipcode) and its binding protein, Zipcode Binding Protein (ZBP1), that is required for the active transport of β-actin mRNA to growth cones in response to neurotrophin (NT-3). Recent evidence indicates that ZBP1 is associated with kinesin, and disruption of kinesin impairs ZBP1 localization. Live cell imaging of transfected neurons showed that EGFP-ZBP1 granules displayed bidirectional movements within the range of fast axonal transport.

Disruption of the formation of the RNP localization complex between β-actin mRNA and ZBP1 resulted in decreased levels of β-actin protein and reduced forward motility of growth cones in response to NT-3. These results suggest a novel mechanism to influence growth cone dynamics involving the regulated transport of a β-actin mRNP localization complex. Neurotrophin regulation of actin-dependent changes in growth cone motility may depend on the signaling of β-actin mRNA transport.

Analysis of genes required for the organization of the neuronal cytoskeleton in *Drosophila*

Christian Klämbt and Sven Bogdan

Institut für Neurobiologie Badestr. 9 48149 Münster

The major axon tracts in the embryonic CNS of *Drosophila* are organized in a simple, ladder-like pattern. In order to understand the molecular logic underlying the correct formation of the commissural tracts we have performed large-scale phenotypic screen for mutations disrupting commissure development. A large group of mutations was found to more affect axonal extension and as further studies showed, identified components required for the regulation of the neuronal cytoskeleton. The analysis of three genes each affecting distinct cytoskeletal components will be presented.

In *futsch* mutant embryos neurite extension as well as dendrite formation is impaired. The Futsch protein localizes to the microtubule compartment of the cell and co-assembles with taxol stabilized bovine microtubules *in vitro*. Both, the N- and C- terminal domains are homologous to the vertebrate MAP1B microtubule associated protein. The central domain of the Futsch protein is highly repetitive and shows sequence similarity to neurofilament proteins of which no *Drosophila* homologs have been reported. Interestingly, glial cells are able to modulate the neuronal expression of *futsch* and thus can influence axonal growth. This may be accomplished by the activation of specific kinases that have been implicated in the regulation of vertebrate MAP1B activity too.

kette is needed for formation of neuronal cell processes, too, and regulates the organization of the F-actin cytoskeleton. It encodes a highly conserved *Drosophila* homolog of the Nck Associated Protein (Hem2/NAP1) that like its mammalian counterparts – interacts with members of the Rho GTPases family (RAC1, CDC42). In *in vivo* as well as in tissue culture models most of the Kette protein is localized in the cytoplasm. Some Kette protein appears to be recruited to the membrane in Ca^{2+} dependent mechanism. To further understand the cellular function of Kette we are currently analyzing several interacting proteins.

A final gene to be discussed is involved in the regulation of *α-spectrin* expression. The different Spectrin proteins make up an important interface between the cytoskeleton and the cell membrane. One the one hand Spectrins bind to Actin and through interactions with proteins such as Ankyrin they are linked to transmembrane proteins. Spectrins were first identified as important determinants defining the shape of erythrocytes but are now recognized as a large class of proteins also present on non-erythrocytes cells.

Regulation of axon extension and navigation by growth-cone calcium transients

Nicholas C. Spitzer

Neurobiology Section, Division of Biology and Center for Molecular Genetics
UCSD, La Jolla CA 92093

What are the functions of neuronal ion channels expressed prior to synaptogenesis that mediate specialized forms of excitability allowing substantial Ca^{2+} influx? Imaging Ca^{2+} in spinal neurons cultured from *Xenopus* embryos demonstrates that these cells generate several distinct types of spontaneous elevations of intracellular Ca^{2+}, which encode developmental information in the frequency with which they are produced.

Spontaneous growth cone Ca^{2+} transients (~30 s duration) are generated by influx and release from stores and regulate the rate of axon outgrowth in cultured neurons by inhibiting axon extension through activation of the Ca^{2+}-dependent phosphatase, calcineurin (CN; Gu and Spitzer, 1995; Lautermilch and Spitzer, 2000). Pharmacological blockers and peptide inhibitors suppress Ca^{2+}-dependent reduction of axon outgrowth. In contrast, expression of a constitutively active form of xCN in the absence of growth cone Ca^{2+} transients results in shorter axon lengths similar to those seen in the presence of these transients. Phosphorylation of GAP-43, which promotes actin polymerization, is reduced by Ca^{2+} transients and enhanced by suppression of CN activity, indicating CN destabilizes the growth cone actin cytoskeleton. The developmental expression pattern of xCN transcripts *in vivo* coincides temporally with axonal pathfinding by spinal neurons, supporting a role of CN in regulating Ca^{2+}-dependent axon extension in the spinal cord.

Consistent with this view, imaging neurons in the embryonic spinal cord demonstrates directly that growth cones generate transient elevations of $[Ca^{2+}]_i$ as they migrate and the rate of axon outgrowth is inversely proportional to the frequency of transients (Gomez and Spitzer, 1999). UV activation of the photolabile Ca^{2+} chelator, diazo-2, leads to suppression of Ca^{2+} transients and accelerated axon growth, while mimicking transients by photorelease of Ca^{2+} from NP-EGTA leads to slowing of otherwise rapid growth. The frequency of Ca^{2+} transients is cell-type specific and depends on the position of growth cones along their pathway. Further, growth cone stalling and axon retraction, that are important aspects of pathfinding, are associated with high frequencies of Ca^{2+} transients. Thus environmentally regulated growth cone Ca^{2+} transients control axon growth in the developing spinal cord.

Spontaneous filopodial Ca^{2+} transients (~0.3 s duration) are highly localized and propagate along these antenna-like structures and stimulate global growth cone Ca^{2+} elevations in cultured neurons (Gomez et al., 2001). They are generated by influx through Ca^{2+} channels activated by integrin receptors, and their frequency is substrate-dependent. These local transients appear to slow neurite outgrowth by increasing filopodial adhesion. They promote turning when asymmetrically generated at a substrate border or when artificially imposed in selected filopodia, and thus serve as both as autonomous regulators of filopodial motility and as frequency-coded signals that are integrated within the growth cone.

References:

Gu, X. and Spitzer, N.C. (1995). Distinct aspects of neuronal differentiation encoded by frequency of spontaneous Ca^{++} transients. *Nature 375:* 784-787.

Gomez, T.M. and Spitzer, N.C. (1999). *In vivo* regulation of axon extension and pathfinding by growth-cone calcium transients. *Nature 397:* 350-355.

Lautermilch, N.J. and Spitzer, N.C. (2000). Regulation of calcineurin by growth cone calcium waves controls neurite extension. *J. Neurosci. 20:* 315-325.

Gomez, T.M., Robles, E., Poo, M.-m. and Spitzer, N.C. (2001) Filopodial calcium transients promote substrate-dependent growth cone turning. *Science 291:* 1983-1987.

Session 5: Pathfinding and targeting
Chair: William A. Harris

Rapid translation is required for chemotropic responses of retinal growth cones

Douglas Campbell and Christine Holt

Department of Anatomy, University of Cambridge, Downing St., Cambridge CB2 3DY

Retinal axons navigate using chemotropic guidance signals in the optic pathway. Retinal growth cones contain mRNA and ribosomes and we have investigated whether translation plays a role in chemotropic guidance. We show that isolated retinal growth cones lose their ability to turn in a chemotropic gradient and to collapse in response to a repellent when translation is blocked. Further, we demonstrate that the guidance factors, semaphorin 3A and netrin-1, stimulate a marked rise in protein synthesis and activate translation initiation factors in isolated growth cones. These effects occur within minutes suggesting that guidance molecules steer axon growth by triggering local translation in growth cones.

In vivo imaging of growth cone and filopodial dynamics support a model of contact-mediated repulsion between sibling processes of an identified cell

M.W. Baker and E.R. Macagno

Division of Biology, University of California, San Diego, La Jolla, CA 92093

During development, the peripheral Comb Cell (CC) of the embryonic leech sends out multiple, parallel growth cone-tipped processes that act as scaffolds for the migrating myocytes of the developing oblique muscle layer. *Hm*LAR2, a receptor protein tyrosine phosphatase that is highly expressed by these growth cones and is capable of homophilic receptor-ligand binding, has been shown to plays a critical role in controlling the outgrowth of these. The results of various perturbations of HmLAR2 function led us to the hypothesis that the non-overlapping projections of this cell results from cell-autonomous signaling by HmLAR2 between sibling processes leading to the retraction of overlapping areas processes (see references below).

We are testing this hypothesis by dynamic imaging of fluorescent dye-filled CCs in the intact developing embryo with a 2-photon confocal microscope. Time-lapse analysis reveals that the growth cones of the CC are highly dynamic, undergoing rapid filopodial extension and retraction. Measurement of filopodial life-span and length shows that most filopodia at the leading edge of the growth cone achieve significantly longer lengths and life spans than lateral filopodia, which are often seen to make apparent contact with adjacent parallel processes and quickly retract. Moreover, selectively cutting single CC processes (using a line scan from a 2-photon laser) leads to undamaged adjacent CC processes sprouting branches into the vacated territory. Together these findings support the hypothesis for the existence of contact-mediated repulsion, mediated by HmLAR2, between neighboring processes of the CC.

References:

RNAi of the Receptor Tyrosine Phosphatase *Hm*LAR2 in a Single Cell of an Intact Leech Embryo Leads to Growth-Cone Collapse. M.W. Baker and E.R. Macagno. *Curr. Biol.* **10**:1071-1074 (2000).

The Role of a LAR-like Receptor Tyrosine Phosphatase in Growth Cone Collapse and Mutual-Avoidance by Sibling Processes. Michael W. Baker and E.R. Macagno. *J. Neurobiol.* **44**:194-203 (2000).

Homophilic Interactions of the Receptor Protein Tyrosine Phosphatase HmLAR2 Contribute to Interbranch Repulsion in a Leech Embryonic Cell. M.W. Baker, S.J. Rauth and E.R. Macagno. *J. Neurobiol.* **45**:47-60 (2000).

Genetic screens and cellular analysis of somite derived signals guiding motor axons

E. Santana, V. Schneider, M. Granato*

Department of Cell and Developmental Biology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6058, USA; * granatom@mail.med.upenn.edu

To connect with their muscle targets, motor growth cones navigate long and complex trajectories. Initially, different motor neuron populations extend their growth cones into a single segmental nerve through which they enter the periphery. In the periphery, motor growth cones a confronted with a series of binary decision at numerous choice points. Experimental studies have shown that these two processes, guidance into the periphery and pathway selection, depend on cues provided by surrounding cells. However, the precise identity of these cells, the identity of the cues they produce and how these cues guide mixed populations of growth cones, but also elicit differential growth cones responses, remains poorly defined

The zebrafish embryo is an excellent model system in which to study the cellular and molecular mechanisms controlling motor axon pathfinding. We have previously identified mutants of two genes, diwanka and unplugged, which appear to act in a coordinated, stepwise manner to guide motor growth cones (1). While the diwanka gene is essential to guide spinal motor axons into the somites, the unplugged controls their subsequent pathway decisions (2, 3). Interestingly, activity of both genes is produced by a small group of mesodermal cells, the adaxial cells. These adaxial cells delineate the motor path before the first growth cone arrives, and begin to migrate radially as the growth cones approach (2). Thus, both genes influence motor growth cones in a contact-independent mechanism, responsible for the initial migration of motor axons into the periphery as well as for their subsequent trajectory choice.

To identify additional components of this somite derived signaling mechanism, we performed a genetic screen using an antibody cocktail to visualize motor axons and adaxial cells. So far, we have screened through ~ 30,000 embryos from 1,680 crosses, representing about 500 genomes. We have identified 19 mutants with striking defects restricted to motor neurons and/or adaxial cells. These defects cover a broad range, including reduced adaxial cell differentiation, defects in adaxial cell migration, reduced/aberrant motor axon outgrowth, aberrant motor axon branching, and formation of multiple segmental nerves. We will present analyses of some of these and other mutants to provide cellular and molecular insights how motor growth cones are guided by multiple, somite derived signals.

References:

1) Granato, M., et al. 1996. Genes controlling and mediating locomotion behavior of the zebrafish embryo and larva, Development 123, 399 - 413.

2) Zeller, J., Granato, M. 1999. The zebrafish diwanka gene controls an early step of motor growth cone migration. Development, 126, 3461- 3472.

3) Zhang, J., Granato, M. 2000. The zebrafish unplugged gene controls motor axon pathway selection. Development, 127, 2099-2111.

Common mechanisms underlying growth cone guidance and axon branching

Katherine Kalil

During development, growth cones direct growing axons into appropriate targets. However, in some cortical pathways target innervation occurs through the development of collateral branches that extend interstitially from the axon shaft. How do such branches form? Direct observations of living cortical brain slices revealed that growth cones of callosal axons pause for many hours beneath their cortical targets prior to the development of interstitial branches. High resolution imaging of dissociated living cortical neurons for many hours showed that the growth cone demarcates sites of future axon branching by lengthy pausing behaviors and enlargement of the growth cone. After a new growth cone forms and resumes forward advance, filopodial and lamellipodial remnants of the large paused growth cone are left behind on the axon shaft from which interstitial branches later emerge. Although axon branching of dissociated cortical neurons occurs in the absence of targets, application of a target derived growth factor, FGF-2 greatly enhances branching.

To investigate how the cytoskeleton reorganizes at axon branch points, microtubules and actin filaments were fluorescently labeled in living cortical neurons and the behaviors of both cytoskeletal elements imaged during new growth from the axon shaft and the growth cone. In both regions microtubules were found to reorganize by splaying apart and fragmenting. Shorter microtubules then invade newly developing branches with anterograde and retrograde movements. Splaying of looped or bundled microtubules is accompanied by focal accumulation of f-actin. Dynamic microtubules were found to co-localize with f-actin in transition regions of growth cones and at axon branch points. In contrast, f-actin is excluded from the central region of the growth cone and the axon shaft. Interactions between dynamic microtubules and f-actin involve their coordinated polymerization. Application of drugs that attenuate either microtubule or f-actin dynamics also inhibits polymerization of the other cytoskeletal element. Importantly, inhibition of microtubule or f-actin dynamics prevents axon branching. Axons are still able to elongate but their outgrowth is undirected. These results show that interactions between dynamic microtubules and f-actin are required for axon branching and directed axon outgrowth. Taken together, these studies demonstrate that growth cone pausing is closely related to axon branching and suggest that common cytoskeletal mechanisms underlie directed axon growth from the terminal growth cone and the axon shaft.

References:

Szebenyi, G., J. Callaway, E. W. Dent and K. Kalil. 1998. Interstitial branches develop from active regions of the axon demarcated by the primary growth cone during pausing behaviors. J. Neuroscience 18: 7930-7940.

Dent, E. W., P.W. Baas and K.Kalil. 1999. Reorganization and movement of microtubules in axonal growth cones and developing interstitial branches. J. Neuroscience 19: 8894-8908.

Kalil, K., G. Szebenyi and E. W.Dent. 2000. Common mechanisms underlying growth cone guidance and axon branching J. Neurobiology 44:145-158.

Szebenyi, G., E.W. Dent, J.L. Callaway, C. Seys, H.Lueth and K. Kalil. 2001. Fibroblast growth factor-2 promotes axon branching of cortical neurons by influencing morphology and behavior of the primary growth cone. J. Neuroscience 21:3932-3941.

Neurotransmitter release and axonal pathfinding in Ariadne mutants of *Drosophila*

Manuel Martínez-Padrón, Miguel Aguilera, Miguel Sancho Ruiz & Alberto Ferrús

Instituto Cajal (CSIC) Ave. Doctor Arce 37, Madrid 28002, Spain

Ariadne-1 is the first member of a conserved new family of genes discovered in *Drosophila*. The protein family is characterized by the string of motives *A*cid-rich, *R*ing-finger, *B*-box, *R*ing-finger and *C*oiled-*C*oil (RBRCC). This string of domains suggests protein-protein interactions that, in conjunction with its ubiquotous expression, make this protein a candidate for a basic component of cell regulation. We have documented that ARI interacts with a novel ubiquitin-conjugating enzyme of *Drosophila*, UbcD10, and that the first R motif is necessary and sufficient to sustain this interaction. Like the protein sequence, this interaction is also conserved since the corresponding mouse homologues, mARI and UbcM4, can substitute their fly counterparts. *Drosophila* null *ari* mutants die during metamorphosis. Occasional (2-5%) adult escapers consistently show major projection defects in the brain and thoracic ganglia with frequently misrouted axonal tracts. Since the peak of transcription occurs at the pupal stage and the four available alleles have a sharp lethal phase at this time, it appears that the protein is at high demand during metamorphosis. This phase of development is characterized by a rapid and extensive reorganization of neural branching and connectivity. Among the structural relatives of ARI, we have identified Parkin, a protein involved in Parkinson's disease (PD) characterized by the RBR motif signature and an ubiquitin-like domain.

Based on the evidences that indicate a role of neurotransmitters in axonal pathfinding, we questioned if the neural projection phenotypes of *ari* mutants could be related to secretion defects. Ideally, the study should be carried out on growth cones during the massive rewiring of the CNS at metamorphosis, the developmental stage when the mutant phenotype becomes evident. However, since electrophysiological recordings from *Drosophila*'s growth cones are not feasible, we attempted to analyze the third instar larvae neuromuscular junction (NMJ) as the best alternative. The NMJ synaptic boutons have been extensively used to characterize the role of a number of proteins in spontaneous and evoked secretion, membrane trafficking, vesicle recycling, etc. Here we show that *ari* mutants present abnormal synaptic release. The frequency of spontaneous release is significantly reduced in all *ari* alleles tested. Also, a point mutation within the R motif that interacts with UbcD10, but not a mutation in the second R motif, results in potentiation of evoked release, likely due to an increased probability of vesicle fusion in response to calcium.

References:

Aguilera et al., (2000) *Ariadne-1*: A vital *Drosophila* gene is required in development and defines a new conserved family of RING finger proteins. **Genetics** *155*, 1231-1244.

POSTERS

Contribution of Netrin-1/DCC and Slit-1 in initiating and directing migration of inferior olivary neurons

Frédéric Causeret, François Danne, Frédéric Ezan, Constantino Sotelo and Evelyne Bloch-Gallego

During the circumferential migration of inferior olivary neurons (ION), the floor-plate constitutes an intermediate target and a source of diffusible factors. We have previously shown that netrin-1 is implied in the survival and migration of ION (Bloch-Gallego et al., 1999; Llambi et al., 2001). We have analysed the role of DCC (Deleted in Colorectal Cancer, a netrin receptor) and slit-1 (another chemotropic molecule produced by the floor-plate) in the multiple actions of netrin-1 during the different steps of ION migration , i.e initiation and ventral positioning of cell bodies without crossing the floor-plate. We have analysed the ION in DCC and netrin-1 deficient mutant embryos: at E12.5, the initiation of ION migration is delayed and at birth, despite important differences in cell survival in both mutants, most of the ION have stopped their migration prematurely and are ectopically located along the submarginal migratory stream. Using *in vitro* confrontation assays with ION explants (E11), we show that netrin-1 accelerates the initiation of axon outgrowth, has tropic effects on ION axon outgrowth and is strictly required for nuclear translocation (nucleokinesis). These effects are mediated by DCC receptor, whereas A2b receptor is not involved. In our *in vitro* assays, slit-1 has a weak effect by itself but when combined to netrin-1, it blocks most of the effects of netrin-1, in particular the chemotropic effect and the nuclear translocation. We show that the slit receptor robo-2 is expressed by the migrating ION, and this expression is maintained once the neurons have arrived and stop close to the floor-plate. Our results indicate that net-1/DCC are strictly required for the migration of ION and are consistent with the notion that Slit-1/robo-2 could silence the chemotropic effect of net1/DCC to cause the arrest of ION at the midline.

Inhibition of protein synthesis prevents chemotropic responses in xenopus retinal growth cones

Douglas S. Campbell and Christine E. Holt

Department of Anatomy, University of Cambridge, Downing Street, Cambridge, CB2 3DY, UK

Axonal growth cones navigate to their correct targets in the embryonic brain using a series of diffusible and membrane bound guidance cues. *Xenopus* retinal growth cones without their cell bodies are able to navigate correctly in vivo suggesting the directional choices made are under local control (Harris et al., Development, 1987, 10:23-33). Growth cones possess mRNA, ribosomes and other components of the translation machinery and have been shown to undergo local protein synthesis (Davis et al., J Neurosci, 1992, 12:467-77), however, the functional role of this protein synthesis has not been investigated. Here we show that pharmacological inhibition of protein synthesis prevents semaphorin 3A (Sema 3A)-induced retinal growth cone collapse and repulsive turning and also inhibits netrin-1-induced turning. The inhibitors act locally at the growth cone since the chemotropic responses of growth cones from severed neurites lacking cell bodies are also blocked. Furthermore, we show that translation initiation proteins become phosphorylated and growth cones undergo protein synthesis in response to Sema 3A and netrin-1. We propose that growth cone responsiveness to guidance signals is under local translational control and, since the collapse and turning responses occur on the time-scale of minutes, require rapid protein synthesis.

Supported by MRC (C.E.H.) and BBSRC (D.S.C.)

Screen for axon guidance genes in *C. elegans*

Catherine Chiu, Katrina Sabater, Ray Squires and Scott Clark

To understand further the molecular mechanisms involved in growth cone guidance, we have undertaken several screens in *C. elegans* to identify new genes involved in axon extension and pathfinding.

The two PVQ neurons are located in the tail and each extends a single axon that runs anteriorly to the nerve ring in the head. Using the sra-6::gfp reporter to visualize the PVQs, we have recovered over ninety mutants with a variety of axon outgrowth, branching and pathfinding defects. We found over thirty mutants with defects in either cell fate, cell migration, cell position or programmed cell death. From this screen and others, we isolated over 150 mutations that define over forty genes, including around twenty-five previously known genes.

Mutations in the new gene zag-1 cause axons to defasciculate and branch inappropriately. zag-1 encodes a protein containing multiple zinc finger domains and a homeodomain. ZAG-1 is similar in sequence and structure to the products of the *Drosophila* zfh-1 gene and the vertebrate ZEB genes and likely acts as a transcriptional repressor. A transcriptional zag-1::gfp transgene is expressed in both neuronal and nonneuronal cells. Zag-1::gfp expression is observed in ventral cord neurons in a zag-1 mutant yet not in wild type, indicating that zag-1 regulates, either directly or indirectly, its own expression in some neurons. Our results suggest that zag-1 regulates the expression of genes involved in axon guidance. We are currently investigating how zag-1 activity is regulated as well as the genes that act downstream of zag-1.

SFRP1 promotes axon outgrowth and retina cell differentiation through GSK3b inhibition

Pilar Esteve, Françoise Trousse, Josana Rodríguez-Sánchez and Paola Bovolenta

Departamento de Neurobiología del Desarrollo, Instituto Cajal, CSIC, Dr. Arce 37, Madrid 28002, Spain

Secreted Frizzled Related Proteins (SFRPs) are soluble molecules capable of binding WNTS and preventing the activation of their signaling cascade. However, the full range of SFRP activities and mechanisms of action are not fully understood. Here, we show both in vitro and in vivo that Sfrp1 has a dual function in the development of the chick retina: it promotes axon outgrowth and increases neuronal differentiation, two novel functions for this molecule. These effects are primarily mediated by the CRD domain of the molecule and involve the partial inhibition of GSK3b activity, without a significant variation in the cytoplasmic protein levels of bcatenin. These results suggest that SFRP1 contributes to retina development with a mechanism that is independent from sequestration of endogenously present Wnt molecules.

Kidins220, a novel neurospecific protein, downstream protein kinase D and neurotrophin and ephrin receptors

Teresa Iglesias

Instituto de Investigaciones Biomédicas "Alberto Sols", CSIC, 28029-Madrid, Spain

Protein Kinase D (PKD, also known as PKCμ) is a kinase that is activated *in vivo* by the Protein Kinase C (PKC) family of proteins, whose members have been involved in processes affecting brain function, such as neuronal development and differentiation, synaptic transmission and plasticity, and axonal regeneration. We have just cloned the first physiological substrate for PKD, Kidins220 (Kinase D interacting substrate, 220 kDa), a novel rat neuroespecific protein, which presents orthologues in *D. Melanogaster*, *C. elegans* and humans. Kidins220 contains eleven ankyrin repeats and four transmembrane domains within the N-terminal region, a specific residue (ser-919) that is phosphorylated by PKD, a splicing cassette (some of which variants present a sterile alpha motif or SAM domain) and a carboxy-terminal PDZ-binding domain. We have shown that Kidins220 is an integral membrane protein highly expressed in brain and in neuronal cells, where it is enriched at the tip of the neurites, as well as in lipid rafts (cholesterol rich microdomains participating on axonal protein sorting and signalling). Interestingly, Kidins220 is a downstream target not only of PKD but also of neurotrophin and ephrin tyrosine kinase receptors, whose stimulation plays a prominent role in nervous system development and morphogenesis, neuronal differentiation, synaptic plasticity and axonal growth and guidance. Although future identification of Kidins220 functions is crucial, our findings suggest the possible involvement of Kidins220 and PKD in neurite extension and signalling at the growth cone.

Promotion of neuritogenesis as a novel functional activity of ephrin B1 on cerebellar neurons

Moreno-Flores M.T., Martín-Aparicio E., Ávila J., Díaz-Nido J., Wandosell F.

Centro de Biología Molecular "Severo Ochoa", Facultad de Ciencias, U.A.M.
28049 Madrid, Spain

Ephrins are developmentally regulated molecules that may contribute to axonal pathfinding through their binding to Eph receptor tyrosine kinases. In many cases, ephrins act as negative molecules that stimulate growth cone collapse, although some forms may promote axonal growth. Here we have addressed the role played by ephrin-B1 during rat postnatal cerebellar development. Ephrin-B1 is expressed by both granule and Purkinje neurons whereas EphB is present in granule neurons in early postnatal cerebellum at a time coincident with axonal and dendrite outgrowth. Stably transfected 3T3 cells overexpressing ephrin-B1 enhance survival and neurite growth from cultured cerebellar granule neurons, an effect which is inhibited by the presence of an excess of a soluble EphB protein. Ephrin-B1-induced neuritogenesis is correlated with an increased expression of certain neuronal-specific microtubule-associated proteins (MAPs). Cerebellar granule neurons plated on stably transfected 3T3 cells overexpressing ephrin-B1 show an up-regulation of the expression of axonal MAPs such as Tau and phosphorylated MAP2C as compared with neurons cultured on control 3T3 cells. The level of expression of these axonal MAPs is similar to that found in neurons plated on poly-L-lysine. Interestingly, there is a noteworthy up-regulation of somatodendritic MAPs such as high-molecular-weight MAP2 and mode II-phosphorylated MAP1B in neurons cultured on stably transfected 3T3 cells overexpressing ephrin-B1 as compared with neurons plated on either control 3T3 cells or poly-L-lysine. The effect could be partially inhibited by H-89 an inhibitor of PKA. In view of these data we suggest that ephrin-B1 supports axonal growth and might favour dendritogenesis of granule neurons during the development of the cerebellum.

Inhibition of glycogen synthase kinase 3b in sensory neurons alters actin and microtubule dynamics in growth cones

R. Owen and P. R. Gordon-Weeks

MRC Centre for Developmental Neurobiology, King's College London, U.K

Neuronal process extension is dependent on the reorganisation of the cytoskeleton, in particular microtubules and microfilaments, and one of the ways in which microtubules are regulated is by a group of associated proteins called MAPs. MAP1B, the first MAP to be expressed in developing neurons, has been shown to play an important role during axonogenesis. Previous work has shown that expression of a GSK3b phosphorylated isoform of MAP1B (MAP1B-P) is associated with a decrease in microtubule stability and a loss of stable microtubules, suggesting that MAP1B phosphorylation by GSK3b may act as a switch that regulates microtubule stability during axonogenesis. To test this idea we have examined the effect of GSK3b inhibition on axonogenesis and microtubule dynamics in cultured dorsal root ganglion neurons. To inhibit GSK3b we used either lithium or SB-216763, a potent small molecule competitive inhibitor of GSK3. Loss of MAP1B-P in these neurons was associated with a decrease in axon elongation rate, the development of giant growth cones and changes in filopodial dynamics. These changes correlated with an increase in stable microtubules in growth cones. The results described here extend a recent study of cultured DRG neurons from MAP1B deficient mice, which showed comparable morphological and cytoskeletal changes to those described here. Together these results implicate a crucial role for MAP1B in the early stages of axonogenesis by maintaining the growth cone cytoskeleton in a dynamically unstable state and also suggest an involvement of MAP1B in coordinating the action of microtubules and microfilaments in growth cones.

We thank GlaxoSmithKline for SB-216763.

Ephexin family of guanine nucleotide exchange factors mediate the modulation of actin cytoskeleton by Eph receptors

M. Sahin, M.Z. Lin, S.M. Shamah, M.E. Greenberg

Division of Neuroscience, Children's Hospital, and Department of Neurobiology, Harvard Medical School, Boston, MA, USA

Eph receptors transduce contact-mediated repulsive signals for axon guidance. Gene knockout experiments have confirmed the roles of individual Eph receptors and their ligands, ephrins, in pathfinding of specific axon tracts. The molecular mechanisms by which Eph receptors control growth cones are less well understood. We have recently identified a novel guanine nucleotide exchange factor (GEF), ephexin, which interacts directly with the EphA4 receptor. Ephexin is expressed only in the central nervous system, is enriched in growth cones and can activate three Rho GTPases (rhoA, rac and cdc42), which are known regulators of the actin cytoskeleton. Upon ephrin stimulation, ephexin's GEF activity toward rhoA is increased and its activity toward rac and cdc42 is decreased, resulting in growth cone collapse. Expression of a dominant-negative form of ephexin renders growth cones less responsive to ephrin stimulation, indicating that ephexin is involved in ephrin-induced growth cone collapse. We are currently investigating the mechanism of regulation of ephexin by EphA receptors, and have obtained evidence demonstrating that ephexin is a phosphoprotein. Furthermore, there are four genes in the mammalian genome with high degree of sequence homology to ephexin. We are in the process of characterizing the expression patterns for each member of the ephexin subfamily and investigating their cellular functions *in vivo* and *in vitro*.

Role of Fyn-Cdk5 phosphorylation cascade in semaphorin-3A signaling

Yukio Sasaki

Semaphorin-3A (Sema3A), a secreted chemorepulsive molecule of the semaphorin family, induces growth cone collapse and axoplasmic transport. To elucidate signaling pathway for Sema3A, we have screened various compounds to affect Sema3A-induced growth cone collapse of chick dorsal root ganglion (DRG) neurons. A tyrosine kinase inhibitor, lavendustin A and a serine/threonine kinase Cdk5 inhibitor, olomoucine effectively suppressed the Sema3A responses. In *src* family tyrosine kinase *fyn*- or *cdk5*-defective mice, Sema3A-induced collapse response was significantly attenuated in DRG neurons. In non-neuronal cells expressing Sema3A receptor complex (plexin-A2 and neuropilin-1) and/or two kinases (Fyn and Cdk5), we have observed that 1) plexin-A2 was associated and phosphorylated with Fyn, 2) Constitutively active Fyn was able to augment kinase activity of Cdk5, accompanied with increased level of Tyr15 phosphorylation of Cdk5, and that 3) Sema3A augmented Cdk5 kinase activity time-dependently. Immunocytochemical analyses using phospho-specific antibodies revealed that, in DRG growth cones, Sema3A increased phosphorylation of Cdk5 (Tyr15) and their substrate, tau (Ser 202 & Thr 205). These findings provide evidence that Fyn-Cdk5 pathway plays a role in mediating Sema3A signaling.

Supported by: CREST & MEXT, Japan.

Human Trio induces neurite outgrowth in PC12 cells: functional analysis using Rho-GEF inhibitors

Schmidt Susanne, Estrach Soline, Méry Jean, Diriong Sylvie and Debant Anne

CRBM-CNRS, UPR 1086, 34293 Montpellier Cedex 5, France

During nervous system development, growth cone-guided neurite outgrowth is an essential process underlying the generation of neuronal connectivity. Within the growth cone, actin cytoskeleton reorganization is mediated by Rho family GTPases which are activated by guanine nucleotide exchange factors (GEFs). Trio is a complex protein comprised of two Rho-GEF domains: GEFD1, activating Rac1 via RhoG, and GEFD2, acting on RhoA. It is thus unique in linking Rac and Rho pathways in vivo (1,2). Genetic analyses in *Drosophila* and *C.elegans* indicate that Trio is a key molecule in axon guidance and cell motility (for example, see 3).

Here we show that expression of human Trio induces neurite outgrowth in PC12 cells, by a process dependent on GEFD1 but not on GEFD2. Moreover this requires the presence of Trio SH3-1 domain and spectrin repeats, suggesting that Trio has to integrate inputs from different signaling pathways to play its role in neuronal morphology. Moreover, we show that Trio is an upstream regulator of RhoG in the NGF differentiation pathway (4).

To elucidate the role of Trio GEFD2, we have used a genetic screen in yeast to select in vivo peptide aptamers that could inhibit GEFD2 activity (5). One peptide, TRIPa (TRio Inhibitory Peptide), specifically inhibits GEFD2 exchange activity on RhoA in vitro, and blocks the activity of ectopically expressed GEFD2 on neuronal morphology in PC12 cells. Interestingly, inhibition of endogenous Trio by TRIPa protects PC12 cells from serum-induced neurite retraction, and thereby implicates TrioGEFD2 as an upstream regulator of RhoA in this pathway (6). Thus, TRIPa represents the first Rho-GEF inhibitor targeting TrioGEFD2 in vivo, and will greatly help to gain insights into Trio function in the coordination of Rho-GTPase mediated actin cytoskeleton remodeling, occurring in cell and growth cone migration.

References:

1. Blangy A. et al. TrioGEF1 controls Rac-and Cdc42-dependent cell structures through the direct activation of RhoG.
J. Cell Sci 113, 729-739 (2000).
2. Bellanger J.M. et al. The two guanine nucleotide exchange factor domains of Trio link the Rac1 and RhoA pathways in vivo. Oncogene 16, 147-152 (1998).
3. Newsome, T.P. et al. Trio combines with dock to regulate Pak activity during photoreceptor axon pathfinding in Drosophila. Cell 101, 283-294. (2000).
4. Schmidt S.,et al. The human GEF Trio induces neurite outgrowth in PC12 cells by a GEFD1 dependent process and is an upstream regulator of RhoG in the NGF pathway. Submitted.
5. Colas, P. et al. Genetic selection of peptide aptamers that recognize and inhibit cyclin-dependent kinase 2. Nature 380, 548-550. (1996).
6. Schmidt S. et al. TRIPa is the first peptide inhibitor of a RhoGEF and prevents PC12 cells from TrioGEFD2-induced neurite retraction. Submitted.

The identification of proteins Th 4 interact with UNC-73/Trio in *C. elegans*

Robert Steven, Terry Kubiseski, Lijia Zhang, Joseph Culotti and Tony Pawson

C. elegans unc-73, also known as trio in mammals and *Drosophila*, was the first gene of this family to be identified with a role in axon pathfinding. The axons of many neurons fail to reach their target tissues and often travel along abnormal pathways in unc-73 mutant animals. Experiments in both *C. elegans* and *Drosophila* reveal thatUNC-73/Trio function involves signaling through the Rac GTPase and its downstream effectors to regulate the cytoskeletal rearrangements necessary for growth cone migrations. UNC-73 has two RhoGEF domains which activate members of the Rho family of GTPases. Interestingly, animals with a mutation that eliminates the activity of the first RhoGEF domain are uncoordinated (Unc), presumably as a result of axon guidance defects, while a deletion of the exons encoding the second RhoGEF domain results in early larval lethality. Two approaches are being used to identify proteins that interact with UNC-73. First, a genetic screen is being set up based on the observation that overexpression of a construct encoding a truncated form of UNC-73 in wild-type animals results in a severe uncoordinated phenotype. A stable transgenic line containing this construct will be used in a screen for non-uncoordinated suppressors. Second, a yeast two hybrid screen was used to identify several proteins that interact with UNC-73. One of the interacting proteins is an actin-binding protein that is localized asymmetrically in the membrane of some *C. elegans* cells. This is consistent with our hypothesis that UNC-73 is involved in the localized regulation of the actin cytoskeleton.

The function of lachesin, a GPI-linked membrane protein of the immunoglobulin superfamily, in the developing *Drosophila* nervous system

Maura Strigini, Michael J. Bastiani* and Domna Karagogeos

IMBB/FORTH, Iraklio, Crete, Greece
* Biology Dept., Utah University, Salt Lake City, Utah

Lachesin is a GPI-linked membrane protein belonging to the immunoglobulin (Ig) superfamily. It has been cloned from fly (*Drosophila melanogaster*) and grasshopper (*Schistocerca americana*). Earlier studies in the grasshopper embryo suggested that Lachesin may be involved in processes of axon outgrowth and guidance, on the basis of its expression pattern and its similarity to vertebrate and invertebrate cell adhesion and signalling molecules (Karlstrom et al., 1993).

Several GPI-linked membrane proteins have been shown to play an important role in axon growth and guidance in both vertebrates and invertebrates. These proteins include Ephrins and Ig superfamily members, such as TAG-1 and alternative variants of transmembrane proteins like N-CAM and FasciclinII. Recently it has become clear that GPI-linked proteins do not only function as cell surface anchored ligands in cell-cell communication, triggering a response in an adjacent cell (forward signalling), but may also initiate signalling in the expressing cell (reverse signalling). Exactly how such reverse signalling is taking place is not fully understood yet.

By studying the function of Lachesin in the developing *Drosophila* nervous system, we intend to establish a system where to investigate signalling by GPI-linked membrane proteins with a combination of in vivo genetic approaches and in vitro biochemical studies.
The expression pattern of *Drosophila* Lachesin has been analyzed by in situ hybridization. In the developing nervous system, Lachesin mRNA is detected in neurogenic cells before germ band shortening and, slightly later, in three distinct clusters of cells in each hemisegment. Later in embryonic development, its mRNA is detected in the dorsal surface of the nervous system, in a region corresponding to the axon scaffold forming commissures and connectives. Whether this staining marks the axons themselves or the surrounding glia has still to be determined.

In vivo functional analyses of *Drosophila* Lachesin is based on gain and loss of function experiments, by means of the UAS/GAL4 system, RNA interference, and generation of a mutant allele. Preliminary results will be presented and future directions discussed.

Type I phosphatidylinositol 4-phosphate 5 kinase activity is required for LPA-induced neurite retraction

Francis van Horck, Wouter Moolenaar and Nullin Divecha

Dept. of Cellular Biochemistry, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

Actomyosin-driven neurite remodeling is regulated by Rho family GTPases and critically depends on cell-matrix interactions. In addition, PIP2 has been implicated as a regulator of cytoskeletal artchitecture. Cellular PIP2 levels are regulated by PIPkinases (type I and II), but little is known about the importance of PIP kinases in determining neurite remodeling. Serum factors such as LPA and S1P induce dramatic neurite retraction and cell rounding in neuronal N1E-115 cells due to activation of the RhoA-ROK pathway leading to actomyosin contraction. Here we show that introduction of PIPkin into N1E-115 cells leads to cell rounding and complete inhibition of neurite outgrowth. Strikingly, PIPkin-induced cell rounding and suppression of neurite outgrowth is not accompanied by RhoA activation, nor is it prevented by dn-N19RhoA or the ROK inhibitor Y-27632. Conversely, expression of kinase-dead PIPkin promotes neurite outgrowth; however, these neurites are completely resistant to LPA and to active V14RhoA. We conclude that PIPkin acts downstream of the RhoA-ROK pathway to induce neurite retraction and cell rounding.

The role of *C. elegans* ephrins in regulating cell organization

Xiangmin Wang, Joseph G. Culotti, and Tony Pawson

The establishment of communications among neurons requires both the proper organization of cells involved (topographic patterning) as well as the proper projection of axons. Originally found in the developing nervous systems, ephrins and their receptors, the Eph Receptor Tyrosine Kinases (RTKs) are found to play important roles in mediating communications among the cells. The mammalian ephrin/Eph signaling networks are very complex, as there are at least 14 Eph receptors and 8 ephrins in mammals. In contrast, there are only 4 ephrins and 1 Eph receptor - VAB-1 in *C. elegans*, which makes it an ideal model system to study a complete ephrins/Eph receptor signaling network.

Using a reverse genetic approach, We isolated mutations in three of the ephrin genes *efn-2*, *efn-3*, *efn-4*. Together with the previously characterized *efn-1* and *vab-1* mutations, we determined that *efn-1*, *efn-2*, and *efn-3* function in the same pathways as that of *vab-1*. Unlike other ephrin genes however, recent work suggested that *efn-4* functions genetically in the *C. elegans* Semaphorin II signaling pathway. Using cell culture based *in vitro* biochemistry assays, we found that all four *C. elegans* ephrins bind to VAB-1. While EFN-1, EFN-2 have been shown to activate the VAB-1 kinase activity *in vitro*, EFN-4 does not even at a much higher concentration. Furthermore, biochemistry studies on worm lysates demonstrated that *efn-1*, *efn-2*, and *efn-3* are needed to activate VAB-1 kinase activity *in vivo*. To further study the signaling mechanisms of *C. elegans* ephrins and the VAB-1 receptor, as well as the role of ENF-4 in Semaphorin II signaling pathways, we will carry out both genetic and biochemistry approaches to identify new proteins that function in the ephrin signaling pathways.

LIST OF INVITED SPEAKERS

Peter W. Baas — Dept. of Neurobiology and Anatomy. MCP Hahnemann University. 2900 Queen Lane, Philadelphia, PA. 19129 (USA). Tel.: 1 215 991 8298. Fax: 1 215 843 9082. E-mail: Peter.W.Baas@drexel.edu

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

Joe Culotti — Samuel Lunenfeld Research Institute. Mount Sinai Hospital. 600 University Av., Toronto, ON. M5G 1X5 (Canada). Tel.: 1 416 586 82 44. Fax: 1 416 586 85 88. E-mail: culotti@mshri.on.ca

Barry J. Dickson — Institute of Molecular Pathology. Dr. Bohr-Gasse 7, 1030 Vienna (Austria). Tel.: 43 1 797 30 421. Fax: 43 1 798 71 53. E-mail: dickson@nt.imp.univie.ac.at

Carlos G. Dotti — Cavalieri Ottolenghi Scientific Institute. Fondazione Cavalieri Ottolenghi. Universita degli Studi di Torino. Regione Gonzole, 10, 10043 Orbassano,Torino (Italy). Tel.: 39 011 670 81 80. Fax: 39 011 670 81 51. E-mail: carlos.dotti@unito.it

Uwe Drescher — MRC Centre for Developmental Neurobiology, King's College London, New Hunts House, SE1 1UL London (U.K.). Tel.: 44 207 848 6411. Fax: 44 207 848 6798. E-mail: uwe.drescher@kcl.ac.uk

Alberto Ferrús — Instituto Cajal (CSIC). Ave. Doctor Arce 37, 28002 Madrid (Spain). Tel.: 34 91 585 47 39. Fax: 34 91 585 47 54. E-mail: aferrus@cajal.csic.es

Marcos A. González-Gaitán — Max-Planck Institute of Molecular Cell Biology and Genetics. Pfotenhauerstrasse 108, 01307 Dresden (Germany). Tel.: 49 351 210 25 39. Fax: 49 351 210 13 89. E-mail: gonzalez@mpi-cbg.de

William A. Harris — Dept. Anatomy, University of Cambridge. Downing Street, CB2 3DY Cambridge (UK). Tel.: 44 1223 333 772. Fax: 44 1223 333 786. E-mail: harris@mole.bio.cam.ac.uk

Christine E. Holt — Dept. of Anatomy, University of Cambridge. Downing Street, Cambridge CB2 3DY (UK). Tel.: 44 1223 766 229. Fax: 44 1223 333 786. E-mail: ceh@mole.bio.cam.ac.uk

Katherine Kalil — Dept. of Anatomy. University of Wisconsin, Madison, WI. 53706 (USA). Tel.: 1 608 262 89 02. Fax: 1 608 262 23 27. E-mail: kakalil@facstaff.wisc.edu

Christian Klämbt — Institut für Neurobiologie. Badestr. 9, 48149 Münster (Germany). Tel.: 49 251 832 11 22. Fax: 49 251 832 46 86. E-mail: klaembt@uni-muenster.de

Paul C. Letourneau — Dept. of Neuroscience, University of Minnesota. 321 Church St. S.E., Minneapolis, MN.55455 (USA). Tel.: 1 612 624 5999. Fax: 1 612 624 81 18. E-mail: letour@lenti.med.umn.edu

Liqun Luo — Dept. of Biological Sciences. Stanford University. 385 Serra Mall, Stanford, CA. 94305 (USA). Tel.: 1 650 723 66 45. Fax: 1 650 723 05 89. E-mail: lluo@stanford.edu

Eduardo R. Macagno — Division of Biology, University of California. 9500 Gilman Drive, San Diego, La Jolla, CA.92093 (USA). Tel.: 1 858 534 42 81. Fax: 1 858 534 7314. E-mail: emacagno@biomail.ucsd.edu

Carol A. Mason — Department of Pathology, Center for Neurobiology and Behavior, Columbia University, College of Physicians and Surgeons. 630 W. 168th Street, New York, NY.10032 (USA). Tel.: 1 212 305 21 05. Fax: 1 212 305 54 98. E-mail: cam4@pop.columbia.edu

Patricia C. Salinas — Department of Biological Sciences, Imperial College of Science, Technology and Medicine. Exhibition Road, London SW7 2AY (UK). Tel.: 44 20 7594 5193. Fax: 44 20 7594 5207. E-mail: p.salinas@ic.ac.uk

Nicholas C. Spitzer — Neurobiology Section, Division of Biology and Center for Molecular Genetics UCSD. 9500 Gilman Drive, La Jolla, CA. 92093 (USA). Tel.: 1 858 534 38 96. Fax: 1 858 534 7309. E-mail: nspitzer@ucsd.edu

David L. Van Vactor — Department of Cell Biology, Program in Neuroscience, Harvard Cancer Center, Harvard Medical School. 240 Longwood Avenue, Boston, MA. 02115 (USA). Tel.: 1 617 432 21 95. Fax: 1 617 432 11 44. E-mail: Davie@hms.harvard.edu

Kai Zinn — Division of Biology, California Institute of Technology. 1200 E. California Blvd., Pasadena, CA. 91125 (USA). Tel.: 1 626 395 83 52. Fax: 1 626 449 06 79. E-mail: zinnk@its.caltech.edu

LIST OF PARTICIPANTS

Gary J. Bassell — Dept. of Neuroscience. Rose F. Kennedy Center for Mental Retardation. Albert Einstein College of Medicine of Yeshiva University. 1410 Pelham Parkway South, Bronx, NY.10461 (USA). Tel.: 1 718 430 3648. Fax: 1 718 430 2960. E-mail: bassell@aecom.yu.edu

Evelyne Bloch-Gallego — INSERM U. 106. Hôpital de la Salpetriere. 47, Bld de l'Hôpital, 75 651 Paris Cedex 13 (France). Tel.: 33 1 42 16 26 81. Fax: 33 1 45 70 99 90. E-mail: gallego@infobiogen.fr

Douglas S. Campbell — Department of Anatomy, University of Cambridge. Downing Street, Cambridge, CB2 3DY (UK). Tel.: 44 1223 766 230. Fax: 44 1223 333 786. E-mail: dsc23@cam.ac.uk

Scott Clark — Molecular Neurobiology Program. Skirball Institute. New York University School of Medicine. 540 First Avenue, New York, NY.10016 (USA). Tel.: 1 212 263 0755. Fax: 1 212 263 8214. E-mail: clark@saturn.med.nyu.edu

Juan Andrés De Carlos — Instituto Cajal (CSIC). Avenida Doctor Arce 37, 28002 Madrid (Spain). Tel.: 34 91 585 47 08. Fax: 34 91 585 47 54. E-mail: decarlos@cajal.csic.es

Flora de Pablo — Centro de Investigaciones Biológicas, CSIC. Velázquez 144, 28006 Madrid (Spain). Tel.: 34 91 564 89 78. Fax: 34 91 564 75 18. E-mail: fdepablo@cib.csic.es

Jean-Marc Devaud — Cajal Institute. CSIC. Avenida Doctor Arce 37, 28002 Madrid (Spain). Tel.: 34 91 585 47 38. Fax: 34 91 585 47 54. E-mail: isrmd94@fresno.csic.es

Pilar Esteve — Departamento de Neurobiología del Desarrollo, Instituto Cajal, CSIC. Dr. Arce 37, Madrid 28002 (Spain). Tel.: 34 91 585 47 17. Fax: 34 91 585 47 54. E-mail: PilarEsteve@cajal.csic.es

Gregory Gasic — 52 Standish Circle, Wellesley, MA. 02481 (USA). E-mail: ggasic@mediaone.net

Michael Granato — Department of Cell and Developmental Biology, University of Pennsylvania School of Medicine, Philadelphia, PA. 19104-6058 (USA). Tel.: 1 215 898 27 45. Fax: 1 215 898 98 71. E-mail: granatom@mail.med.upenn.edu

Teresa Iglesias	Instituto de Investigaciones Biomédicas "Alberto Sols", CSIC. Arturo Duperier, 4, 28029 Madrid (Spain). Tel.: 34 91 585 46 37. Fax: 34 91 585 45 87. E-mail: tiglesias@iib.uam.es
Avihu Klar	Dept. of Anatomy and Cell Biology, Hebrew University - Hadassah Medical School, Jerusalem 91120 (Israel). Tel.: 972 2 675 71 33. Fax: 972 2 675 74 51. E-mail: avihu@cc.huji.ac.il
Juan Carlos López	Nature Reviews Neuroscience. 4 Crinan St., London N1 9XW (UK). Tel.: 44 207 843 36 08. Fax: 44 207 843 36 29. E-mail: j.lopez@nature.com
Laura López-Mascaraque	Instituto Cajal. Avenida Dr. Arce, 37, 28002 Madrid (Spain). Tel.: 34 91 585 47 08. Fax: 34 91 585 47 54. E-mail: mascaraque@cajal.csic.es
Javier López-Rios	Instituto Cajal-CSIC. Avenida Dr. Arce 37, 28002 Madrid (Spain). Tel.: 34 91 585 47 17. Fax: 34 91 585 47 54. E-mail: isrl321@cajal.csic.es
Emilie Marcus	Neuron/Cell press. 1100 Massachusetts Avenue, Cambridge, MA.02138 (USA). Tel.: 1 617 397 28 39. Fax: 1 617 397 28 19. E-mail: emarcus@cell.com
Guillermo Marqués	HHMI/University of Minnesota. 6-160 Jakson Hall, 321 Church Street SE, Minneapolis MN. 55455 (USA). Tel.: 1 612 625 86 02. Fax: 1 612 625 54 02. E-mail: marques@umn.edu
Mª Teresa Moreno-Flores	Centro de Biología Molecular "Severo Ochoa", Facultad de Ciencias, U.A.M., 28049 Madrid (Spain). Tel.: 34 91 397 84 75. Fax: 34 91 397 47 99. E-mail: mtmoreno@cbm.uam.es
Rebecca Owen	MRC Centre for Developmental Neurobiology, King's College London. New Hunts House, Guys Campus, London SE1 1UL (UK). Tel.: 44 20 7848 6426. Fax: 44 20 7848 6798. E-mail: rebecca.owen@kcl.ac.uk
Mustafa Sahin	Division of Neuroscience, Children's Hospital, and Department of Neurobiology, Harvard Medical School, Boston, MA. 02115 (USA). Tel.: 1 617 355 63 32. Fax: 1 617 738 15 42. E-mail: mustafa.sahin@tch.harvard.edu
Yukio Sasaki	Dept. of Pharmacology. Yokohama City University School of Medicine. 3-9 Fukuura, Kanazawa-ku, 236-0004 Yokohama (Japan). Tel.: 81 45 787 2595. Fax: 81 45 785 3645. E-mail: sasakyu1@med.yokohama-cu.ac.jp

Susanne Schmidt — CRBM-CNRS, UPR 1086. 1919, route de Mende, 34293 Montpellier Cedex 5 (France). Tel.: 33 467 61 33 57. Fax: 33 467 52 15 59. E-mail: schmidt@crbm.cnrs-mop.fr

Robert Steven — The Samuel Lunenfeld Research Institute. Mount Sinai Hospital. 600 University Ave., M5G 1X5 Toronto, ON. (Canada). Tel.: 1 416 586 45 24. Fax: 1 416 586 88 69. E-mail: steven@mshri.on.ca

Maura Strigini — IMBB/FORTH. Vassilika Vouton, 71110 Iraklio, Crete (Greece). Tel.: 30 81 391158. Fax: 30 81 391104. E-mail: strigini@imbb.forth.gr

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

Francis van Horck — Dept. of Cellular Biochemistry, The Netherlands Cancer Institute. Plesmanlaan 121, 1066 CX Amsterdam (The Netherlands). Tel.: 31 20 512 1978. Fax: 31 20 512 19 89. E-mail: frhorck@nki.nl

Francisco Wandosell — Centro de Biología Molecular "Severo Ochoa", Facultad de Ciencias, U.A.M., 28049 Madrid (Spain). Tel.: 34 91 397 87 10. Fax: 34 91 397 47 99. E-mail: fwandosell@cbm.uam.es

Xiangmin Wang — The Samuel Lunenfeld Research Institute. Mount Sinai Hospital. 600 University Ave., Toronto, ON. M5G 1X5 (Canada). Tel.: 1 416 586 4524. Fax: 1 416 586 8869. E-mail: wang@ mshri.on.ca

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.

---

*: 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 2000,
a total of 149 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 8th through the 10th of October, 2001, at the Instituto Juan March.

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

There is a limited edition of 450 copies of this volume, available free of charge.