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Functional diversity and mechanisms of action of the semaphorins

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Summary

The second EMBO workshop on 'Semaphorin function and mechanisms of action', held in the gorgeous surroundings of the 12th Century Abbaye des Vaulx de Cernay near Paris, France this May, brought together a wide range of scientists working in diverse systems with a common interest: the semaphorins. Emerging new themes discussed at the meeting included the recognition of an increasingly complex way in which different cells regulate responsiveness, and the significance of considering semaphorins in the pathology of various diseases.

Introduction

At the outset of scientific interest in the semaphorins, they tended to be thought of as growth cone 'collapsing factors' or as inhibitory guidance cues that are essential for nervous system development. This important, but somewhat limited, view of these molecules has since evolved, as semaphorins also play pivotal roles in the immune and vascular system, control the movement of neural crest cells, regulate cardiac and skeletal development, and are involved in tumour growth and cancer cell metastasis. Correspondingly, research into semaphorins has expanded significantly over recent years, and, only 10 years after the founding members of this group 'collapsin' and 'fasciclin IV' were identified (Kolodkin et al., 1992; Luo et al., 1993), the first EMBO workshop was held on Corsica in 2003. In May 2008, Valerie Castellani (University of Lyon, France), Alain Chedotal (Institute de la Vision, Paris, France) and Alex Kolodkin (Johns Hopkins University School of Medicine, Baltimore, MD, USA) organised the next EMBO meeting in this field and gathered together scientists involved in analysing the various aspects of semaphorin biology in the beautiful setting of the Abbaye des Vaulx de Cernay (see Fig. 1).

The closing lecture of the meeting given by Hajime Fujisawa provided a very insightful account of the discovery of the semaphorin receptors, neuropilin and plexin, and their function in neural development. Now a Professor Emeritus of Nagoya University, Fujisawa has played a central role in the foundation of research into these membrane proteins. When neuropilin 1 was identified as an essential binding receptor for Class III semaphorins (He and Tessier-Lavigne, 1997; Kolodkin et al., 1997), Fujisawa had already recognized its function as a cell surface protein (the A5 antigen) that is expressed on specific subsets of axons in the developing *Xenopus* nervous system (Fujisawa et al., 1990; Takagi et al., 1991; Takagi et al., 1987). As it turned out, his B2 antigen was later identified as a plexin. Since then, more components of functional semaphorin receptor complexes have been identified, mediating a diverse range of responses in different cell types, which was a recurring topic of discussion at this interesting meeting.

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Regulating responsiveness at the receptor level

Fanny Mann (Development Biology Institute of Marseille, Luminy, France) reported on the divergent cellular responses evoked by Sema3E (see Fig. 2 for more on semaphorin nomenclature), which is known to require the plexin D1 receptor and can function – unusually for Class III semaphorins – independently of neuropilins. Her previous work in mice indicates that neurons of the subiculum, which form the major output tract of the hippocampus, and cortical neurons use plexin D1 to transduce Sema3E signalling. However, the presence of neuropilin 1 in subicular neurons robustly transforms the repulsive signal mediated by plexin D1 into an attractive one (Chauvet et al., 2007). A convincing investigation by Mann into the downstream signalling stirred interest in this curious Class III semaphorin. She reported that, in cortical neurons, repellent Sema3E antagonises the phosphorylation of Akt and of Gsk3 (glycogen synthase kinase 3) through the downregulation of PI3K (phosphoinositide 3-kinase), involving the intrinsic RasGAP (Ras GTPase activating protein) activity of plexin D1. In subiculum neurons, however, Mann found that the effect of Sema3E on increasing neurite length appeared to be independent of the plexin-RasGAP activity, which may highlight the presence of an alternative transducing receptor for Sema3E attractive responses. Her favoured candidate is Vegfr2 (vascular endothelial growth factor receptor 2), which she showed is readily expressed in the neurons of the subiculum. Evidence for a further bifunctional role of Class III semaphorins was presented by Jeroen Pasterkamp (University Medical Centre Utrecht, The Netherlands). His careful analysis of the formation of the mesodiencephalic dopamine system in mice revealed that neurons of the ventral tegmental area (VTA) appear to be distinct, not only on the anatomical, but also on the molecular, level. For example, whilst rostrally located VTA neurons are repelled by Sema3C, Sema3F evokes attractive responses in this specific neuronal population. By contrast, Sema3F mediates the repulsion of caudally located VTA neurons. Not only do Pasterkamp's results exemplify another semaphorin that exerts dual responses, they will undoubtedly create invaluable information on the development of a key neuronal circuit that is affected in neurodegenerative and neurodevelopmental diseases, such as Parkinson's disease and schizophrenia. Oded Behar (The Hebrew University, Israel) focused his talk on a completely different branch of responses that can be evoked by semaphorins, the induction of neuronal apoptosis. In mice, loss of plexin A3 causes the complete failure of Sema3A to induce death responses in dorsal root ganglion (DRG) neurons, whilst – at the same time – Sema3A responses in the collapse assay are preserved. His work suggests that Sema3A-mediated cell death and guidance in the same cell type requires different functional receptor complexes.

Repulsive semaphorins expressed in the vicinity of larger bundles of axons, the axonal fascicles, have classically been thought to function by creating an inhibitory territory, which actively pushes axons to extend in tight bundles. It was surprising, then, to hear Mary Halloran (University of Wisconsin-Madison, WI, USA) introduce the idea that Sema3D regulates axon-axon interaction in a slightly different way. She investigates the pathfinding of axons

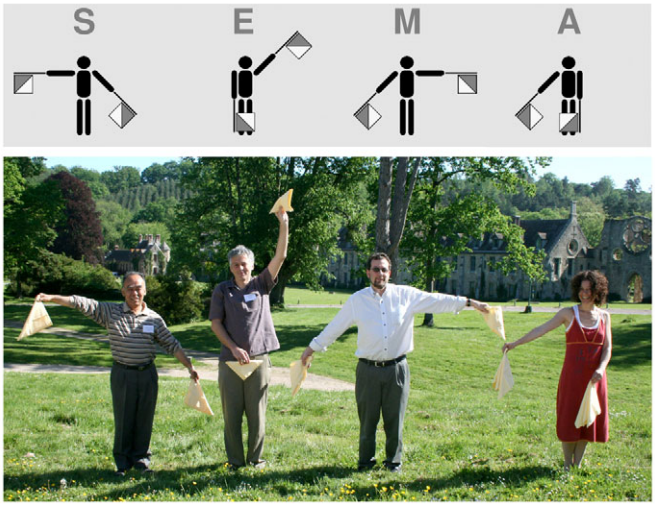

Fig. 1. Flagging the Sema code. From the left, Hajime Fujisawa and the three meeting organisers Alain Chedotal, Alex Kolodkin and Valerie Castellani in front of the Abbaye des Vaulx de Cernay, where the second EMBO workshop on 'Semaphorin function and mechanisms of action' was held. Photo courtesy of B.J.E.

that form the medial longitudinal fascicle (MLF, see Fig. 3D) in zebrafish, a major axon tract that connects the midbrain and the spinal cord. In this system, posteriorly located neurons extend axons first, whereas subsequent axons move on preformed fascicles. MLF neurons express the zebrafish neuropilin 1A and the cell adhesion molecule (CAM) L1, whilst Sema3D expression borders their trajectory. This expression pattern has led to the idea that Sema3D might promote MLF fasciculation by repulsion. Unexpectedly, patches of Sema3D, expressed ectopically in the MLF path, were insufficient to divert axons. Halloran's analysis reveals that Sema3D affects fasciculation by regulating the levels of L1 expression and axon-axon adhesion. Whilst morpholino knockdown of Sema3D reduces L1 expression, its overexpression increases the surface expression of L1 in MLF axons (Wolman et al., 2007). In addition to L1, other CAMs, such as Tag1 (transiently expressed axonal glycoprotein 1), might be required for proper axon-axon interactions and for the guidance of MLF neurons (Wolman et al., 2008), demonstrating that the formation of even a relatively simple tract is governed by the concerted action of numerous components. Halloran's work is undoubtedly of wider significance, as it promises to inform the investigation of other systems. Improper fasciculation may turn out to be the underlying cause of several defects frequently referred to as 'guidance defects'.

The midline never fails to attract

It is not surprising that guidance mechanisms involved at the ventral midline of the developing spinal cord have become a focus of the molecular analysis of semaphorin function. Axons of the dorsally located commissural neurons travel ventrally, leading them to an intermediate target – the floor plate – before turning sharply in the ventral funiculus of the spinal cord white matter. Secreted Sema3B, which is expressed in the floor plate, has previously been shown to be essential for regulating the proper guidance of commissural axons during and after their crossing of the floor plate (Zou et al., 2000), and work presented by Homaira Nawabi (University of Lyon, France) focussed on the cellular regulation of Sema3B in the mouse spinal cord. Based on the observation that commissural neurons gain sensitivity to Sema3B only following incubation with floor plate-conditioned medium, Nawabi analysed expression of the receptors that are likely to be involved in this switch. Her data show that plexin A1 appears to be present in the distal segment of commissural axons only, precisely from the moment when axons traverse the floor plate, which indicates a potential involvement of this plexin in increasing the sensitivity of axons to Sema3B. In her search for an underlying mechanism that controls this localised expression, Nawabi's work suggests that plexin A1 is a target of intra-neuronal proteolytic cleavage in pre-crossing axons. Sharply at the floor plate, this cleavage activity is inhibited, and commissural neurons gain plexin A1 expression and responsiveness to Sema3B.

Work presented by Greg Bashaw (University of Pennsylvania School of Medicine, PA, USA) highlighted another way in which midline crossing is co-ordinated by a tightly woven network of different cellular events. In the *Drosophila* midline, Commissureless (Comm) controls the midline crossing of commissural neurons through the regulation of the Roundabout (Robo) receptor on pre-crossing axons. Much progress has been made in the analysis of Comm expression and function, and the effect it has on Robo. By contrast, much less is known concerning the regulation of Comm itself. Here, Bashaw made the intriguing observation that mRNA levels of Comm are affected in flies deficient in the attractive Netrin receptor Frazzled. As a consequence, one would naturally propose that the Netrin/Frazzled interaction positively regulates Comm transcription and midline crossing. However – in a finding that shows the midline remains full of surprises – *NetA*/*NetB* double mutant flies exhibit no reduction in Commissureless mRNA, suggesting that the Netrin receptor Frazzled fulfils a dual purpose, evoking Netrin-dependent responses (axon attraction) and Netrin-independent responses (activation of Commissureless transcription). One wonders if Semaphorins or their receptors will also be identified as being mediators of transcriptional activity in the context of axon guidance.

Compartmentalisation of semaphorin transducers in time and space

Much of what we currently understand about the signalling mechanisms that are activated by semaphorins is based on extended biochemical analysis and, in many cases, the analysis of cellular systems that exploit techniques that force the overexpression (activation) or the loss of expression (activity) of specific components of a signalling pathway. Along this vein, the work presented by Manabu Negishi (Kyoto University, Japan) analysed the signalling events downstream of Sema4D/plexin B1 hippocampal neurons of the rat. Following ligand stimulation, he reported on the rapid loss of (activatory) phosphorylated Akt and (inhibitory) phosphorylated Gsk3, which regulates Crmp2 (collapse response mediator protein 2), thereby potentially affecting microtubule dynamics (Ito et al., 2006). Although it has been proposed that similar signalling relationships mediate responses by Sema3A (Chadborn et al., 2006; Eickholt et al., 2002) and Sema3E (as presented in the talk by Fanny Mann), the question of how Akt/Gsk3 is controlled provoked some controversy at the meeting. Unquestionably, the intrinsic GTPase activity of plexin B1 (which is found in all plexin family members A-D) and the activity of its substrate R-Ras are crucial in antagonising PI3K upstream of Akt. However, all three (Negishi, Eickholt and Mann) agreed that the 3-phosphatase PTEN is also an essential component of the regulation of semaphorin signalling and its functional responses. Whilst Negishi's work proposes that C-terminal phosphorylation of PTEN regulates the activity of the phosphatase in his specific system,

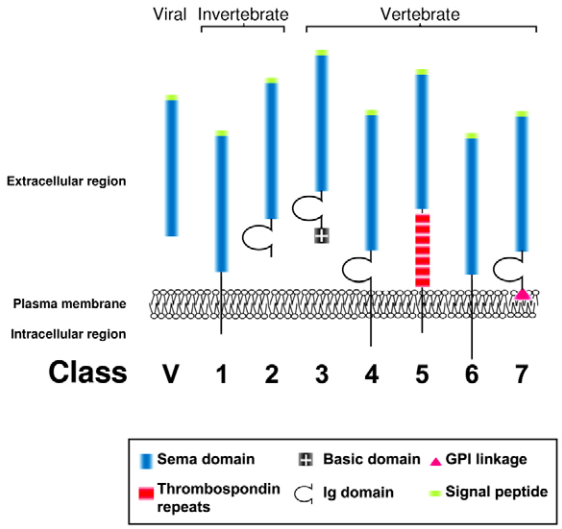

Fig. 2. The semaphorin family of proteins. The known members of the semaphorin family have been categorised into 8 classes (V-7). All semaphorins share a ~500 amino acid semaphorin (Sema) domain, which is followed, in some classes, by a single Ig-like domain. Several members of the semaphorin family are secreted molecules with no membrane attachment site (for example Class 2 and Class 3 semaphorins), whereas others are linked to the cell surface by a transmembrane domain or by a GPI anchor. One subfamily, the Class 5 semaphorins, contains a set of thrombospondin type I repeats. Adapted, with permission, from the Semaphorin Nomenclature Committee (Semaphorin Nomenclature Committee, 1999).

Eickholt's presentation proposed a model in which changes in the subcellular distribution of PTEN is important in regulating responsiveness. Clearly, further analysis is required to fully comprehend the control of PTEN in the semaphorin pathway, which promises to be an exciting avenue for future work. James Zheng (University of Medicine and Dentistry of New Jersey, NJ, USA) presented a case for the compartmentalisation of signalling being crucial for semaphorin responses. He concentrated on the 'A kinase anchoring proteins' (AKAPs), which function as a molecular scaffold and can anchor enzymes, bringing them into close proximity with their respective effectors (and/or affectors). Zheng has shown previously, for example, that the spatial targeting of PKA to growth cone filopodia is mediated by AKAP and that interference with this association impairs cAMP-mediated attractive turning responses (Han et al., 2007). AKAP is also important for the Sema3A-mediated repulsion of *Xenopus* growth cones. However, Zheng's result suggests that its involvement is independent of PKA and potentially involves the ERM (ezrin, radixin and moesin) proteins.

Semaphorins control protein synthesis in axon guidance and morphogenesis

Two talks demonstrated the ability of semaphorins to regulate the translation of specific subsets of mRNA, and it was striking that in two fairly divergent systems – *Xenopus* retinal ganglion cell growth cones and epithelial rays of the nematode worm – a common functional target of semaphorin function is the cell's translational machinery. In the first of these talks, Christine Holt (Cambridge University, UK) investigated the possibility that specific sets of protein are translated in the growth cone in response to guidance cues, including Sema3A. Indeed, her earlier work has provided evidence that protein synthesis is required for Sema3A growth cone collapse (Piper et al., 2006). One likely target of localised Sema3A-induced translation in growth cones is the actin filament severing factor ADF/Cofilin. She suggests that the Sema3A-induced, spatially restricted synthesis of ADF/Cofilin may distort normal actin dynamics sufficiently in order to induce collapse responses. Akira Nukazuka (Nagoya University, Japan) discussed similar findings from his work on semaphorin function during ray morphogenesis in *Caenorhabditis elegans*. Each ray is composed of four cells, the hypodermis, a structural cell and two neuronal cells, and ray assembly requires the two worm semaphorins SMP-1/SMP-2 and their Plexin receptor, Plexin 1. From an unbiased screen for suppressors of the *plexin 1*$^{-/-}$ ray phenotype, Nukazuka isolated a negative regulator of translation initiation, GCN-1, which inhibits mRNA translation initiation by participating in the phosphorylation of eIF2α (eukaryotic translation initiation factor 2α). To investigate whether semaphorin regulates eIF2α phosphorylation in vivo, he then used the power of worm genetics. He expressed Flag-eIF2α specifically in rays and analysed phosphorylation levels following the retrieval of the Flag-tagged proteins. His results show that *plexin 1/Smp-1/Smp-2* mutants have substantially elevated levels of phosphorylated eIF2α in their rays compared with wild-type worms (Nukazuka et al., 2008). As the knockdown of ADF/cofilin phenocopies *plexin 1/Smp-1/Smp-2* mutants, it appears that the actin-severing protein may be a key target of semaphorin-induced translation, as in the research presented by Holt. Although it is difficult to see how rapid changes in growth cone dynamics and a morphogenic programme use similar signalling systems, the overlap in results is compelling. It remains to be seen, however, if the translation of specific subsets of mRNA, especially those involved in cytoskeletal regulation and the control of growth cone motility, contribute to the assembly of neuronal circuits in vivo.

Shaping neuronal circuits

The talks discussed thus far portrayed the multifaceted way in which semaphorins use cellular mechanisms to guide axons to their appropriate synaptic targets. But their involvement in assembling proper neuronal circuits does not stop there. It is apparent that semaphorins also regulate synaptogenesis, dendrite morphogenesis, and the removal (pruning) of excess axons. In this context, David Ginty (Johns Hopkins University School of Medicine, USA) provided invaluable information on the function of Sema3A and Sema3F during cortical and hippocampal circuit formation. His work – a collaboration with Alex Kolodkin – shows that *Sema3a*$^{-/-}$ and neuropilin 1$^{-/-}$ mice exhibit defects in the elaboration of basal dendrites in the cortex, whilst apical dendrites appear normal. Similar phenotypes are seen in plexin A4$^{-/-}$ mice, suggesting that Sema3A exerts its function on cortical dendrite development through a neuropilin 1/plexin A4 receptor complex. This is in contrast to the defects that occur in *Sema3f*$^{-/-}$ or neuropilin 2$^{-/-}$ mice, which exhibit striking increases in the number and length of dendritic spines in granule cell neurons of the hippocampus, a phenotype also present in layer-5 pyramidal neurons of the cortex. Electron microscopy analysis reveals the presence of spines with enlarged post-synaptic densities that appear to form multiple synapses. The loss of plexin A3 phenocopies this defect, revealing that Sema3F controls synapse development through neuropilin 2 and plexin A3. Thus, Sema3A appears to control cortical neuronal morphogenesis, regulating appropriate basal dendrite development, whereas Sema3F signalling restricts the growth of dendritic spines.

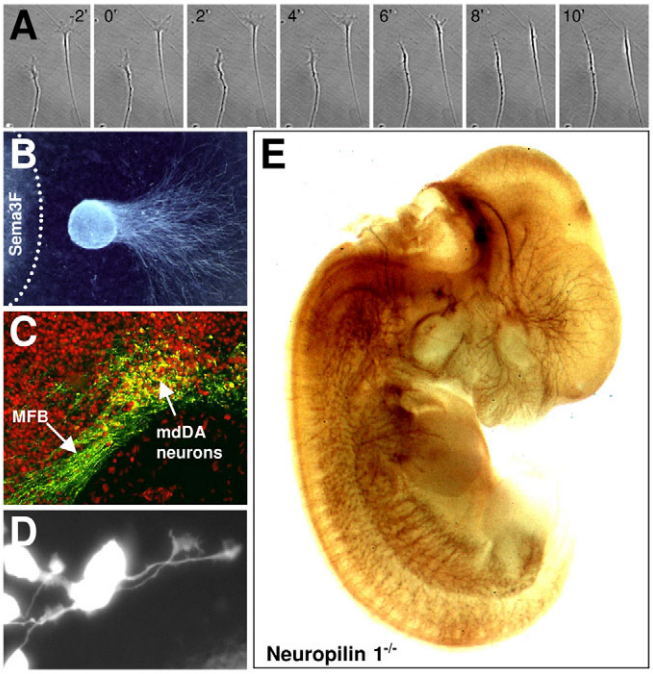

Fig. 3. Semaphorin responses in different neuronal systems. (**A**) Bath application of embryonic chick dorsal root ganglion (DRG) neurons with Sema3A induces a rapid growth cone collapse response. Picture sequence shows images that were taken 2 minutes before and every 2 minutes after the application of Sema3A at 0 minutes (0′). Image courtesy of B.J.E. (**B**) Sema3F, expressed in Cos-cells, induces strong repulsion of axons extending from a rat hippocampal explant in a collagen gel assay. Image courtesy of A. Chedotal. (**C**) Mesodiencephalic dopaminergic (mdDA) neurons in the ventral tegmental area project their axons (green) in the medial forebrain bundle (MFB) rostroventrally through the developing diencephalon and telencephalon to innervate the prefrontal cortex (PFC). Innervation of the PFC is controlled by Class III semaphorins and their neuropilin receptors. Image courtesy of J. Pasterkamp. (**D**) Growth cones of the medial longitudinal fascicle (MLF) imaged in live transgenic zebrafish embryos. Axons extend normally in tightly bundled fascicles, whereas loss of either Sema3D or Tag1 disrupts fasciculation. Shown here is a morpholino knockdown of Tag1. Image courtesy of M. Halloran. (**E**) Side view of a neuropilin $1^{-/-}$ mouse embryo at E12.5 labeled with anti-neurofilament antibody. Loss of neuropilin 1 results in abnormal targetting of efferent projecting neurons. Image courtesy of H. Fujisawa.

The talk presented by Hwai-Jong Cheng (University of California at Davis, CA, USA) provided a neat example of how different semaphorins function in a context-dependent fashion that is important at later stages of development during circuit formation. His model system, the corticospinal tract (CST) of mice, is characterised by the presence of several transitory connections that are established in inappropriate locations. For example, a transient component of the developing CST arises in the visual cortex, whereas pyramidal neurons from the motor cortex make connections with the superior colliculus of the visual system. Cheng finds that in plexin A3/plexin A4 double mutant mice, the visual CST fails to be pruned, with un-pruned neurons maintaining synaptic contacts in the spinal cord. By contrast, the pruning of the motor corticospinal component in these mice is not affected. His results further suggest that the stereotyped pruning of the visual CST is likely to be regulated by Sema3F (Low et al., 2008).

The development of two other circuits – the limbic and the cortical circuits – was discussed by Kevin Mitchell (Trinity College Dublin, Ireland). He performed a careful comparative expression analysis of Sema6A, plexin A2 and plexin A4, and related these data with the phenotypes that occur as a result of loss of these components in mice. His data indicate that Sema6A and plexin A2/plexin A4 are often co-expressed, and that the phenotypes of Sema6A and plexin A2/plexin A4 mutant mice are not always suggestive of a classical ligand-receptor relationship. Indeed, there is evidence that Sema6A may be involved in bidirectional signaling and that interactions in cis may be important in vivo. Single plexin A mutant phenotypes also tend to reflect Sema6 function more than Sema3 function. Sema6A mutants exhibit widespread defects in cell migration and axon guidance, including some that directly parallel pathological changes observed in schizophrenia, for example, a reduction and decreased fasciculation of the fornix, and altered thalamocortical connectivity, which leads Mitchell to propose that Sema6A mutants might serve as a model for the study of this psychiatric disorder. Using EEG recordings, Mitchell showed that Sema6A-null mice exhibit increased brain activity, which is blocked by clozapine, an antipsychotic drug for treating schizophrenia. Behavioral defects in these mice also include hyperlocomotion (again reversible by clozapine), altered social interaction and decreased anxiety.

Liqun Luo (Stanford University, CA, USA) summarised the function of Sema-1a and Sema-2a in the wiring of olfactory circuits in *Drosophila*. In this system, olfactory sensory receptor neurons (ORNs) in the antenna project to the antennal lobe in a highly organized fashion, and connect with distinct synaptic glomeruli in the central nervous system. His work demonstrates that the transmembrane semaphorin Sema-1a is required for the proper axon targeting of a subset of ORNs, mediating axonal segregation most likely through axon-axon repulsion (Sweeney et al., 2007). In addition, there exists a graded distribution of Sema-1a in the antennal lobe, where it acts as a receptor and instructs the targeting of the dendrites of olfactory projection neuron (PNs) (Komiyama et al., 2007). Brain-derived secreted Sema-2a, however, appears to be required for ORN axon targeting.

Functions of semaphorins in cancer and the immune system

Several participants discussed the role of semaphorins in tumour progression, cancer cell metastasis, and in immune responses, which will be summarised – given the interests of the readership of *Development* – only briefly here. Semaphorins have become a major target for the development of therapeutics for treating malignancies and autoimmune diseases. Gera Neufeld's talk (Israel Institute of Technology, Israel) was oriented by questions as to why Sema3B, expressed in HEK293 or cancer cells, exerts relatively weak repulsion on endothelial cells. He finds that the weak response is caused by an inactivation of Sema3B through furin-like pro-convertase-dependent cleavage of the protein. A furin-resistant Sema3B exerts inhibitory function on endothelial cell tube formation, which identifies Sema3B as an anti-angiogenic factor. Given that furin activities are increased in a number of cancers, this work highlights an example in which cancer cells have adopted strategies that enable tumour progression by overcoming factors that inhibit angiogenesis. Neufeld's presented work also identified additional Class III semaphorins, including Sema3A, Sema3D, Sema3E and Sema3G, as being anti-tumorigenic factors with anti-angiogenic properties.

In his presentation, Luca Tamagnone (University of Torino, Italy) considered a mechanism that clarified other questions concerning the function of Sema3B. While Sema3B has been classified as a putative tumour suppressor gene, there is no clear correlation between loss of Sema3B and tumour development. As a matter of fact, he finds that although Sema3B overexpression delays tumour growth in nude mice, it actually increases metastatic dissemination to the lungs. Notably, Sema3B is ineffective in increasing the motility or invasiveness of cancer cells in vitro. Here, an indirect mechanism, involving Sema3B-induced changes of the tumour microenvironment solves the controversy. Tamagnone shows that Sema3B expression increases the production of Il8 (interleukin 8) by cancer cells, a cytokine that is known to regulate infiltrating leucocytes and endothelial cells in the tumour stroma and to promote metastatic progression (Rolny et al., 2008).

Anil Bagri (Genentech, CA, USA) presented an exceptionally promising therapeutic approach for the treatment of malignancies. The lymphatic vasculature is an important route for the distribution of metastasising cancer cells, and a key factor that controls the sprouting of lymphatic vessels is Vegfc. Neuropilin 2 functions as a Vegfc co-receptor and, thus, interfering with this receptor was hypothesised to impede the formation of lymphatics that is associated with tumours. As the Vegf association with neuropilin 2 is mediated by the b1/b2 domain of neuropilin 2 (which is not targeted by semaphorins), the Genentech group generated a high-affinity antibody specific to this domain. The results offered by Bagri demonstrate that anti-neuropilin 2 treatment is effective in inhibiting the formation of functional lymphatics associated with tumours in mice, thereby attenuating the development of metastasis (Caunt et al., 2008). However, because treatment with the antibody did not cause a significant reduction in tumor size, a combined use of this therapeutic tool in association with tumor growth-inhibiting drugs may be warranted. This notwithstanding, the talk signifies that neuropilin 2 is an excellent target for modulating metastasis in humans.

Hitoshi Kikutani (Osaka University, Japan) presented his work on investigating the immune responses that are mediated by Sema4A and the GPI-anchored Sema7A – an, as yet, less-characterised semaphorin that has previously been found to promote axon outgrowth through β1 integrin receptors (Pasterkamp et al., 2003). Through functional α1/β1 integrins, Sema7A functions as a potent stimulator of monocytes and macrophages. Kikutani demonstrates further that α1 integrin-deficient macrophages exhibit reduced responses to Sema7A, and that *Sema7A*$^{-/-}$ mice are defective in cell-mediated immune responses, including experimental autoimmune encephalomyelitis, in the presence of normal T-cell development and migration. Because *Sema7A*$^{-/-}$ T-cells fail to induce contact hypersensitivity, Kikutani's work suggests that Sema7A functions locally at the site of inflammation (Suzuki et al., 2007). The loss of Sema4A in immune cells, by contrast, leads to the impaired differentiation of type 1 helper T-lymphocytes, and a fraction of *Sema4A*$^{-/-}$ mice spontaneously develop atopic dermatitis-like skin lesions.

Conclusion

In conclusion, the organisers of the meeting provided an excellent and stimulating programme, which clearly highlighted the current and emerging trends in the field. Undoubtedly, they achieved their goal in fostering discussion, exchanging ideas and facilitating the establishment of new collaborations among scientists working on different experimental systems involving semaphorins. Such 'mixed system – same molecule' conferences are extremely valuable in this respect, and also support the development and the dissemination of tools in the field. We certainly look forward to the next semaphorin workshop, not least because it might involve clarification of some of the interesting and divergent findings and views prefigured by this one.

I would like to thank all of the participants in this conference for their stimulating discussions. I am very grateful to all of the speakers discussed for their permission to reproduce their work and for their helpful feedback. Unfortunately, due to space limitations, I was unable to include all of the presentations in this report. Special thanks are due to Luca Tamagnone and Alex Kolodkin for commenting on the text.

References

Caunt, M., Mak, J., Liang, W. C., Stawicki, S., Pan, Q., Tong, R. K., Kowalski, J., Ho, C., Reslan, H. B., Ross, J. et al. (2008). Blocking neuropilin-2 function inhibits tumor cell metastasis. *Cancer Cell* **13**, 331-342.

Chadborn, N. H., Ahmed, A. I., Holt, M. R., Prinjha, R., Dunn, G. A., Jones, G. E. and Eickholt, B. J. (2006). PTEN couples Sema3A signalling to growth cone collapse. *J. Cell Sci.* **119**, 951-957.

Chauvet, S., Cohen, S., Yoshida, Y., Fekrane, L., Livet, J., Gayet, O., Segu, L., Buhot, M. C., Jessell, T. M., Henderson, C. E. et al. (2007). Gating of Sema3E/PlexinD1 signaling by neuropilin-1 switches axonal repulsion to attraction during brain development. *Neuron* **56**, 807-822.

Eickholt, B. J., Walsh, F. S. and Doherty, P. (2002). An inactive pool of GSK-3 at the leading edge of growth cones is implicated in Semaphorin 3A signaling. *J. Cell Biol.* **157**, 211-217.

Fujisawa, H., Takagi, S. and Hirata, T. (1990). Cell surface molecule A5: a putative involvement in retinal central connection. *Neurosci Res. Suppl.* **13**, S11-S17.

Han, J., Han, L., Tiwari, P., Wen, Z. and Zheng, J. Q. (2007). Spatial targeting of type II protein kinase A to filopodia mediates the regulation of growth cone guidance by cAMP. *J. Cell Biol.* **176**, 101-111.

He, Z. and Tessier-Lavigne, M. (1997). Neuropilin is a receptor for the axonal chemorepellent Semaphorin III. *Cell* **90**, 739-751.

Ito, Y., Oinuma, I., Katoh, H., Kaibuchi, K. and Negishi, M. (2006). Sema4D/plexin-B1 activates GSK-3beta through R-Ras GAP activity, inducing growth cone collapse. *EMBO Rep.* **7**, 704-709.

Kolodkin, A. L., Matthes, D. J., O'Connor, T. P., Patel, N. H., Admon, A., Bentley, D. and Goodman, C. S. (1992). Fasciclin IV: sequence, expression, and function during growth cone guidance in the grasshopper embryo. *Neuron* **9**, 831-845.

Kolodkin, A. L., Levengood, D. V., Rowe, E. G., Tai, Y. T., Giger, R. J. and Ginty, D. D. (1997). Neuropilin is a semaphorin III receptor. *Cell* **90**, 753-762.

Komiyama, T., Sweeney, L. B., Schuldiner, O., Garcia, K. C. and Luo, L. (2007). Graded expression of semaphorin-1a cell-autonomously directs dendritic targeting of olfactory projection neurons. *Cell* **128**, 399-410.

Low, L. K., Liu, X.-B., Faulkner, R. L., Coble, J. and Cheng, H.-J. (2008). Plexin signaling selectively regulates the stereotyped pruning of corticospinal axons from visual cortex. *Proc. Natl. Acad. Sci. USA* **23**, 8136-8141.

Luo, Y., Raible, D. and Raper, J. A. (1993). Collapsin: a protein in brain that induces the collapse and paralysis of neuronal growth cones. *Cell* **75**, 217-227.

Nukazuka, A., Fujisawa, H., Inada, T., Oda, Y. and Takagi, S. (2008). Semaphorin controls epidermal morphogenesis by stimulating mRNA translation via eIF2alpha in Caenorhabditis elegans. *Genes Dev.* **22**, 1025-1036.

Pasterkamp, R. J., Peschon, J. J., Spriggs, M. K. and Kolodkin, A. L. (2003). Semaphorin 7A promotes axon outgrowth through integrins and MAPKs. *Nature* **424**, 398-405.

Piper, M., Anderson, R., Dwivedy, A., Weinl, C., van Horck, F., Leung, K. M., Cogill, E. and Holt, C. (2006). Signaling mechanisms underlying Slit2-induced collapse of Xenopus retinal growth cones. *Neuron* **49**, 215-228.

Rolny, C., Capparuccia, L., Casazza, A., Mazzone, M., Vallario, A., Cignetti, A., Medico, E., Carmeliet, P., Comoglio, P. M. and Tamagnone, L. (2008). The tumor suppressor semaphorin 3B triggers a prometastatic program mediated by interleukin 8 and the tumor microenvironment. *J. Exp. Med.* **205**, 1155-1171.

Semaphorin Nomenclature Committee (1999). Unified nomenclature for the semaphorins/collapsins. *Cell* **97**, 551-552.

Suzuki, K., Okuno, T., Yamamoto, M., Pasterkamp, R. J., Takegahara, N., Takamatsu, H., Kitao, T., Takagi, J., Rennert, P. D., Kolodkin, A. L. et al. (2007). Semaphorin 7A initiates T-cell-mediated inflammatory responses through alpha1beta1 integrin. *Nature* **446**, 680-684.

Sweeney, L. B., Couto, A., Chou, Y. H., Berdnik, D., Dickson, B. J., Luo, L. and Komiyama, T. (2007). Temporal target restriction of olfactory receptor neurons by Semaphorin-1a/PlexinA-mediated axon-axon interactions. *Neuron* **53**, 185-200.

Takagi, S., Tsuji, T., Amagai, T., Takamatsu, T. and Fujisawa, H. (1987). Specific cell surface labels in the visual centers of Xenopus laevis tadpole identified using monoclonal antibodies. *Dev. Biol.* **122**, 90-100.

Takagi, S., Hirata, T., Agata, K., Mochii, M., Eguchi, G. and Fujisawa, H. (1991). The A5 antigen, a candidate for the neuronal recognition molecule, has homologies to complement components and coagulation factors. *Neuron* **7**, 295-307.

Wolman, M. A., Regnery, A. M., Becker, T., Becker, C. G. and Halloran, M. C. (2007). Semaphorin3D regulates axon axon interactions by modulating levels of L1 cell adhesion molecule. *J. Neurosci.* **27**, 9653-9663.

Wolman, M. A., Sittaramane, V. K., Essner, J. J., Yost, H. J., Chandrasekhar, A. and Halloran, M. C. (2008). Transient axonal glycoprotein-1 (TAG-1) and laminin-alpha1 regulate dynamic growth cone behaviors and initial axon direction in vivo. *Neural Develop.* **3**, 6.

Zou, Y., Stoeckli, E., Chen, H. and Tessier-Lavigne, M. (2000). Squeezing axons out of the gray matter: a role for slit and semaphorin proteins from midline and ventral spinal cord. *Cell* **102**, 363-375.