Review

Weaving the neuronal net with target-derived fibroblast growth factors

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Fibroblast growth factors (FGFs) are a large family of secreted growth factors that are involved in the development, regeneration and repair of various tissues. In the nervous system, FGFs have been implicated in early developmental processes, such as neural induction, proliferation and patterning. Accumulating data indicate that FGFs are also important for the formation of functional neural networks. The role of FGFs in axon guidance, target recognition and synaptic differentiation as target-derived factors, and how they cooperate with cell adhesion molecules that are also involved in the wiring of the nervous system are the focus of this review.

Key words: axon guidance, cell adhesion molecules, fibroblast growth factors, synaptic differentiation, target recognition.

Introduction

Fibroblast growth factors (FGFs) are a family of inter-cellular signaling molecules (encoded by 22 genes in humans and mice) that signal through a set of four distinct receptor tyrosine kinases (FGFRs; reviewed in Ornitz & Itoh 2001). FGFs have been implicated in the regulation of a wide range of processes, including cell proliferation, migration, differentiation, tissue repair, and response to injury in almost all organs (reviewed in Ornitz & Itoh 2001; Thisse & Thisse 2005). In the nervous system, at least 14 FGFs and all four FGFRs are expressed, and they have been shown to play critical roles during early development, including neural induction, neural patterning, neuronal proliferation and survival, neuroprotection and placode development (reviewed in Dono 2003; Reuss & von Bohlen und Halbach 2003; Sato *et al*. 2004; Mason 2007).

This variety of FGF functions appears to be mediated by a collaboration of various factors: (i) expression of 22 FGFs; (ii) seven main receptors, which are generated by alternative splicing. FGFs exhibit different but

Table 1. Members of fibroblast growth factor (FGF) family and their high affinity FGF receptors (FGFRs). FGFRs contain three immunoglobulin (Ig) domains in the extracellular region and a tyrosine kinase domain in the intracellular region. The third Ig domain is alternatively spliced to generate b or c isoforms. Receptors that showed strong binding to FGFs are shown in the table (Ornitz *et al*. 1996; Zhang *et al*. 2006) FGF11-14 do not bind to any FGFRs and are now called FGF homologous factors (FHFs)

FGF	High affinity FGFR
1, 2	All
3, 7, 10, 22	2b, 1b
4, 5, 6	1c, 2c, 4
8, 17, 18	3c, 4, 2c, 1c
9, 16, 20	3c, 2c, 3b, 1c
15/19, 21, 23	All (weak)
11, 12, 13, 14 (FHF1-4)	No binding

overlapping receptor binding specificity (Table 1; Ornitz *et al*. 1996; Zhang *et al*. 2006); (iii) heparan sulfate proteoglycans (HSPGs). HSPGs are obligate co-factors that modify affinity and specificity of FGF binding to the receptor (Mason 2007); (iv) modifications of FGF and FGFR by glycosylation (e.g. Beer *et al*. 1997 for FGF10 glycosylation); (v) FGFR-interacting molecules such as N-Cadherin, NCAM, L1-CAM (see below), EphA4 (Yokote *et al*. 2005) and neuropillin (West *et al*. 2005) that modify FGFR activity; as well as (vi) multiple intracellular signaling pathways, which result in different gene expression events. Thus, the activity of FGFs and the cellular responses to FGFs are regulated at multiple

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levels by a number of different factors. This variety makes FGFs prime candidates to control specific synaptic connections in the nervous system.

In the nervous system, neurons connect with their target cells (neurons, muscles, etc.) to transmit information. The sites of information transduction are called synapses. Information is transferred when neurotransmitters released from presynaptic vesicles are received by receptors in the postsynaptic membrane. Alterations in synaptic formation and strength are critical for neural plasticity, both under adaptive conditions, such as learning and memory, and under maladaptive states, such as neurological disorders. Thus, proper and specific synapse formation is essential for the optimal functioning of the nervous system.

Several steps are involved for generating the complex and specific patterns of neural connections (Fig. 1). First, axons from presynaptic neurons are guided and attracted to their postsynaptic target cells (Fig. 1A; Axon guidance). After the axon reaches the target, signals from the postsynaptic cell instruct the axon to recognize and adhere to the correct area of the target cell (Fig. 1B; Target recognition; reviewed in Benson *et al*. 2001; Yamagata *et al*. 2003). If a specific connection between appropriate synaptic partners is confirmed, synaptic differentiation will commence. Signals from the presynaptic axon and the target cell induce the differentiation of their respective synaptic partner cells so that each side of the synapse can carry out its role during synaptic transmission (Fig. 1C; Synaptic differentiation; reviewed in Scheiffele 2003; Ziv & Garner 2004; Fox & Umemori 2006). After this step, the synapse becomes functional. Subsequently, the synaptic connections are further refined by neural activity (reviewed in Goda & Davis 2003; Waites *et al*. 2005), resulting in their maturation, and are maintained. Each step is controlled by developmental signals between the pre- and postsynaptic cells to assure a proper connection. Recent findings indicate that target-derived FGFs are critical for these steps (Fig. 1).

FGF in axon guidance

After neurons are born in the nervous system, they migrate to their appropriate locations, polarize to form axons and dendrites, and start to send axons toward their targets. Various molecules, which are derived from the intermediate and final target cell, guide axons to their correct target. FGFs represent one type of such guidance molecules (Fig. 1A).

A role for FGF8 in axon guidance was suggested in the avian isthmus at the boundary between the mid- and hindbrain (MHB). Trochlear motor axons (IVth cranial nerve axons) in the hindbrain grow dorsally along the
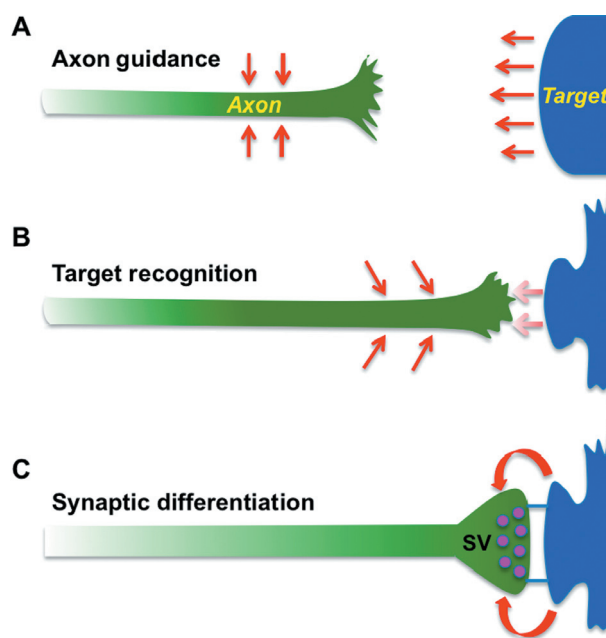

Fig. 1. Steps of synapse formation and the role of fibroblast growth factors (FGFs). For the formation of specific synaptic connections, three separate steps are involved: (A) axon guidance, (B) target recognition, and (C) synaptic differentiation. FGFs play important roles in each of these steps. (A) Axons are guided toward their target cells. FGFs participate in this step in two ways (red arrows): (i) FGFs direct the axon as *en passant* guiding molecules (e.g. FGF8); and (ii) FGFs attract axons as target-derived chemoattractants (e.g. FGF2, 4, 8, 9). (B) Axons recognize their appropriate targets as they come close to the target cells. Levels of FGFs from the target, relative to those in the axon pathway, are important for this step. In the Xenopus retinotectal system, retinal ganglion cells (RGCs) extend axons through the optic tract and form connections with neurons in the tectum. FGF2 is abundant in the optic tract, but not in the optic tectum. Axonal growth of RGCs is supported by FGF2 expressed through the optic tract. When RGC axons reach the tectum, diminished levels of FGF2 in the tectum stop RGC axons to recognize their target, the tectum. (C) Molecules are exchanged between presynaptic axons and postsynaptic target cells to form functional synapses. Postsynaptic cell-derived 'presynaptic organizers', including FGFs (e.g. FGF2, 7, 10, 22; red arrows) and cell adhesion molecules (blue lines), induce presynaptic differentiation. Synaptic vesicles (SV) are recruited to the terminal and multiple active zones (neurotransmitter release sites) are formed. The postsynaptic apparatus contains clustered neurotransmitter receptors and scaffolding molecules.

MHB and exit from the neural tube. FGF8 release in the isthmus appears to guide trochlear motor axons along the MHB by redirecting the growth of axons as an *en passant* attracting molecule (Irving *et al*. 2002).

A chemoattractant activity of target-derived FGFs was demonstrated for developing mouse motor axons (Shirasaki *et al*. 2006). The dermomyotome, from which axial muscles are formed, and FGF2, FGF4, FGF8 and

FGF9, attracted medial-class spinal motor neuron (MMCm) axons *in vitro*. These effects are blocked by an FGFR kinase inhibitor (SU5402). Expression of FGFR1, which can bind to FGF2, FGF4, FGF8 and FGF9, is restricted to MMCm motor neurons, and this expression is regulated by the LIM transcription factor Lhx3. Conditional deletion of FGFR1 in neural progenitor cells (using Nestin-Cre) resulted in motor axon guidance defects. These results indicate that FGFs serve as target (dermomyotome)-derived factors to attract specific motor axons.

A role of FGFR1 in axon guidance is also supported by work on mouse commissure formation. Commissures connect cerebral hemispheres by commissural axon tracts (corpus callosum, hippocampal commissure, anterior commissure). In telencephalon-specific knockout mice of FGFR1 (using Foxg1-Cre), commissural neurons are still present and initially project their axons, but they did not cross the midline, and the hemispheres are separated (Tole *et al*. 2006). This is due to a defect in radial glia translocation, because the commissure is disrupted in the telencephalic radial glia-specific FGFR1 deletion (using GFAP-Cre), but not in the telencephalic neural precursor-specific knockout (using Nestin-Cre) (Smith *et al*. 2006). Interestingly, *acerebellar* (*ace*) mutant zebrafish, which generate a truncated and non-functional FGF8 protein, also display defects in commissural axon (anterior commissure and postoptic commissure) pathfinding (Shanmugalingam *et al*. 2000). The exact role of FGFs in commissure formation needs to be elucidated.

In addition, in zebrafish, FGF19 is implicated in axon pathfinding. Knockdown of FGF19 caused incorrect retinal ganglion cell (RGC) guidance (Nakayama *et al*. 2008). The precise developmental expression pattern of FGF19 and its role in the RGC axon guidance are not known.

FGF in target recognition

As the axon reaches the target cell, the axon and its specific target need to recognize each other to identify that they are the right partners to connect. FGFs (more accurately, changes in the local FGF concentration) play important roles in this step (Fig. 1B) for Xenopus retinal ganglion cells (RGCs) to recognize their target, the optic tectum. RGC axons extend from retina through the optic tract to the tectum, where they form synaptic connections with tectal neurons. FGF2 is abundant in the optic tract (the pathway of RGCs), but not in the optic tectum (the target of RGCs). When FGF2 was overexpressed in the tectum, RGC axons did not stop at the tectum and bypassed it (McFarlane *et al*. 1995). This suggests that a change in FGF2 levels at the

border between the optic tract and the optic tectum serves as a target recognition cue for RGC axons. FGF2 in the optic tract supports the RGC axon growth, and when RGC axons reach the tectum, diminished levels of FGF2 slow down the growth of RGC axons to recognize the tectum. This requires FGFR kinase activity in RGCs, because transfection of a dominant-negative FGFR, which lacks the tyrosine kinase domain, into RGCs made them bypass the tectum (McFarlane *et al*. 1996). A similar bypass phenotype was observed by exogenously applying FGF2-binding heparan sulfate to the optic pathway thereby competitively inhibiting FGF2 activity. Removal of endogenous heparan sulfate also induces mis-targeting of RGC axons, suggesting that FGF2 and heparan sulfate cooperate for the target recognition (Walz *et al*. 1997). *In vitro* assays revealed that a local application of FGF2 repels RGC growth cones, supporting the idea that RGC axons sense changes in local FGF2 concentration to find their target. This repulsive activity is dependent on the phospholipase pathways that are mediated by FGFR (Webber *et al*. 2003, 2005).

FGF in synaptic differentiation

Functional presynaptic terminals accumulate synaptic vesicles, concentrate mitochondria, and possess active zones at which vesicles fuse to release neurotransmitters. Postsynaptic densities, where neurotransmitter receptors are concentrated, are found just opposed to these active zones (Fox & Umemori 2006). This perfect arrangement between active zones and postsynaptic densities, as well as the fact that presynaptic differentiation occurs only after the growth cone touches the target cell (e.g. Takahashi *et al*. 1987; Buchanan *et al*. 1989), indicate that there are postsynaptic cell-derived factors that organize presynaptic differentiation (Fig. 1C). FGFs appear to be one class of such factors.

In one *in vitro* study using Xenopus spinal cord neurons, FGF2-coated beads induced synaptic vesicle aggregations at the contact site with neurites. This was shown by both immunostaining and electron microscopy (Dai & Peng 1995). This inductive event involves tyrosine kinase activity and calcium influx (Dai & Peng 1995). FGF2 applied to rat hippocampal cultures also increased the number of puncta for presynaptic and postsynaptic proteins, without affecting the size and staining intensity of each puncta. Since FGF2 also induced neurite elongation and the branching of hippocampal neurons, the induction of synapses by FGF2 might have been the result of increased contacts between neurites (Li *et al*. 2002). Although FGF2 appears to have important roles in the proliferation and survival of various cells, FGF2-knockout mice have grossly normal

organogenesis (Zhou et al. 1998). However, neocortices of FGF2-mutant mice contain fewer neurons, possibly because of defects in the proliferation of neural progenitor cells (Dono et al. 1998; Ortega et al. 1998; Vaccarino et al. 1999). FGF2-knockout mice show a reduction in cortical glutamatergic pyramidal neurons, mainly in the prefrontal and parietal cortex (Korada et al. 2002). A similar phenotype was also observed in transgenic mice expressing a tyrosine kinase domain-deficient FGFR1 (Shin et al. 2004), suggesting that the function of the FGF2/FGFR1 system is crucial for the correct maturation of the cerebral cortex. The in vivo role of FGF2 in synapse formation still remains unclear.

Based on a candidate molecule approach, several presynaptic organizing molecules had been proposed in addition to FGF2. These include Laminin β2 at the neuromuscular junction (NMJ; Hunter et al. 1989), and Neuroligin (Scheiffele et al. 2000), Wnt-7a (Hall et al. 2000), and SynCAM (Biederer et al. 2002) in the central nervous system. As the differentiation of nerve terminals was compromised in the Laminin β2 mutants, Laminin β2 is an important presynaptic organizer at the NMJ. However, these mutant mice still form functional NMJs (Noakes et al. 1995). Neuroligin, Wnt-7a and SynCAM were shown to have appropriate bioactivities when tested in vitro. However, knockout mice for Neuroligin or Wnt-7a have functional synapses in the brain (for a review, see Fox & Umemori 2006). Thus, these results indicate that there are additional presynaptic organizers that play important roles in vivo. Therefore, we tried to find such critical presynaptic organizers by carrying out an unbiased search.

FGF22 as a presynaptic organizer in the cerebellum

To identify presynaptic organizers, we biochemically purified active molecules from developing mouse brain. We used their ability to cluster synaptic vesicles in cultured neurons as a specific bioassay. After > 1000-fold purification, we isolated a fraction that induces synaptic vesicle aggregation and axon branching. Protein sequence analysis identified the active component as FGF22. In the developing brain, we found that FGF22 is highly expressed by granule cells in the cerebellum. Its main receptor FGFR2 is expressed by pontine neurons, whose axons, called mossy fibers, form synapses on granule cells. This pattern suggested that FGF22 released from granule cells induces the presynaptic differentiation of mossy fiber terminals. We carried out four experiments that support this hypothesis: (i) recombinant FGF22 induced the formation of functional synaptic vesicle aggregates in cultured pontine axons; (ii) inactivating FGF22 with a blocking reagent (sFGFR2bAP, a soluble form of the extracellular domain of FGFR2b fused to alkaline phosphatase) markedly reduced synapse formation between pontine axons and granule cells in culture without affecting the total level of vesicle proteins; (iii) similarly, inactivation of FGF22 in vivo reduced the formation of synapses on granule cells during nervous system development in mice; and (iv) mutants lacking FGFR2 have a similar defect in pontine axon-granule cell synapses in vivo. These results indicate that FGF22 is an important presynaptic organizer in the cerebellum (Umemori et al. 2004).

We wondered whether the presynaptic effects of FGF22 are specific for FGF22 or are shared by other FGFs. To address this issue, we tested a panel of 12 FGFs on cultured neurons, assaying three activities: synaptic vesicle aggregation, axon branching, and neurite elongation. We identified four functionally distinct groups of FGFs: (i) FGF7/10/22, with vesicle aggregation and axon branching activities; (ii) FGF4/6/9, with vesicle aggregation and neurite elongation activities; (iii) FGF17/18, with branching activity; and (iv) FGF1/2/5/23, with no significant activity (Umemori et al. 2004). In addition, FGF14, a member of the FGF homologous factors (FHFs) that do not bind FGFR, also appears to be involved in synapse formation. The FGF14-knockout mice show a reduction of docked synaptic vesicles and active zones in the hippocampus (Xiao et al. 2007).

FGF7, FGF10 and FGF22 as presynaptic organizers at the neuromuscular junction

The NMJ represents the synapse linking motor neurons and muscles (Sanes & Lichtman 1999). By searching for presynaptic organizers at the NMJ, we found that FGF7, FGF10 and FGF22 have activities to induce presynaptic differentiation in motor neuron cultures. These FGFs are all expressed by muscles. To investigate their in vivo function, we analyzed the neuromuscular phenotype in mice lacking their common receptor, FGFR2. For these experiments, we used two mutant mouse lines: a deletion mutant of their specific receptor, FGFR2b (Eswarakumar et al. 2002), and the conditional FGFR2 mutant (Yu et al. 2003), which was crossed with a motor-neuron specific Cre line (HB9-Cre; Pun et al. 2002). In FGFR2b knockouts (embryonic day 18), synaptic vesicles are diffusely distributed throughout the axon, whereas vesicles are normally concentrated at synaptic sites in control animals (Fig. 2). The same phenotype was also observed in motor-neuron-specific deletion mutants of FGFR2. These results indicate that FGF7/10/22 are embryonic presynaptic organizers that recruit vesicles to the motor nerve terminal during the formation of functional motor nerve terminals (Fox et al. 2007).

Fig. 2. FGF7/10/22 signaling is critical for synaptic vesicle recruitment to the motor neuron terminals. Whole mount staining of intercostal muscles from wild-type (A) or FGFR2b-deficient (B) E18 embryos (left panels). Staining for synaptic vesicles (synaptophysin; green) and axons (neurofilament; red). Right panels are schematic illustrations of the staining, with the location of target muscles. At the wild type neuromuscular junction (A), synaptic vesicles are aggregated at the nerve terminal to form synapses with the target muscles, but in FGFR2b-knockout animals (B), vesicles remain in the preterminal (arrowheads), indicating that the FGF signaling is involved in the synaptic vesicle (SV) recruitment to the terminal. Total levels of synaptophysin were not significantly different from each other (Fox *et al*. 2007).
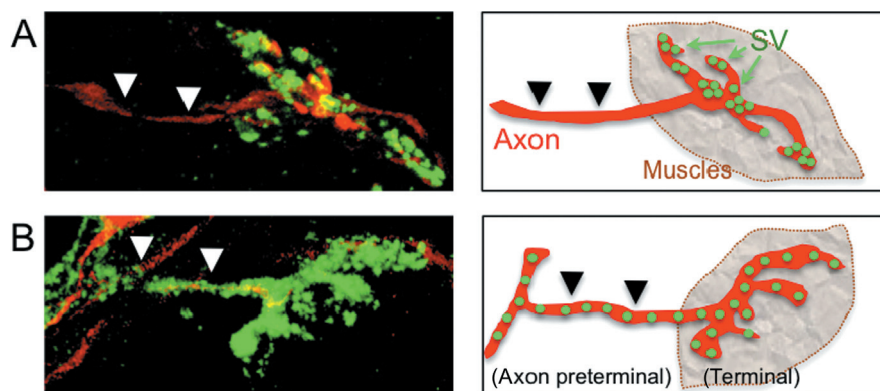
Cooperation of FGF with cell-adhesion molecules in neural wiring

Cell adhesion molecules (CAMs) play critical roles in axon guidance, target recognition and synaptic differentiation. Accumulating evidence suggests that CAMs and FGFs cooperate in these steps to form neural connections. Here I describe two kinds of such cooperation: crosstalk (Fig. 3A) and sequential cooperation (Fig. 3B).

Crosstalk

NCAM, N-Cadherin and L1-type proteins are CAMs that promote axon elongation of various neurons. These CAMs are activated by homophilic adhesion, and their neurite outgrowth activities require tyrosine kinase activity and calcium influx into the neuron (Williams *et al*. 1992, 1994). The FGFR kinase is proposed to be mediating the axon elongation activity of these CAMs. All FGFRs have three extracellular Immunoglobulin (Ig) domains. Antibodies generated to a sequence in the second Ig domain of FGFR inhibited neurite elongation of cerebellar neurons induced by NCAM, L1-CAM and N-Cadherin (Williams *et al*. 1994). NCAM- and L1-CAM-mediated adhesion induces the phosphorylation of FGFR, and a kinase dead FGFR1 inhibits axon elongation in response to NCAM, N-Cadherin and L1-CAM (Saffell *et al*. 1997). The interaction of NCAM and N-Cadherin with FGFR1 appears to involve the FGFR acid box, which is right upstream of the second Ig domain (Sanchez-Heras *et al*. 2006). In addition, another sequence in FGFR that binds to NCAM was identified by NMR (Kiselyov *et al*. 2003). This sequence is located in the third Ig domain of FGFR. The sequence in NCAM that binds to the third Ig domain of FGFR
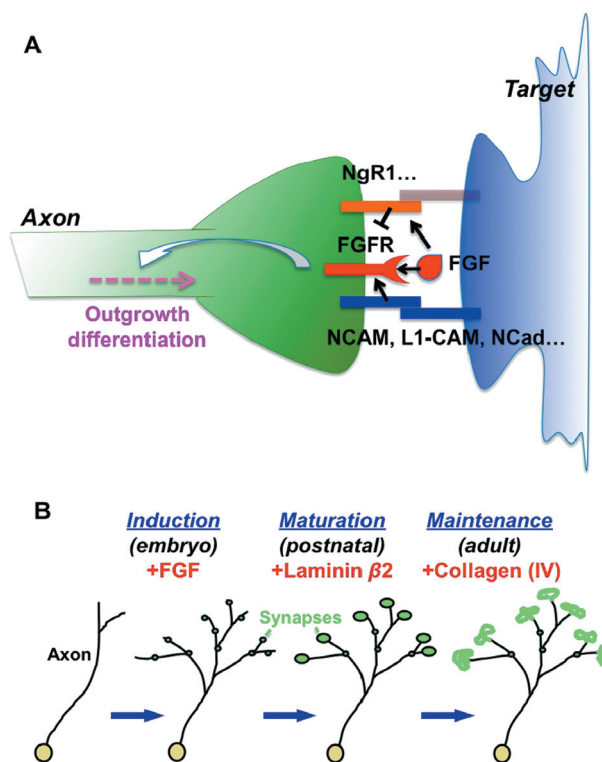

Fig. 3. Cooperation of fibroblast growth factor (FGF) and FGF receptor (FGFR) with cell-adhesion molecules in neural wiring. (A) Schematic illustration of crosstalk between FGF/FGFR and various cell adhesion molecules. NCAM, L1-type CAMs and N-Cadherin (NCad) signal through FGFR to induce axon outgrowth and differentiation. Nogo-66 receptor 1 (NgR1) binds FGF and negatively regulates FGF signaling in the brain. (B) A model of sequential cooperation by different synaptic organizers. FGF7/10/22 cluster synaptic vesicles in embryos. β2 laminins promote the postnatal maturation of nerve terminals. Collagen IV is required for synaptic maintenance in adults.

was located in the second fibronectin domain of NCAM (FG loop). Interestingly, this FG loop also binds to ATP, and accordingly ATP prevents NCAM from binding to FGFR. Neurite outgrowth, which is induced by the FG loop peptide, is inhibited by ATP, suggesting that ATP regulates the effect of NCAM by regulating the NCAM-FGFR interaction (Kiselyov *et al*. 2003). FG loop peptides enhanced neurotransmitter release in hippocampal cultures, which is inhibited by the FGFR inhibitor (SU5402). Injection of the FG loop peptide into the ventricle of the brain enhanced synapse formation and memory consolidation (Cambon *et al*. 2004).

The crosstalk between FGFR and L1-type proteins has also been demonstrated in Drosophila. Loss-of-function conditions for Neuroglian, the Drosophila L1-type gene (Bieber *et al*. 1989), and for Heartless (one of two Drosophila FGFR genes) display a similar phenotype, abnormal guidance and mis-targeting of sensory neuron axons. The Neuroglian phenotype can be suppressed by the overexpression or constitutive activation of the heartless FGFR, suggesting the presence of functional interaction between L1-type proteins and FGFR (García-Alonso *et al*. 2000).

Recently, a crosstalk between FGF and another CAM, the Nogo-66 receptor 1 (NgR1), was reported. NgR1 is expressed in neurons and binds to molecules expressed by the myelin sheath, such as Nogo-A, MAG (myelin-associated glycoprotein) and OMgp (oligodendrocyte myelin glycoprotein). NgR1 is involved in the growth-cone-collapse activity that is induced by these myelin constituents (Xie & Zheng 2008). Using a cell-based binding and a pull down assay, Lee *et al*. found that FGF1 and FGF2 physically interact with NgR1 (Lee *et al*. 2008). NgR1 and FGFR1 are colocalized at synapses, and an overexpression of NgR1 in rat cortical neurons blocked axon branching, which is induced by FGF2. Thus, it appears that NgR1 negatively regulates the FGF signaling by binding to FGFs (Fig. 3A; Lee *et al*. 2008). Interestingly, FGFR and NgR1 are also colocalized at postsynaptic sites and regulate spine morphology.

Sequential cooperation

In addition to FGF7/10/22, we have identified two additional candidates that might act as presynaptic organizers for motor neurons: Collagen IV and Laminin β2 (Hunter *et al*. 1989; Fox *et al*. 2007). Both proteins are expressed by muscles and induce presynaptic differentiation when applied to cultured motor neurons. Why are there three distinct presynaptic organizers at the NMJ? There are several potential explanations: (i) different organizers act at different synapses; (ii) different

organizers are responsible for different synaptic structures; (iii) they act redundantly, or compensate for each other; (iv) they are part of the same signaling pathway; or (v) they act sequentially to pattern the synapse. To answer this question, we analyzed the neuromuscular phenotype in the knockout mice of each presynaptic organizer. We found the following results: (i) in Laminin β2 knockout mice, NMJs were normal at birth, but presynaptic differentiation fails during the first postnatal week (Nishimune *et al*. 2004; Fox *et al*. 2007), suggesting that Laminin β2 is a neonatal presynaptic organizer; and (ii) in collagen IV knockout animals, NMJs form and show defects only in adulthood, indicating that collagen IV is an adult presynaptic organizer. These results suggest that multiple target-derived molecules act sequentially to organize presynaptic differentiation, with FGF7/10/22, Laminin β2 and collagen IV playing predominant roles in the induction, maturation and maintenance of the motor nerve terminals, respectively (see Fig. 3B for the proposed model). Thus, FGFs temporally cooperate with CAMs to pattern the NMJ (Fox *et al*. 2007).

Conclusions

The biological roles of FGFs are profound. In the nervous system, in addition to their roles during early development, they are involved in neural wiring, differentiation and functioning. FGFs exhibit a variety of effects during the patterning of our bodies, and their functions often involve a crosstalk and cooperation with various CAMs. The precise mechanisms that govern the differential functions of FGFs during development remain an important question that still needs to be addressed.

Genetic alterations of FGFs and FGFRs have been found in various diseases including skeletal disorders, cancers (reviewed in Eswarakumar *et al*. 2005) and psychiatric disorders such as major depressive disorders (Evans *et al*. 2004). By analyzing the specific functional roles of FGFs in the nervous system, we will understand how FGFs are involved in these disorders. Hopefully, this will eventually result in effective treatments and/or the prevention of these diseases.

Update

Recent work demonstrated another FGF crosstalk: the crosstalk between FGFR and the adenosine A_{2A} receptor (A_{2A}R; Flajolet *et al*. 2008). The intracellular domains of FGFR and A_{2A}R directly interact with each other. When both receptors are stimulated together, they induce MAPK activation, neurite and spine formation, and cortico-striatal plasticity.

Acknowledgments

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