

Neural Plate Patterning by Secreted Signals

The patterning of the CNS relies on the interaction of multiple signaling molecules such as Sonic Hedgehog, Wnts, and BMPs and their antagonists Chordin and Noggin. The identification of the secreted molecule Tiarin (Tsuda et al., 2002, this issue of *Neuron*), produced by the nonneural ectoderm at border of the anterior and lateral neural plate, now introduces a novel signaling pathway participating in CNS development.

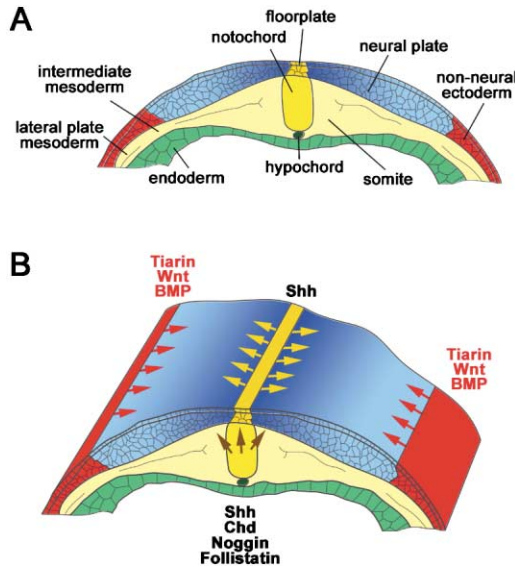
The generation of the central nervous system (CNS) depends on the formation of multiple neuronal cell types arranged in appropriate numbers and precise position. This patterning process is initiated during early embryonic development. Over the past years, important advances have been made showing that neural induction relies on complex interactions between the FGF, bone morphogenetic protein (BMP), and Wnt signaling pathways (Wilson and Edlund, 2001; Streit et al., 2000). In *Xenopus*, neural induction can be traced back to the nuclear accumulation of β -catenin triggered by fertilization (Baker et al., 1999) and the expression at the blastula stage of BMP antagonists such as *Chordin* in a dorsal pre-organizer region (Wessely et al., 2001). The patterning of the neural plate at later stages is also a complex and highly regulated process. As shown in the first Figure, two opposing signaling centers impart polarity to the neural plate. The floor plate and the notochord are required for ventralizing the neural tube and secrete the signaling molecule Sonic hedgehog (Shh) and the BMP antagonists Chordin and Noggin (Briscoe and Ericson, 2001). The opposite type of signals emanates from the roof plate of the CNS: when the roof plate is ablated genetically by introducing the gene encoding the diphtheria toxin into the *Gdf7* locus, the neural tube loses its dorsal identity. The neural tube is said to be ventralized, since dorsal neural progenitors are absent and a more ventral class of interneurons is expanded (Lee et al., 2000). The two known families of signals secreted by the dorsal epidermal ectoderm are Wnts (Wnt1 and Wnt3a) as well as members of the TGF- β gene superfamily, such as BMP-2, -4, -7, and GDF-7 (Lee and Jessell, 1999).

The group of Yoshiki Sasai has now identified a novel pathway able to pattern the embryonic neural plate (Tsuda et al., 2002 [this issue of *Neuron*]). The work provides an excellent illustration of the power of *Xenopus* embryonic assays for the identification of new patterning molecules. Tiarin is expressed as a tiara or horse-shoe in the nonneural ectoderm surrounding the anterior neural plate (see first Figure). It is a secreted glycoprotein of 467 amino acids with an Olfactomedin-like domain (OLF, see <http://www.ebi.ac.uk/interpro>). This mo-

tif of as yet unknown function is found in many extracellular proteins such as Olfactomedin, a protein secreted by the olfactory epithelium, and Noelin, a secreted protein implicated in the generation of the neural crest (Moreno and Bronner-Fraser, 2001). When *Tiarin* synthetic mRNA was microinjected into blastomeres fated to become neural plate, striking patterning changes were detected. Dorsal CNS markers such as *Pax3*, *Gli3*, and Rohon-Beard sensory neurons were expanded, while ventral markers such as *Nkx2.2*, motoneurons and floor plate markers (e.g., *Kiellin*) were lost. Interestingly, this phenotypic alteration occurred with almost no effect on the neighboring tissues. For example, *Shh* expression, although lost from the floor plate, was unaffected in the notochord. Similarly, the anterior endoderm continued to express the Wnt antagonist *Frzb-1*.

These results suggested that Tiarin plays an important role in the patterning of the neural plate. But how does it function? To better understand Tiarin's mechanism of action, the authors turned to a favorite *Xenopus* assay. Ectodermal explants (called animal caps) can easily be excised at blastula and cultured in saline solution until cell differentiation occurs at later stages of development. *Tiarin* was unable to induce neural tissue on its own, and the animal caps remained as epidermis. If, however, *Tiarin* was coinjected with the neural inducer *Chordin*, the resulting CNS tissue formed was skewed toward a dorsal type. In addition, *Tiarin* antagonized the ventralizing effect of Shh. Upon injection of *Chordin* and *Shh* mRNA, floor plate markers such as *Kiellin* were induced, and this induction was abolished by *Tiarin* mRNA. Is *Tiarin* therefore a Shh antagonist? The answer to this question is negative, for *Tiarin* could not inhibit expression of the Shh downstream target genes *Patched* or *Gli1*. Finally, other *Xenopus* embryo assays showed that Tiarin does not affect BMP or Wnt signaling levels. Thus, it appears that Tiarin patterns the neural plate independently of Shh, BMP, and Wnt signals. Tiarin therefore may provide an entry point for the identification of a new CNS patterning pathway. What is the nature of this novel pathway? This is not known, but a clue is perhaps provided by the observation that the Olfactomedin-like domain (OLF) of Tiarin can also be found in the extracellular domain of the α -Latrotoxin receptor (see second Figure, panel B). This seven-transmembrane receptor shows considerable similarity to the secretin family of G protein-coupled receptors. It acts as a receptor for the toxin of the venom of black or brown widow spiders of the genus *Latrodectus* (Südhof, 2001). The presence of the OLF domain in both a seven-transmembrane receptor and multiple secreted proteins is analogous to the case of the Frizzled seven-transmembrane Wnt receptors, and the family of secreted Wnt binding antagonists called Frzbs or sFRPs (see second Figure, panel A; Leyns et al., 1997).

For those more molecularly minded readers, the cloning of Tiarin offers interesting technical aspects. To identify secreted proteins expressed during early neural development, Tsuda et al. (2002) combined differential screening and signal sequence-trap cloning in a very



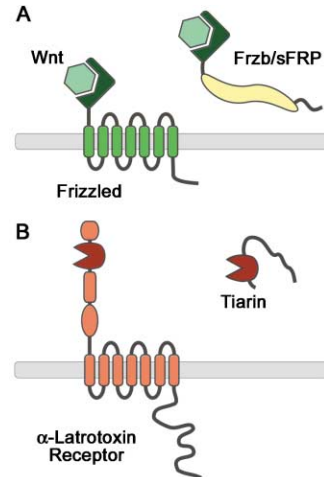
Patterning of the Neural Plate by Secreted Signals

(A) Schematic representation of a cross-section of a *Xenopus* early neurula at the time *Tiarin* is expressed. In the ectodermal germ layer the nonneural ectoderm is shown in red, the neural plate in blue, and the floor plate in orange. Note that at the neural plate stage the dorsal region of the closed neural tube corresponds to the lateral regions, and that the future ventral neural tube corresponds to the medial neural plate. The mesoderm (yellow) is subdivided into four independent regions: the notochord, the somite giving rise mostly to muscle, the intermediate mesoderm giving rise to kidney, and the lateral plate mesoderm, which will give rise to the somatic and visceral mesodermal sheets once the coelomic cavity forms. The endodermal germ layer is shown in green and the hypochord, its dorsal-most derivative, is indicated.

(B) The dorsoventral polarity of the future neural tube is established via the interaction of multiple signaling pathways. The floor plate secretes Shh and the notochord Shh and the BMP antagonists Chordin, Noggin, and Follistatin. The activity of these midline signals is opposed by factors secreted by the dorsal most nonneural ectoderm. Wnts, BMPs, and the newly identified Tiarin lead to the formation of dorsal cell types in the neural tube.

imaginative way. Neural plate cDNAs were selected for fragments containing the 300–500 nucleotides of the 5'-most portion using a PCR-based selection protocol and cloned into a vector encoding a signal-peptide-less Interleukin-2 receptor (Tashiro et al., 1999). This trick resulted in a mini-library enriched in sequences which—when derived from a secreted or transmembrane protein—restore the cell membrane localization of the Interleukin-2 receptor. Twenty thousand of these cDNA colonies were screened by differential hybridization for genes expressed in the anterior neural plate, but absent in nonneural ventral marginal zone explants of the same stage. The remaining 6,000 neural-enriched clones were transfected into COS cells in pools of 25 and screened by immunofluorescence for the expression of the Interleukin-2 receptor at the cell surface. Using this nice screening approach, Tsuda et al. (2002) isolated nine different extracellular proteins.

The discovery of Tiarin is a reminder that we can still expect many surprises in the study of neural development. From the discovery of a novel signaling pathway



Hypothetical Model for the Function of OLF Domain Containing Proteins

(A) Wnt receptor Frizzled and the family of secreted Wnt antagonists of the Frzb/sFRP class contain a CRD domain that binds Wnt. In the case of the receptor, interaction with the Wnt ligand leads to the activation of the seven-transmembrane receptor. In the case of the Frzb/sFRP, Wnt binding removes the ligand from the signaling pool and antagonizes Wnt signaling (after Leyns et al., 1997).

(B) Tiarin and the α -Latrotoxin receptor share a common protein domain. Although the molecular mechanism of action of Tiarin is unresolved, the presence of the olfactomedin-like domain (OLF) in Tiarin and in the extracellular region of the receptor is suggestive. The natural endogenous ligand of the α -Latrotoxin receptor is unknown, and the OLF domain could be involved in signaling by either binding a putative ligand or by interacting with extracellular matrix components.

involved in neural patterning to the fine methods for identifying secreted proteins, the Tsuda et al. (2002) paper is worthwhile reading.

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Is Synaptic Homeostasis Just *wishful thinking*?

The bone morphogenetic proteins (BMPs) are secreted polypeptides of the TGF- β family, whose diverse functions include primary neural induction and the dorsoventral patterning of the neural tube. In this issue of *Neuron*, Aberle et al. (2002) and Marqués et al. (2002) present evidence that BMP receptors may also influence the development of synapses. The results suggest a novel mechanism for regulating neuronal growth and synaptic homeostasis during development.

Structure and function are concepts forever wedded in biology, and nowhere is this more true than in the nervous system. A good example of how neuronal morphology and function are linked involves synaptic growth during development. As nervous systems enlarge, dendritic and axonal arbors must grow appropriately, so that synaptic signals remain physiologically effective, a phenomenon termed synaptic homeostasis. A delicate balance is maintained, so that postsynaptic signals remain of sufficient amplitude to drive action potentials. Evidence from *Drosophila* suggests that synaptic homeostasis depends on signals exchanged between synaptic partners, but the molecular players have remained largely unknown (Koh et al., 2000).

The larval neuromuscular junction (NMJ) of *Drosophila* is a favorable system for examining synaptogenesis and synaptic homeostasis. *Drosophila* offers the advantages of working in a model organism with a fully sequenced genome and powerful genetic tools. Each abdominal hemisegment possesses exactly 30 individually specified muscle fibers, innervated by 35 motoneurons. Each motoneuron projects to specific muscle fibers, making this one of the best characterized arrays of synapses in any organism. Neurons and muscle fibers are uniquely identifiable, allowing one to pose experimental questions with single cell resolution. For example, the GAL4/UAS bipartite expression method may be used to drive the expression of molecular constructs in specific cells and to either side of the synapse.

The *Drosophila* NMJ is a marvel of morphological growth. The animals grow 10-fold in length during the 4 days of larval life. Each day the muscles double in length, yielding a 100-fold increase in surface area by the time of pupation. To maintain their ability to drive contractions, motoneuron arbors grow in parallel. The number of synaptic boutons increases 100-fold, and the boutons themselves become more complex, with a 20-fold increase in the number of active zones. Synaptic homeostasis involves a fine regulation of the quantal content of the NMJ. In experiments where the postsyn-

aptic sensitivity has been reduced, the motoneuron responds by increasing its release of transmitter. This regulation of release has been demonstrated by reducing the density or function of neurotransmitter receptors, as well as by inhibiting the ability of the muscle to depolarize through the expression of shunting potassium channels (Paradis et al., 2001; White et al., 2001).

How does a motoneuron know how effective it is? It is thought that a feedback signal from the muscle is monitored by the motoneuron terminals, regulating arbor growth, bouton formation, and neurotransmitter release. Two papers in this issue of *Neuron* (Aberle et al., 2002; Marqués et al., 2002) provide tantalizing evidence for a role of BMP signaling as part of the feedback mechanism. Both studies show that mutations of the BMP type II receptor gene *wishful thinking* (*wit*) result in motor endings with fewer synaptic boutons than normal. Since the phenotype becomes progressively more severe as the larva grows, it is proposed that the defect is due to a reduced ability to add or maintain synaptic boutons. In short, the motoneuron cannot keep up with the growth of the muscle. By contrast, events that occur in the embryo are normal in *wit* mutants, including motoneuronal specification, axonal outgrowth, and target selection. The *wit* mutations are 100% penetrant and result in mortality during the pupal stage. The *Wit* gene is expressed in subsets of larval neurons, including motoneurons. Mosaic experiments show that the mutant phenotypes can be rescued by the presynaptic expression of wild-type *Wit* protein in the motoneurons, indicating that a major function of the gene is in those cells.

The discovery of the *wishful thinking* gene by the O'Connor and Goodman laboratories depended on two distinct experimental strategies that illustrate the range of approaches available to *Drosophila* researchers. Marqués et al. (2002) took a reverse genetic approach. They scanned the *Drosophila* genome and noted sequences homologous to previously characterized mammalian BMP receptors. In addition, they identified the gene through low stringency homology screening using probes for type II receptors, and then made mutants. By contrast, Aberle et al. (2002) followed a forward genetic approach and identified the same gene through a screen for mutations that alter the morphology of larval NMJs, an approach that reflects the interest in the Goodman lab in synaptogenesis. The screen used a GFP-tagged reporter construct (CD8-GFP-Sh) that labels synapses. One can visualize mutant synapses through the transparent skin of the living larva, bypassing the need to do dissections and staining. Remarkably, NMJs may today be viewed as externally visible genetic markers, not unlike the wings, bristles, and eye phenotypes of the classical era of *Drosophila* genetics.

Consistent with their smaller bouton numbers, *wit* mutants release much less neurotransmitter than normal. The defect is associated with ultrastructural changes in the boutons, including detachment from the postsynaptic membrane, aberrant membrane ruffling, and aberrant T bar-like structures. Adhesion molecules, such as the NCAM homolog Fasciclin II, are downregulated at the NMJ. *Wit*'s identity as a BMP type II receptor suggests that the neuromuscular phenotypes arise as the result of altered expression of a battery of genes that are downstream of BMP receptor activation.