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Neural Plate Patterning by Secreted Signals

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Abstract

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

Selected Reading

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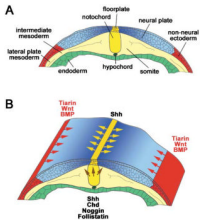

Figure 1. Patterning of the Neural Plate by Secreted Signals

(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.

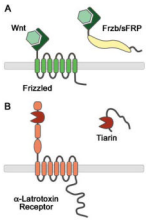

Figure 2. Hypothetical Model for the Function of OLF Domain Containing Proteins

(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.