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Pax-2 in the chiasm

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Abstract. *Pax* genes are expressed in specific patterns in the nervous system during development and in the adult. Recent findings suggest a link between the expression of *Pax-2* and axonal guidance. Mice with a targeted deletion of *Pax-2* are an excellent tool for studying axonal pathfinding at the molecular level, especially with respect to the optic chiasm. The date reviewed here suggest that *Pax-2* regulates the expression of surface molecules involved in contact attraction and that the mutual regulation of the expression of *Pax-2* and the *Sonic hedgehog* gene is of importance in the formation of the chiasm region.

Key words: *Pax-2* – Optic chiasm – Axonal guidance – *Sonic hedgehog* – Mutant mice – Zebrafish

Introduction

The *Pax* gene family encodes a group of nine transcription factors (*Pax-1* to *Pax-9*) that share a highly conserved DNA stretch of 384 base pairs named the paired box (reviewed in Walther et al. 1991; Noll 1993; Stuart et al. 1993). Some family members encode a second DNA-binding domain called the paired-type homeobox. The *Pax* genes are expressed in dynamic, spatially, and temporally restricted patterns in the nervous system during development and in the adult (except for *Pax-1* and *Pax-9*). It has been suggested that *Pax* genes and other transcription factors with restricted expression in the developing nervous system are involved in the regionalization and then in the differentiation of the neural primordium (see, for instance, Stoykova and Gruss 1994). Nevertheless, the cellular mechanisms by which these nuclear proteins regulate the development of the brain remain elusive. Recent findings, however, suggest a link between the expression of one of the *Pax* genes and a specific function at the cellular level: axonal guidance. Analysis of mouse embryos with a targeted deletion of *Pax-2* (Torres et al. 1996) shows important defects of the optic chiasm that provide insights into the development of this region. These mutants are an excellent tool for studying the mechanisms of axonal navigation at the molecular level.

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The optic chiasm: to cross or not to cross

In vertebrate species, many or all of the axons from the retina cross to the opposite side of the brain at the optic chiasm, which is located at the ventral side of the forebrain. The formation of the optic chiasm requires a choice by the axonal growth cones: to cross or not to cross the midline. Because of the apparent simplicity of this decision, the formation of the optic chiasm constitutes a favorite model for the study of the cellular and molecular basis of axonal pathfinding. The current state of research in the field has been extensively reviewed (see, for instance, Sretavan 1993; Guillery et al. 1995). The processes of chiasm formation have been dissected into a succession of minute steps that can, up to a point, be independently analyzed. One of these steps is the development of the chiasm region itself, i.e., the formation of the precisely defined region in the ventral forebrain neuroepithelium that will offer the retinal axons many of the clues for the "cross/do not cross" decision. This is precisely the context, the specification of the chiasm region, at which *Pax-2* expression seems to be crucial (Torres et al. 1996).

Pax-2 in the formation of the chiasm region in the mouse

Early in the morphogenesis of the eye, *Pax-2* expression is found in the ventral half of the optic vesicle. When the future neural retina invaginates, expression is lost in most of its cells but remains in the borders of the choroid fissure, from where it extends to the ventral half of the optic

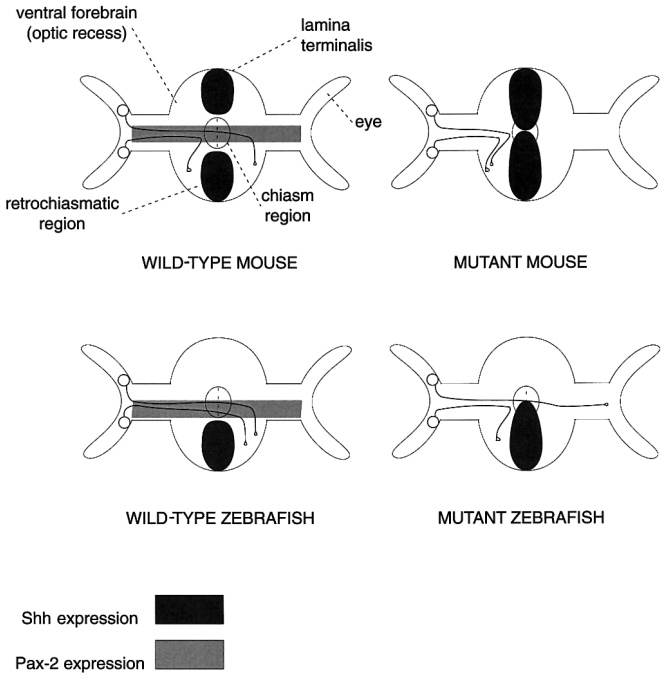

Fig. 1. Diagram summarizing the pattern of expression of *Shh* and *Pax-2* in the chiasm region of wild-type and *Pax-2* mutant mouse embryos at E11, and zebrafish. Redrawn and simplified from Torres et al. (1996)

stalk by embryonic day 9 (E9). At the same stage in development, the ventral forebrain midline, i.e., the region where the optic chiasm region and then the crossing itself will develop, is formed by *Sonic hedgehog (Shh)*-expressing neuroepithelial cells (Echelard et al. 1993; Torres et al. 1996). The first optic fibers leave the retina and reach the brain wrapped in the *Pax-2*-expressing optic stalk around E11. By this time, a transverse band of *Pax-2*-expressing neuroepithelium, continuous with the *Pax-2*-expressing optic stalk, extends from side to side of the ventral forebrain (Fig. 1). In the region where this band of tissue cuts across the midline, *Shh* is no longer expressed (Torres et al. 1996). This is the chiasm region, a patch of neuroepithelium expressing molecular determinants that will induce some axons to proceed across the midline (the axons from the nasal side of the retina, about 95% in the mouse) and some to turn to the ipsilateral optic tract. The optical axons that cross can safely navigate through the midline "perils" inside the *Pax-2*-expressing pathway (Torres et al. 1996). Certain hypothalamic neurons may collaborate with *Pax-2*-expressing cells in funneling the optic fibers through the chiasm region. Early differentiating neurons of the retrochiasmatic region of the hypothalamus form the caudal border of the chiasm region and have been shown to express CD44 and also L1, cell surface molecules known to influence retinal axon growth and whose interactions with the incoming optic fibers have been suggested to form an "anatomical template" for the chiasm (Sretavan et al. 1994). One of the earliest specific markers for this region is the POU-III homeodomain transcription factor *Tst-1*, which is expressed only by retrochiasmatic cells at comparable stages in rat development (Alvarez-Bolado et al. 1995). The key constituent of the chiasm region appears to be a specialized group of glial cells straddling the midline and uniquely expressing a number of markers (McKanna 1993). The turning of the axons from the temporal side of the retina occurs upon reaching these particular glial cells (Marcus et al. 1995), which could be the source of the inhibitory signal that exists in the chiasm region of the midline (Wizenmann et al. 1993; Wang et al. 1995). In the *Pax-2* null mutant mouse, the neuroepithelial cells normally forming the optic stalk are substituted by pigmented retinal cells. The axons of the retinal ganglion cells, however, are still able to leave the eye primordium, enter the optic stalk, and reach the ventral forebrain. In these embryos, a proper chiasm region is never formed, since *Shh* expression is continuous and uninterrupted throughout the optic recess. A detailed analysis of the cellular composition of the chiasm region in these mutant embryos has not yet been performed, so neither the fate of the midline glia nor their relationship to the *Shh*-expressing cells are known. However, it seems that, in the *Pax-2* mutants, no retinal axons cross the midline, and all of them enter the ipsilateral optic tract (Torres et al. 1996). Different hypothesis can be offered to explain this phenotype. As has been proposed previously, hedgehog proteins might regulate the expression of *Pax-2* and *Pax-6* in the developing eye (reviewed in Macdonald and Wilson 1996); furthermore, *Shh* could activate the expression of *Pax-2*, and *Pax-2* inhibit the expression of *Shh* (Macdonald et al. 1997). According to this scenario, expression of *Shh* would remain activated in the absence of *Pax-2* in the ventral forebrain. If this were the case, it would follow that *Shh*-expressing cells do not support the growth of retinal axons, or that, as a consequence of retained *Shh* expression, some cells in the chiasm cannot differentiate. Another possibility is that *Pax-2*-expressing cells display the guidance cues that lead the retinal axons from the retina to the very place in the midline where decisions are made. In the absence of *Pax-2*, optic fibers would simply be unable to reach the molecular cues expressed in the chiasm region.

Pax-2 in the formation of the chiasm region in zebrafish

Comparisons with the development of the chiasm in *Pax-2* mutants of other species might contribute to clarifying which of these hypothesis is more likely. Human mutants for *Pax-2* are known to have coloboma and to suffer from serious visual problems (Sanyanusin et al. 1995). Unfortunately, no data are available on the fibers in the optic chiasm of these patients. In contrast, alterations in chiasm formation have been extensively analyzed in *noi* mutants (Macdonald et al. 1997); *noi* is a zebrafish gene highly homologous in sequence to *Pax-2* and showing similar expression domains (Brand et al. 1996). However, since *Pax-2* has two paralogous genes in mammals (*Pax-5* and *Pax-8*), it cannot be said with certainty that *noi* is the ortholog of *Pax-2* (this subject has been discussed in detail by Brand et al. 1996). We will refer to

noi as *noi/Pax-2* for the sake of simplicity. In zebrafish, the growth cones entering the ventral forebrain find the neuroepithelium precisely organized into transverse bands of cells expressing various molecular markers. These bands of neuroepithelium are confined by two transverse bundles of axons, i.e., rostrally by the anterior commissure, and caudally by the postoptic commissure (corresponding to the supraoptic commissures of mammals). As in the mouse, the optic fibers navigate over one of these bands that is formed by *noi/Pax-2*-expressing cells. This substrate seems to encourage the growth of optic fibers and funnel them straight across the midline, preventing them from accessing other substrates. However, in both species, once the axons have crossed the midline, they are able to abandon the *Pax-2* pathway and head toward the optic tract. This behavior is reminiscent of that shown by another extensively researched commissural system. In the developing spinal cord, commissural axons are attracted toward the floor plate. Once there, most of them cross the midline; the majority of these fibers then change direction and turn to course longitudinally. Indeed, axons regulate their responsiveness to local guidance cues by changing the expression of surface molecules as they progress (see, for instance, Bastiani et al. 1987; Dodd et al. 1988; reviewed in Tessier-Lavigne and Goodman 1996).

In the *noi/Pax-2* mutant fish, as in *Pax-2* mutant mice, the retinal axons show pathfinding defects. However, instead of merely entering the ipsilateral optic tract (as in the mouse mutants), they exhibit a number of navigational mistakes, such as entering the opposite optic nerve or joining the ipsilateral optic tract. In some cases, the majority of the retinal axons enter the ipsilateral optic tract, as in the mouse *Pax-2* mutant. What is the relationship between *Shh* and *noi/Pax-2* expression? In zebrafish, the *noi/Pax-2* cells do not open the way through a previously *Shh*-expressing midline (as they have been suggested to do in mouse; Torres et al. 1996). Even so, in the absence of *noi/Pax-2*, *Shh* expression shows a more rostral boundary, invading the chiasm region, as in the mouse *Pax-2* mutants. However, some of the zebrafish optic fibers are apparently still able to cross the midline by growing over the *Shh*-expressing cells, which demonstrates that *Shh* does not inhibit axonal growth in the fish, and which suggests that it does not do so in the mouse either. The pathfinding errors of the retinal fibers in the optic chiasm region of the *noi/Pax-2* mutant fish seem however to support the hypothesis that *Pax-2*-expressing cells lead retinal axons to the midline, toward the relevant molecular cues. At the same time, *Pax-2*-expressing cells keep other axons from entering the pathway of the retinal fibers.

The most remarkable difference in the chiasmatic phenotypes of the fish and mouse *Pax-2* mutants is the pathway chosen by the retinal axons. In the mouse, they choose the ipsilateral optic tract, whereas in fish, they show a variety of behavioral patterns, including crossing the midline. It is not clear why the possible routes are more reduced for mouse retinal axons; one of the reasons could be that all axons cross the midline in fish, whereas the fibers coming from the temporal side of the retina are ipsilateral in mouse. The nasal axons would then choose the ipsilateral optic tract as a default pathway.

Concluding remarks

The role of *Pax-2* in the formation of the optic chiasm offers the first example to date of a *Pax* family member being involved in axon guidance. The exact role of *Pax-2* in the guidance of retinal axons to the ventral forebrain midline is not yet clear, although the data reviewed suggest that it could regulate the expression of surface molecules involved in contact attraction On the other hand, analysis of chiasm formation in mouse and fish *Pax-2* mutants suggests that the mutual regulation of the expression of *Pax-2* and *Shh* could also be of importance.

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