<Further Development 15.05>

Specifying the Brain Boundaries

The anterior-posterior identity of each of the mammalian brain vesicles is specified during gastrulation by the prechordal mesoderm and notochord. This specification appears to be stabilized at the neural plate stage by interactions within the plane of the ectoderm. Only the major molecules involved in forebrain and midbrain specification will be discussed here, and the details of hindbrain and spinal cord specification by the *Hox* genes has been discussed in Chapter 12.

I. Pax2/5 and Pax6 transcription factors subdivide the early neural tube into three divisions

The expression patterns of *Pax2* and *5* and *Pax6* genes demarcate the midbrain and forebrain primordia at the neural plate stage. In knockout mice that lack *Pax5* and *Pax2*, the entire mesencephalic primordium is absent. Instead, the hindbrain connects directly with the forebrain. In these animals, the tectum (the dorsal region of the midbrain) and cerebellum (derived from the dorsal region of the metencephalon) are absent. *Pax 6* expression expands both rostrally from the hindbrain region and caudally from the prosencephalon (Schwarz et al., 1999; Figure 1).

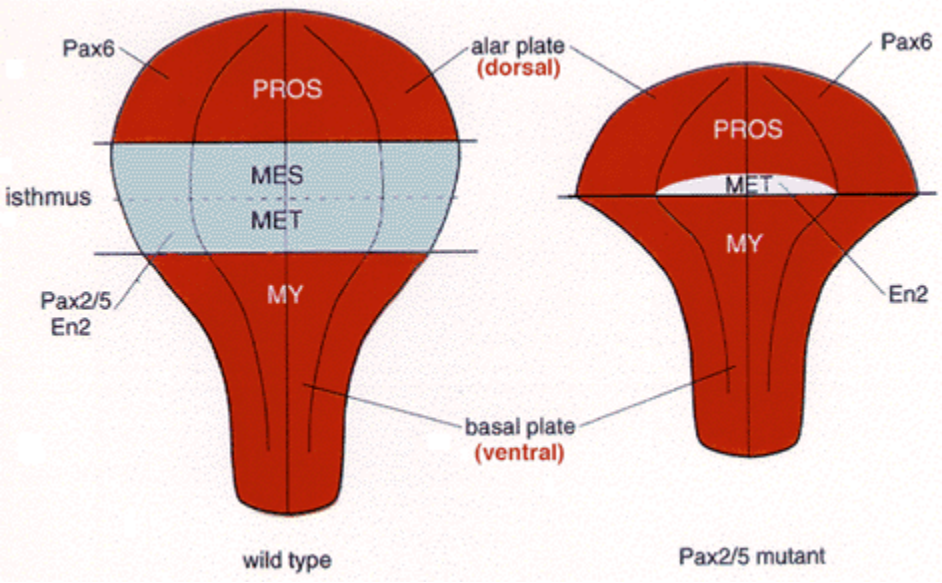

Figure 1 Schematic representation of the alterations in the *Pax2/5* compound mutant. In the wild-type neural plate, expression of *Pax6* (red) and *Pax2* and *5* (blue) delimit three domains--the prosencephalon (PROS), mesencephalon/myelencephalon (MSE/MET) and hindbrain (MY). In the *Pax2/5* double mutant, the middle subdivison is absent, its only remnant being a small ventral region (light blue) that expresses *engrailed-2* and corresponds to the basal region of the metencephalon. (After Schwarz et al; 1999.)

II. Border specification by paracrine factors Sonic Hedgehog and Fgf8

The forebrain and midbrain regions are defined by the underlying prechordal mesoderm and anterior notochord. Two genes that are expressed in these anterior mesodermal tissues are *Lim1* and *Otx2*. If either one is missing, the embryo does not form a forebrain or midbrain. Caudal to rhombomere 2, the embryos appear to be normal (Acampora et al., 1995; Shawlot and Behringer, 1995). Rubenstein and Puelles (1994) have proposed that the forebrain is composed of six neuromeric regions called prosomeres. Prosomeres p1-p3 comprise the diencephalon, whereas prosomeres p4-p6 comprise the hypothalamus (ventrally) and the telencephalon (dorsally). The prosomeric boundaries coincide with the expression boundaries of several genes that are thought to be important in neural specification. They are also seen to be the boundaries that limit the responses to certain external stimuli. The p2/p3 boundary may be critical in patterning the forebrain region. This boundary corresponds to the zona limitans. It is also a source of Sonic hedgehog, a diffusible protein known to induce patterning during gastrulation and limb formation (Rubenstein and Puelles,1994).

One of the critical regions for midbrain development is the metencephalon/mesencephalon border that will normally give rise to the tissues of the isthmus. No morphological boundary can be seen here, but it is marked by the most posterior portion of *Otx2* gene expression. When mid-to-anterior mesencephalon tissue is transplanted to the diencephalon or rhombencephalon, it induces the cells surrounding it to develop mesencephalonic fates (in the diencephalon) or cerebellar fates (in the rhombencephalon) (Figure 2A; Bally-Cuif and Wassef, 1994; Marin and Puelles, 1994). When rotated, a "triplication" can ensue, since tissues on both sides of the graft are induced (Figure 2B).

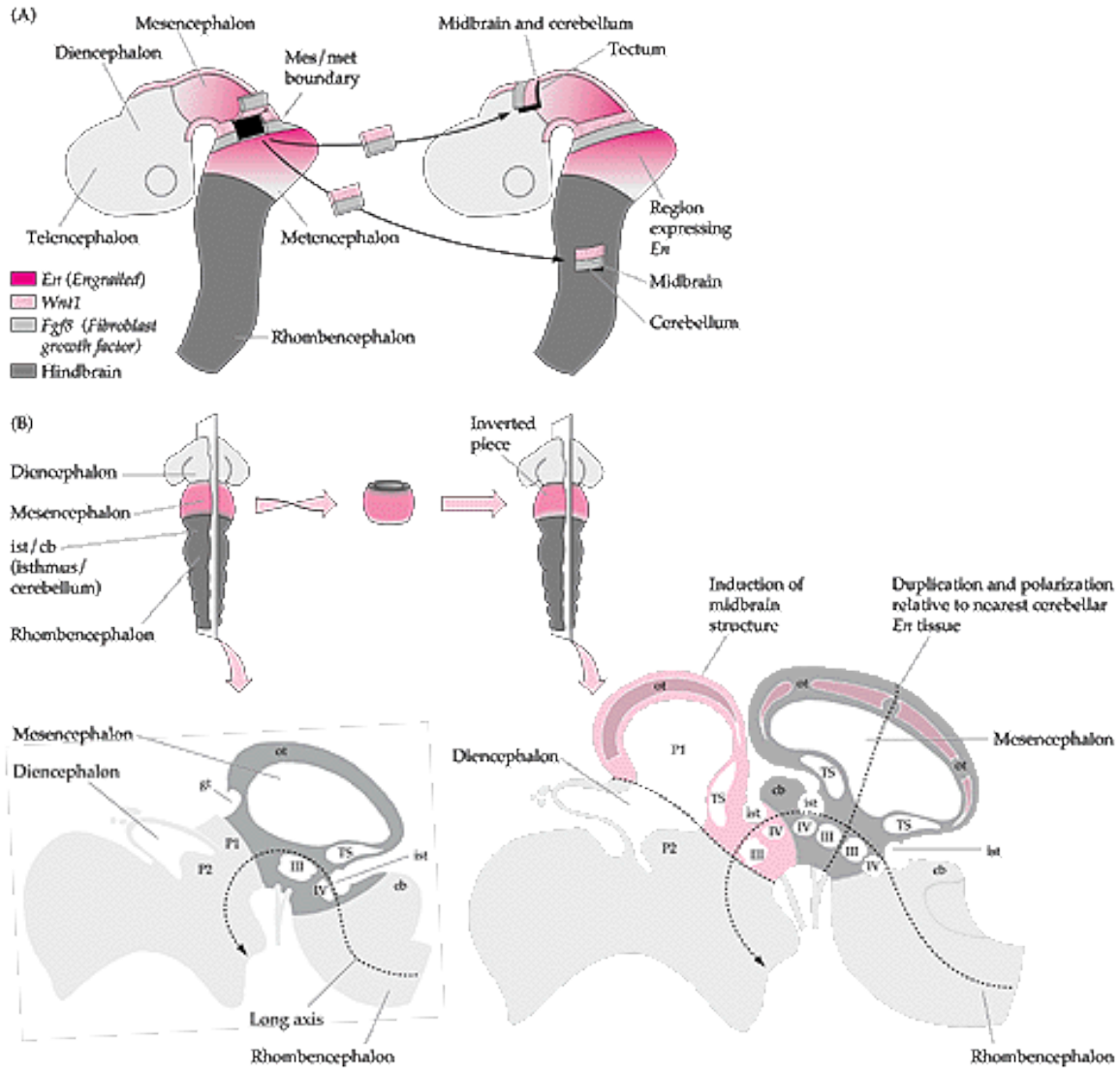

Figure 2 The mesencephalon/metencephalon ("mes/met") junction region can act as an inducer of midbrain development and *engrailed* expression when rotated or transplanted to other regions of the brain. (A) Transplantation of the mes/met junction results in the induction of *engrailed* gene expression and midbrain and cerebellar structures in ectopic positions. (B) Rotation of the mes/met junction causes "triplications" of certain structures, such as the optic tectum. Abbreviations: gt, griseum tectale; TS, torus semicircularis; P1, pretectal segment; P2, dorsal thalamic segment; cb, cerebellum; ot, optic tectum; ist, isthmus; III, third cranial, or oculomotor, nerve; IV, fourth cranial, or trochlear, nerve. The postulated polarity is represented by arrows. (B after Rubenstein and Puelles, 1994.)

This mes/met-inducing region appears to be controlled by fibroblast growth factor 8 (FGF8). Crossley and colleagues (1996) found that this isthmus-forming tissue secreted FGF8. Moreover, when they transplanted FGF8-containing beads into the diencephalon or rhombencephalon, they obtained the same duplicated midbrain structures. Control beads soaked in saline did not show any such duplications. The FGF8 beads also induced the expression of three genes in the surrounding tissues–*Wnt1*, *Engrailed-2*, and *Fgf8* itself. These three genes are normally expressed in the isthmus region. *Wnt1* and *Engrailed* are known to be important in the formation of the cerebellum. Even though the cerebellum does not express *Wnt1* genes, mice deficient in *Wnt1* lack their midbrain regions as well as the cerebellum (McMahon and Bradley, 1990; Thomas and Cappecchi,

1990). *Wnt1* appears to maintain *Engrailed* gene expression in the cerebellar precursor cells, enabling the cells to proliferate (Dickinson et al., 1994; Danielian and McMahon, 1996).

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