EARLY ANTERIOR/POSTERIOR PATTERNING OF THE MIDBRAIN AND CEREBELLUM

Aimin Liu1 and Alexandra L Joyner1,2
Howard Hughes Medical Institute and Developmental Genetics Program, Skirball Institute of Biomolecular Medicine, Departments of 1Cell Biology and 2Physiology & Neuroscience, New York University School of Medicine, New York, NY10016;
e-mail: liua02@saturn.med.nyu.edu, joyner@saturn.med.nyu.edu

Key Words  midbrain/hindbrain organizer, Fibroblast growth factor, Otx2, Gbx2, Engrailed

Abstract  Transplantation studies performed in chicken embryos indicated that early anterior/posterior patterning of the vertebrate midbrain and cerebellum might be regulated by an organizing center at the junction between the midbrain and hindbrain. More than a decade of molecular and genetic studies have shown that such an organizer is indeed central to development of the midbrain and anterior hindbrain. Furthermore, a complicated molecular network that includes multiple positive and negative feedback loops underlies the establishment and refinement of a mid/hindbrain organizer, as well as the subsequent function of the organizer. In this review, we first introduce the expression patterns of the genes known to be involved in this patterning process and the quail-chick transplantation experiments that have provided the foundation for understanding the genetic pathways regulating mid/hindbrain patterning. Subsequently, we discuss the molecular genetic studies that have revealed the roles for many genes in normal early patterning of this region. Finally, some of the remaining questions and future directions are discussed.

INTRODUCTION

Regionalization of the central nervous system (CNS) is a critical early event in vertebrate neural development. By embryonic day 9.5 (E9.5), the main regions of mouse CNS can be clearly distinguished morphologically along the anterior/posterior (A/P) axis (Figure 1). The forebrain consists of the telencephalon situated at the most rostral end of the neural tube and the more posterior diencephalon, which abuts the midbrain (mesencephalon). The forebrain is divided into several prosomeres, with prosomere 1 being the most caudal. The hindbrain is separated from the midbrain by a constriction that is called the mid/hindbrain junction or isthmus and is divided into the rostral metencephalon and the caudal myelencephalon. The hindbrain, also called rhombencephalon, is further divided
into eight rhombomeres. The metencephalon consists of rhombomere 1 (r1), which gives rise to the cerebellum and pons (Millet et al. 1996, Wingate & Hatten 1999), and r2. In this review we refer to the region that gives rise to the midbrain and cerebellum and that includes the mesencephalon and anterior metencephalon (r1) as the mes/met region.

Quail-chick transplantation experiments showed that development of the mes/met region could be regulated by a group of organizer cells located at the mid/hindbrain junction (for review, see Alvarado-Mallart 1993). Subsequently, several secreted growth factor genes were found to be expressed in the isthmus, and a number of genes encoding transcription factors were found to be expressed more broadly in the mes/met (Wassef & Joyner 1997, Joyner et al. 2000) (Figure 1). Gain- and loss-of-function studies over the past 10 years have demonstrated that these genes are indeed critical regulators of vertebrate mes/met development. Significantly, Fibroblast growth factor-8 (FGF8), which is expressed in the isthmus, is both necessary for mes/met development and can induce ectopic midbrain or cerebellum development. Furthermore, the junction between the expression domains of two transcription factors, Otx2 and Gbx2, defines the position of the cells with organizer function. Finally, a complicated set of genetic interactions involving these and other growth factors and transcription factors regulate both establishment of the mes/met and subsequent A/P patterning of the midbrain and cerebellum.

EXPRESSION PATTERNS OF MANY MES/MET GENES ARE CONSISTENT WITH THE MID/HINDBRAIN JUNCTION CONTAINING AN ORGANIZER

An organizer, such as the well-known Spemann’s organizer, is a group of cells that produce signals that can “organize” neighboring cells into well-patterned and functional structures (for a review, see Nieto 1999). One important characteristic shared by all known organizers is that they can direct competent tissues to take on new fates that are normally taken only by their native neighbors. Meinhardt (1983) studied the formation of several well-known organizers and found that they were all formed at points of apposition of two or more differentially specified tissues. Therefore, he proposed a model in which determinants from two (or more) adjoining compartments acting on the same border cells lead to the induction of expression in the border cells of a patterning signal(s), which in turn, function(s) to sharpen the border and guide the patterning of the adjacent compartments.

The expression patterns of a number of mes/met genes during early development are consistent with patterning of the region being regulated in a manner similar to that predicted by the model proposed by Meinhardt (1983). Two differentially specified regions of the neural plate, anteriorly located Otx2-expressing tissue and posteriorly located Gbx2-expressing tissue, are found to confront each other in
mice as early as E7.5 (Wassarman et al 1997). Expression of another transcription factor gene \( Pax2 \) is then initiated at the presomite stage (E7.5) surrounding the site of confrontation, followed by similar expression of several transcription factors (Figure 1, see color insert) (for a review, see Wassef & Joyner 1997, Joyner et al 2000). Most interesting, the expression patterns of the two secreted molecules, FGF8 and WNT1, become highly restricted to adjacent narrow transverse bands at the \( Otx2/Gbx2 \) border (Wilkinson et al 1987, Crossley et al 1995). A relatively fuzzy boundary between \( Gbx2 \) and \( Otx2 \) expression domains also becomes sharp at the 4- to 6-somite stage, and this boundary colocalizes with the mid/hindbrain junction that in mice is morphologically clear by E9.5. Genes such as \( En1, En2, \) and \( Pax5 \) continue to be expressed in both the mesencephalon and the metencephalon after E9.5 (Figure 1). This scenario of gene expression correlates well with the “organizer model” proposed by Meinhardt (1983). Indeed, numerous transplantation experiments with chicks have shown that the mid/hindbrain junction has an organizing activity (for a review, see Alvarado-Mallart 1993, Joyner et al 2000). We review in more detail the expression patterns of various mes/met genes mainly in mice in this section and then discuss the experiments that revealed the organizing function of the isthmus.

**Otx2 and Gbx2**

The transcription factors OTX1 and -2 are homologues of the Drosophila orthodenticle protein (Simeone et al 1992). Prior to gastrulation, \( Otx2 \) is expressed throughout the epiblast and in the anterior visceral endoderm (Simeone et al 1992, 1993). As the primitive streak forms, \( Otx2 \) expression becomes progressively restricted to an anterior region of the mouse embryo in all three germ layers and in the visceral endoderm. \( Otx1 \) expression begins at the 1- to 3-somite stage in the anterior neuroectoderm. Subsequently, the caudal boundaries of \( Otx1 \) and -2 expression are located in the isthmic constriction in mouse embryos. Fate mapping experiments with chicks have provided evidence that the caudal limit of \( Otx2 \) expression as early as Hamburger & Hamilton (HH) stage 10 (10 somites) (Hamburger & Hamilton 1992) marks the boundary between the midbrain and hindbrain (Millet et al 1996).

At E7.5, the homeobox gene \( Gbx2 \) is expressed in all three germ layers in the posterior region of a mouse embryo (Bouillet et al 1995, Wassarman et al 1997). Subsequently, its expression in the CNS becomes restricted to the anterior hindbrain by E8.5. At E9.5, \( Gbx2 \) expression is restricted to the anterior metencephalon and four longitudinal lateral stripes through the entire length of the hindbrain and spinal cord. Starting at E10.0, \( Gbx2 \) expression in the CNS is also found in precursors of the thalamus in the dorsal diencephalon.

**Fgf8, Fgf17, and Fgf18**

\( Fgf8 \) is expressed in many locations of a developing mouse embryo at different stages, which suggests that it has multiple roles in regulating cell proliferation and
differentiation in diverse processes (Heikinheimo et al 1994, Crossley & Martin 1995). In mouse mes/met development, Fgf8 is initially expressed in a broad region in the prospective anterior hindbrain starting at the 3-somite stage. This expression quickly becomes more intense and more restricted to a narrow ring just caudal to the mid/hindbrain junction and persists until E12.5.

More recently, it has been reported that two additional mouse Fgf genes, Fgf17 and Fgf18, which share very high sequence homology with Fgf8 (63.7% and 56.8%, respectively, at the amino acid level), are also expressed in the anterior hindbrain region at E8.5 (Maruoka et al 1998, Xu et al 1999). However, it seems that their expression levels at early somite stages are weaker than that of Fgf8. Furthermore, Fgf17 expression is broader than that of Fgf8, it occurs in both the mesencephalon and the metencephalon, and it persists until at least E14.5, a stage when Fgf8 is no longer expressed. The fact that Fgf8 expression seems stronger at early somite stages and the fact of the severe mes/met phenotype of Fgf8 loss-of-function mutants (see below) suggest that FGF8 is the major FGF molecule that is essential for early patterning of this region.

**Wnt1, Lmx1b, En1/2, and Pax2/5**

Wnt1 is a homologue of the Drosophila segmentation gene wingless and encodes a secreted signaling molecule (for a review, see Nusse & Varmus 1992). En1 and En2 are homologues of the Drosophila segmentation gene engrailed and encode homeodomain-containing transcription factors (Joyner & Martin 1987). Pax2 and Pax5 are the homologues of the Drosophila pair-rule gene paired and encode paired-domain-containing transcription factors (Gruss & Walther 1992). Lmx1b is a gene that encodes a LIM homeodomain-containing transcription factor. Lmx1b expression has been described in detail only from chick brain (Yuan & Schoenwolf 1999, Adams et al 2000). It is first expressed broadly in the caudal forebrain, midbrain, and hindbrain. By HH stage 10, the expression is restricted to the dorsal and ventral midline of the midbrain, overlapping with Wnt1 expression. Lmx1b is also expressed in the isthmus at this stage, and its expression overlaps with both the Wnt1 and Fgf8 domains.

Pax2 expression in mice begins at the presomite stage in cells surrounding the Otx2/Gbx2 boundary (Rowitch & McMahon 1995). Wnt1 and En1 expression then initiates within the Pax2 domain at the 1-somite stage, with Wnt1 expression restricted to Otx2-positive midbrain cells, and En1 expression in the entire mes/met (Davis & Joyner 1988, Rowitch & McMahon 1995, Liu & Joyner 2001). En2 and Pax5 expression initiates around the 5-somite stage across the Otx2/Gbx2 boundary (Davis & Joyner 1988, Adams et al 1992, Song et al 1996). The expression patterns of all these genes are dynamic in the mes/met region. For example, Wnt1 is expressed in the entire mesencephalon at early somite stages and soon becomes restricted to a narrow ring rostral to the mid/hindbrain junction, as well as being expressed along the dorsal midline of the midbrain and caudal diencephalon, caudal hindbrain, and spinal cord. Transient expression of Wnt1 in the ventral midline
of the midbrain and diencephalon is also seen at E9.5–E10.5. Pax2 expression also becomes restricted to the isthmus by E9, just caudal to the Wnt1 expression domain. In contrast, the expression of En1, En2, and Pax5 remains relatively broad on both sides of the mid/hindbrain junction (Figure 1)
Figure 2  The isthmic region can act like a midbrain/cerebellum organizer. (A) When the entire mesencephalic vesicle is inverted in the chick at HH stage 10, a bicaudal midbrain is formed, and the adjacent caudal forebrain is transformed into an ectopic midbrain. (B) Transplantation of isthmic tissue can induce ectopic expression of mes/met genes, such as En2 and ectopic mes/met development. The strongest endogenous En2 expression is in the mid/hindbrain junction, and it decreased on both sides of the junction. (C) The apposition of caudal rhombomere 1 (r1) tissue and midbrain or caudal forebrain tissue leads to induction of Fgf8 expression. T, telencephalon; D, diencephalon; Mes, mesencephalon; Met, metencephalon; My, myelencephalon.
These results suggest that formation of an isthmic organizer might result from an interaction between midbrain and anterior hindbrain tissues. Furthermore, Otx2 and Gbx2 might be involved in this process, given the correlation between the isthmic organizer and the Otx2/Gbx2 border. Nevertheless, Irving & Mason (1999) suggested that there must be other genes involved in Fgf8 induction because when r2 tissue, which also expresses Gbx2, is placed adjacent to midbrain tissue, no Fgf8 is induced at the border. However, compared with expression in r1, Gbx2 expression in r2 appears weaker and restricted dorsally and, thus, may not be strong enough to induce Fgf8 expression.

Hidalgo-Sanchez et al (1999) also addressed whether juxtaposition of Otx2-positive and Gbx2-positive cells could induce an organizer using transplants. They showed that when Otx2-positive diencephalic tissue is transplanted into Gbx2-positive r1, Gbx2 is induced and Otx2 is repressed in the grafted cells that contact the Gbx2-positive host r1 tissue. Fgf8, En2, Wnt1, and Pax2 are also induced in the grafts at the new Gbx2/Otx2 border. The only complication with these studies, as mentioned by the authors, is that transient contact between the graft and Fgf8-expressing host cells in the isthmus is hard to avoid. Therefore, it is difficult to distinguish whether the mes/met gene expression that is induced in grafted cells is solely due to the initial confrontation between Gbx2- and Otx2-expressing cells and/or to the inductive function of Fgf8 in the host.

FIBROBLAST GROWTH FACTORS ARE CRITICAL FOR MIDBRAIN AND CEREBELLAR DEVELOPMENT

General Properties of Fibroblast Growth Factors and Their Receptors

The mouse fibroblast growth factor (FGF) family currently consists of at least 19 members, named FGF1–19 (for a review, see Basilico & Moscatelli 1992, Coulier et al 1997). Most FGFs are secreted proteins and are expressed not only in transformed tumor cells but also in many signaling centers during normal vertebrate embryonic development. It has been shown in Xenopus that FGFs are involved in inducing mesoderm formation and posterior neural fate (for a review, see Slack et al 1996). The importance of the Fgf genes in mouse development has been demonstrated by the analysis of null mutants for several Fgf genes (Mansour et al 1993, Hebert et al 1994, Feldman et al 1995, Min et al 1998, Sekine et al 1999, Sun et al 1999).

The best-characterized FGF receptors are high-affinity, signal-transducing tyrosine kinase coupled receptors, which include at least four members, called FGFR1–4. Many FGFR isoforms are generated by alternative splicing of the mRNAs. The dimerization of the FGFRs on ligand binding triggers the activation of the receptors and downstream signaling events. Each kind of FGF molecule is a preferential ligand for a specific FGFR, and each kind of FGFR can bind several different FGF
molecules (Basilico & Moscatelli 1992). It has been suggested that heparan-sulfate proteoglycans may help to stabilize the FGF-FGFR complex, and structural analysis has provided evidence for such a function (Plotnikov et al 1999).

Comparative expression studies have been done in medaka fish and in chicks to investigate the expression patterns of different Fgfr genes as a means of gaining information as to which gene(s) could be involved in mes/met development. In medaka fish, Fgfr2, 3, and 4 are expressed in the midbrain and anterior hindbrain; however, Fgfr2 is the only gene that is expressed in cells adjacent to, and on both sides of, the mid/hindbrain junction at early somite stages (Carl & Wittbrodt 1999). In contrast, chick Fgfr1, 2, and 3 are all expressed throughout the presumptive neural plate during early neural induction (∼HH stage 5) and elevated in the anterior neural plate before neuromeres form (∼HH stage 8) (Walshe & Mason 2000). However, when neuromeres form (HH stage 9–11), only Fgfr1 is expressed in the mes/met region. Detailed comparative study of the expression patterns of Fgfrs in mouse brain at early stages has not been reported, although it has been shown that Fgfr1 and -2, but not -4, are expressed in embryonic brain (Stark et al 1991; Yamaguchi et al 1992; Orr-Urtreger et al 1993; Peters et al 1992, 1993). Our preliminary studies, however, show that in mice Fgfr1 is weakly expressed throughout the CNS and Fgfr2 and -3 are largely excluded from the posterior midbrain and r1 at E8.5–E9.5 (A Liu, AL Joyner, unpublished data). Taken together, the expression patterns of the FGF receptors are dynamic, and at least FGFR1 and 2 could be involved in mes/met development. All four Fgfr genes have been mutated in mice by gene targeting, but mes/met patterning defects have not been reported for any Fgfr mutant, either in mutant embryos or in chimeras containing mutant cells in the mes/met. It is possible that the FGFRs have overlapping functions (Deng et al 1994, 1996; Yamaguchi et al 1994; Colvin et al 1996; Ciruna et al 1997; Arman et al 1998, 1999; Weinstein et al 1998; Xu et al 1998).

Fgf8 was first identified as an oncogene responsible for androgen-dependent growth of mammary gland carcinoma cells and was initially called androgen-induced growth factor (Tanaka et al 1992). FGF8 has seven isoforms (Crossley & Martin 1995, MacArthur et al 1995b), and biochemical and cell transformation assays have demonstrated that most of the FGF8 isoforms activate the c isoforms of FGFR2, -3, and -4 and can transform NIH3T3 cells in culture. Among the FGF8 isoforms, FGF8b has the strongest affinity for the three receptors and has the strongest ability to transform NIH3T3 cells, whereas FGF8a shows little affinity to the receptors and very weak transforming activity (MacArthur et al 1995a,b; Blunt et al 1997).

Fgf8 is Required for Normal Development of the Vertebrate Mes/Met Region

A series of mouse Fgf8 mutant alleles have been generated by gene targeting in ES cells (Meyers et al 1998). A null mutation in which the second and third exons are deleted [Fgf8Δ2,3/Δ2,3] leads to a gastrulation defect (Meyers et al 1998, Sun et al 1999). Mice homozygous for a hypomorphic allele, Fgf8neo, which contains
an insertion of a neo cassette into the first intron, or trans-heterozygous for the hypomorphic and null alleles, lack most of the midbrain and cerebellum, indicating Fgf8 is required for mes/met development (Meyers et al 1998). However, the loss of mes/met tissue either could be due to the Fgf8 expression in the metencephalon or could be secondary to an early mild gastrulation defect. This will be addressed by analyzing conditional mutants in which the Fgf8 gene is mutated only in mes/met precursor cells.

Zebrafish ace (acerebellar) mutants lack the isthmus and cerebellum and have a point mutation in a zebrafish homologue of Fgf8 that leads to production of a truncated form of protein (Brand et al 1996, Reifers et al 1998). The midbrain in ace mutants is expanded caudally and has impaired polarity along both the A/P and dorsal/ventral axes (Picker et al 1999). Analysis of marker gene expression showed that mes/met expression of eng, wnt1, and pax2.1 was initiated properly at the end of gastrulation but was then progressively lost. The apparent milder phenotype in ace mutants compared with that of Fgf8 hypomorphic mouse mutants could be because either the ace mutation is a hypomorphic Fgf8 allele or another Fgf family member can partially compensate for the loss of Fgf8 in fish.

FGF8b-Soaked Beads Can Induce Mes/Met Genes and Ectopic Midbrain and Cerebellar Structures in Regions of Chick Brain

A striking finding is that FGF8b-soaked beads inserted into either the posterior diencephalon (prosomere 1 and 2) or anterior mesencephalon of stage 9–12 chicken embryos induce En1, En2, Wnt1, and Pax2 expression within 24–48 h (Crossley et al 1996, Shamim et al 1999). An interesting recent finding is that Sprouty2, which encodes a putative FGF antagonist, is also induced by FGF8-soaked beads, but more rapidly and within an hour (Minowada et al 1999, Chambers et al 2000). Normally, Sprouty1 and -2 are expressed around the isthmus, and thus they could normally play a role in modulating FGF signaling from r1 (Minowada et al 1999, Chambers et al 2000, Chambers & Mason 2000).

The long-term consequence of insertion of FGF8-soaked beads into certain brain regions is induction of ectopic mes/met-derived structures. Both the rostral mesencephalon and caudal diencephalon can be transformed into caudal mesencephalic structures, with an ectopic isthmus close to the FGF8 bead (Figure 3A). FGF8b-soaked beads placed in prosomere 1 or the anterior midbrain were found to induce Gbx2 expression and repress Otx2 in cells around the beads (Irving & Mason 1999, Martinez et al 1999). Furthermore, in some cases the Otx2-negative cells in contact with the beads can form an outgrowth that protrudes from the neural tube (Martinez et al 1999). The outgrowth later develops proximally into isthmic nuclei and distally into cerebellum-like structures, which suggests that one of the normal functions for FGF8 might be to repress Otx2 expression in the anterior hindbrain and allow, or induce, cerebellum development (Figure 3A). Recently, it was shown that although FGF8-soaked beads can induce En2 and Pax2 in r2, it does not lead to formation of ectopic structures (Irving & Mason 2000) (Figure 3A).
Figure 3  FGF8 can induce ectopic mid/hindbrain development in chicks and mice. In the diencephalon and midbrain of both chicken embryos and mouse brain explants, FGF8-soaked beads can induce mid/hindbrain gene expression and, in chicks, ectopic development of midbrain and cerebellar structures. FGF8-soaked beads inserted into the anterior forebrain do not induce mes/met gene expression or mes/met structures. In the anterior hindbrain, FGF8 can induce the expression of En2, Pax2, and Wnt1 but no induction of mes/met structures. p, prosomere; r, rhombomere.

It is interesting that Shamim et al (1999) found that both FGF4- and FGF8-soaked beads when implanted into the midbrain can induce Fgf8 expression, with FGF4 inducing expression in a broader region. In contrast to what was described by Crossley et al (1996), endogenous Fgf8 was not found to be induced by FGF4 or -8 in the posterior forebrain. However, weak expression of Fgf8 was induced in the anterior midbrain when FGF4-, but not FGF8-, soaked beads were inserted into the caudal forebrain. Taken together, these chick studies using FGF-soaked beads show that FGF8 is sufficient to induce midbrain, isthmic, and cerebellar structures. However, not all brain regions are equally competent to respond to FGF8.

Wnt1-Fgf8b Transgenic Mouse Embryos Show an Early Transformation of the Midbrain and Posterior Forebrain into an Anterior Hindbrain Fate

To study the role of Fgf8 in mouse mes/met patterning, transgenic embryos (Wnt1-Fgf8) have been produced in which Fgf8 is expressed under the control of a Wnt1 regulatory element such that Fgf8 expression is extended into the entire mesencephalon at early somite stages and along the dorsal midline into the caudal diencephalon after E9.0 (Lee et al 1997, Liu et al 1999). Fgf8b-expressing embryos show severe exencephaly and die shortly after E15.5 (Liu et al 1999). Detailed
marker gene expression studies at E9.5 indicate that the midbrain and posterior forebrain do not form in such embryos, and the hindbrain is found adjacent to the remaining anterior forebrain. This phenotype appears to result from an early transformation of the midbrain and posterior forebrain into an anterior hindbrain fate, as indicated by an expansion of Gbx2 expression, repression of Otx2 expression, and a rostral shift of Fgf8 expression by the 5- to 7-somite stage, soon after the transgene is first expressed. In contrast, ectopic expression of Fgf8a mainly causes overproliferation of the midbrain and caudal diencephalon and up-regulation of En2, as well as EphrinA2, a prospective downstream target of En2 (Logan et al 1996) in the anterior dorsal midbrain (Lee et al 1997, Liu et al 1999). This difference between the phenotypes is consistent with results obtained in tissue culture systems demonstrating that FGF8b is a stronger isoform than FGF8a.

The patterning function of FGF8 in mouse mid/hindbrain development has also been investigated in brain explant culture systems (Shimamura & Rubenstein 1997, Liu et al 1999, Liu & Joyner 2001). Similar to the in vivo experiments in mice, FGF8 can induce En1, En2, Pax5, and Gbx2, but not Fgf8, in both midbrain and forebrain explants. Pax6, a diencephalon gene (Walther & Gruss 1991), is repressed by FGF8 in caudal forebrain explants but Otx2 is repressed only in midbrain explants. It is interesting that both Lmx1b and Wnt1 are initially induced broadly by FGF8 in midbrain explants, but subsequently Wnt1 is excluded from cells adjacent to the FGF8 source. These explant assays provide a simple in vitro model system to study the early responses of mes/met genes to FGF8 in normal and mutant brain tissue.

Fgf17 Collaborates with Fgf8 in Patterning the Anterior Cerebellum

As discussed above, following initiation of Fgf8 expression, Fgf17 and Fgf18 are also expressed in the mid/hindbrain region. Mice lacking Fgf17 have only a mild cerebellar defect, with a decrease in precursor cell proliferation in the medial part of the cerebellum after E11.5 (Xu et al 2000). Fgf8Δ2,3/+; Fgf17−/− mutant embryos have a more severe phenotype than do Fgf17−/− mutants, indicating that FGF17 and FGF8 have partially overlapping functions in development of the anterior hindbrain.

OTX1, OTX2, AND GBX2 ARE REQUIRED TO POSITION THE ISTHMUS ORGANIZER AND FOR LATER MIDBRAIN OR CEREBELLUM DEVELOPMENT, RESPECTIVELY

Multiple Essential Roles for Otx2 in Anterior Patterning

Otx2 null mutant embryos have severe gastrulation defects resulting in deletion of the rostral part of the neural tube anterior to r3 (Acampora et al 1995, Matsuo et al
1995, Ang et al 1996, Rhinn et al 1998) (Figure 4B). The expression patterns of marker genes in Otx2 mutants revealed that the forebrain and midbrain are absent as early as E7.75 and the metencephalon is deleted by E8.5. Chimeric embryos were made to distinguish the functions of OTX2 in the visceral endoderm and epiblast (Rhinn et al 1998). Chimeras composed mostly of wild-type cells in the epiblast and Otx2−/− cells in the visceral endoderm have a phenotype similar to

Figure 4  Otx2 is required in the visceral endoderm for normal gastrulation and initiation of anterior brain development and in the epiblast for the maintenance of anterior brain structures. (A) Otx2 is expressed in both the epiblast and visceral endoderm of wild-type embryos, and it guides normal development of anterior structures. (B) Otx2−/− embryos that lack Otx2 expression in both the epiblast and visceral endoderm fail to gastrulate normally and lose structures anterior to rhombomere (r)3 at an early stage. (C) In chimeric embryos in which Otx2 is only expressed in the visceral endoderm, or in embryos in which hOTX1 replaces Otx2 and is present only in the visceral endoderm, forebrain and midbrain structures initially form at early stages, but are lost subsequently.
that of Otx2 null mutants. In contrast, chimeras in which the epiblast is mostly composed of Otx2−/− cells and the visceral endoderm wild-type cells undergo nearly normal gastrulation but fail to maintain the forebrain and midbrain after E8 (Figure 4C). Taken together, these studies show that the first requirement for Otx2 is in the extraembryonic endoderm and that Otx2 plays a cell nonautonomous role in the anterior visceral endoderm in inducing the formation of anterior head structures. This role of Otx2 likely reflects a function in generating an organizing center in the anterior visceral endoderm (for a review, see Beddington & Robertson 1999). Otx2 also has a second role in the epiblast, where it is required to maintain the survival and/or identity of rostral brain structures.

Distinct functions for Otx2 in the visceral endoderm and the epiblast were further demonstrated by analyzing a mouse mutant in which a human OTX1 cDNA was inserted in place of the endogenous Otx2 gene, such that OTX1 mRNA is only translated in the visceral endoderm and not the epiblast. Embryos homozygous mutant for this Otx2 allele undergo nearly normal gastrulation and initial anterior brain development but subsequently lose anterior brain structures, including the forebrain and midbrain by E8.5 (Acampora et al 1998; Y Li, AL Joyner, unpublished data) (Figure 4C). Nevertheless, Fgf8 and other mes/met genes are expressed even at late stages, but in overlapping domains at the rostral tip of the mutant embryo.

Otx1+/−; Otx2+/− (Suda et al 1997) or Otx1−/−; Otx2+/− (Acampora et al 1997) mouse embryos have the striking phenotype that the midbrain and caudal forebrain are replaced with cerebellar tissue. Gene expression analysis in such mutants indicates that at early somite stages, expression of all the mes/met genes is initiated normally. However, Fgf8 expression soon fails to be restricted to the rostral hindbrain region and expands into more rostral brain regions (Figure 5B). This expansion is followed by a retraction of Otx2 expression and a rostral shift in the En1/2, Wnt1, and Gbx2 domains. Therefore, the midbrain and caudal forebrain are transformed into an anterior hindbrain fate during early neural patterning, when the dose of OTX proteins is not sufficient to maintain their normal fate. These studies indicate that the caudal limit of Otx2 could be critical for determining the position of the isthmic organizer, or the Fgf8 expression domain, and a high level of Otx2 is required for midbrain development.

The above Otx1/2 compound mutant phenotype showed that both Otx genes have overlapping functions late in development of the midbrain and caudal forebrain. In contrast, when mouse Otx1 is inserted into the Otx2 locus such that it is expressed like Otx2 in the embryo, Otx1 can rescue the gastrulation defect of Otx2 mutant embryos but not the deletion of most of the rostral brain region at early somite stages (Suda et al 1999). Thus, the two OTX proteins appear to have different functions during early specification of forebrain and midbrain precursors.

Finally, recent gain-of-function studies in which Otx2 was ectopically expressed in the dorsal metencephalon from early somite stages provided further evidence that Otx2 is not only required to position the organizer and direct midbrain development, it also is sufficient to direct both these processes. When Otx2 was expressed in the
Figure 5  *Otx2* and *Gbx2* regulate mes/met development differently. (A) Schematic representation of a wild-type E9.5 mouse brain. *B* In *Otx1<sup>+/−</sup>; *Otx2<sup>+/−</sup>* embryos, the midbrain and posterior forebrain are transformed into an anterior hindbrain. (C) Ectopic expression of *Otx2* in the dorsal metencephalon from the *En1* locus results in a caudal expansion of the midbrain and partial deletion of the metencephalon. (D) In the absence of *Gbx2*, rhombomeres (r)1–r3 do not form and the midbrain expands caudally, such that the midbrain directly abuts r4. (E) Ectopic expression of *Gbx2* in the posterior midbrain of transgenics from E8.5 leads to a smaller midbrain and an enlarged metencephalon at E9.5.
dorsal anterior hindbrain of mouse embryos, the caudal midbrain was extended and the cerebellum partially deleted (Broccoli et al 1999) (Figure 5C). These morphological changes were accompanied by a repression of Fgf8 and Gbx2 in the dorsal anterior most hindbrain from early stages. Similar results were obtained in chicken embryos by electroporating anterior hindbrain tissue with an Otx2 expression construct (Katahira et al 2000).

**Gbx2 is Required for Normal Isthmic Organizer Function and Position as well as Anterior Hindbrain Development**

Mouse Gbx2 null mutants die at birth with loss of anterior hindbrain (r1-r3) derivatives, which suggests that Gbx2 might be involved in maintaining the identity and/or survival of the anterior hindbrain (Wassarman et al 1997). Gene expression analysis between E9.5 and E12.5 showed that the caudal Otx2 expression border was adjacent to that of the r4 marker Hoxb1, showing an early deletion of the anterior hindbrain. Furthermore, unlike in wild-type embryos, where Fgf8 expression does not overlap with that of Otx2 and Wnt1 in the isthmus, the expression domains of the three genes overlap in Gbx2 mutant embryos. More interesting, the morphology of the posterior midbrain is not normal, which suggests that Gbx2 also contributes to midbrain patterning, possibly indirectly through a function in regulating the organizer.

More recently, it has been shown that in Gbx2/−/− embryos, the Otx2 domain is greatly expanded caudally by the 4- to 6-somite stage, and its caudal boundary is never sharp (Millet et al 1999) (Figure 5D). The Wnt1 and Fgf8 expression domains also shift caudally and Fgf8 overlaps Wnt1 and Otx2. These expression studies demonstrated that an early phenotype in Gbx2 mutants is a transformation of presumptive r1–r3 cells into a midbrain fate. In a complementary manner, misexpression of Gbx2 in the midbrain from a Wnt1 promoter (Wnt1-Gbx2 transgenics) at early somite stages results in a repression of Otx2 expression and a rostral shift of isthmic Wnt1 and Fgf8 expression (Millet et al 1999) (Figure 5E). Endogenous Gbx2 expression is also induced by the transgene. The consequence of the early changes in gene expression is a reduction of the midbrain and expansion of the hindbrain at E9.5. Taken together, these two types of mutant studies suggest GBX2 functions to antagonize Otx2 expression and midbrain development, in addition to being involved in positioning the isthmic organizer.

It is striking that midbrain development begins to recover soon after the transgene expression is lost at E9.5, and by E12.5 the brains of Wnt1-Gbx2 embryos look indistinguishable from those of wild types. Thus, the midbrain is still plastic at E9.5 in mice and can recover from early transient Gbx2 misexpression. In contrast, transient ectopic expression of Gbx2 in the chick midbrain at HH stage 10 leads to repression of Otx2 expression only transiently in rostral midbrain regions but permanently in caudal regions. Furthermore, caudal midbrain misexpression of Gbx2 results in a rostral shift of the mid/hindbrain junction and a smaller midbrain at late stages (Katahira et al 2000).
Taken together, the gain-of-function and loss-of-function studies of both Gbx2 and Otx2 mutants provide strong evidence that these genes act antagonistically, and that they are also required for anterior hindbrain and midbrain development, respectively. Furthermore, the two genes are involved in positioning and normal functioning of the isthmic organizer. However, the two genes are not required to initiate expression of Fgf8 and other mes/met genes because in embryos lacking both Otx2 and Gbx2, all the genes are expressed in the rostral CNS (Y Li, AL Joyner, manuscript in preparation).

WNT1, EN1/2, AND PAX2/5 ARE ESSENTIAL FOR NORMAL MES/MET DEVELOPMENT

**Wnt1 is Required for Mes/Met Development and Positioning of the Caudal Otx2 Border**

Loss of Wnt1 function leads to death at birth with an early large-scale deletion first of the mesencephalon and then of r1 (McMahon & Bradley 1990, Thomas & Capecchi 1990, Mastick et al 1996). Furthermore, Enl expression is initiated normally in Wnt1 mutants but is lost by the 27-somite stage, before the loss of the r1 tissue (McMahon et al 1992). One possible interpretation of these studies is that one function of Wnt1 is to maintain En1 expression. Consistent with this, a transgene with a Wnt1 enhancer driving En1 expression was found to rescue most of the Wnt1 mutant phenotype (Danielian & McMahon 1996). However, in Wnt1 mutants, Fgf8 expression in the metencephalon is lost by the 14-somite stage, which suggests that WNT1 more directly regulates Fgf8 expression in this region and that loss of En1 could be secondary to the loss of Fgf8 (Lee et al 1997). Because En1 misexpression in chicks leads to induction of Fgf8 in both midbrain and diencephalon (see below), transgene driven En1 expression in Wnt1 mutants might lead to maintenance of Fgf8 expression and, thus, rescue of the phenotype.

Analysis of a Wnt1 mutant allele, Swaying, that appears to be a hypomorph has revealed a function for Wnt1 in maintaining a stable mid/hindbrain junction (Thomas et al 1991, Bally-Cuif et al 1995). Swaying contains a frame-shift mutation that causes premature termination of WNT1 protein, such that a large portion of the C terminus of WNT1 is deleted. Wnt1<sup>sw/sw</sup> mutant mice have a less-severe brain phenotype compared with targeted Wnt1 null mutants, with the midbrain and hindbrain regions being only partially reduced. It is striking that analysis of the relatively late (E9.0 and onward) midbrain and hindbrain phenotype of Swaying mutants showed that formation of the straight Otx2 and Wnt1 caudal expression borders was perturbed, and small ectopic islands of Otx2-positive (mes) or Otx2-negative (met) cells were located inappropriately within the hindbrain or midbrain, respectively. Moreover, Wnt1 expression was induced at the border of the ectopic mes and met islands, consistent with the idea that
interactions between mes (Otx2 positive) and met (Otx2 negative) cells positively regulate organizer gene expression, as reflected by Wnt1 expression at the boundary.

Wnt1 gain-of-function studies have failed to demonstrate that Wnt1 is sufficient to alter mes/met patterning. In chicks, Wnt1 misexpression in the midbrain at HH stage 9–12 does not result in any phenotype, although in the telencephalon it causes overproliferation (Adams et al 2000). Furthermore, ectopic expression of Wnt1 in the ventricular zone of the neural tube posterior to the r6/r7 boundary between E8.5 and E10.5 results only in expansion of the ventricular zone and has no obvious changes in patterning (Dickinson et al 1994).

A recent study showed that ectopic Lmx1b expression leads to up-regulation of Wnt1 expression in chick midbrain (Adams et al 2000). Furthermore, Lmx1b is induced in chick caudal forebrain by FGF8-soaked beads. This study suggests that Lmx1b might be in the same pathway as Wnt1 downstream of Fgf8 and could play a role in early mes/met patterning. The requirement for Lmx1b in mes/met development has not been reported, although Lmx1b mouse mutants have been generated (Chen et al 1998).

**En** Genes are Required for Mes/Met Development and Likely Repress Forebrain Development

En1 mutant mice have a deletion including most of the midbrain and cerebellum, a phenotype similar to, but milder than, that of Wnt1 null mutants (Wurst et al 1994). Unlike En1 mutants, En2 mutants are viable and show only subtle defects in cerebellar development, which include an early reduction in size of the cerebellar anlage and later abnormal foliation (Joyner et al 1991; Millen et al 1994, 1995). This suggests at least one role for En2 is in patterning the cerebellum. Two experiments have demonstrated that the two EN proteins can carry out similar functions in the brain, given correct temporal and spatial expression of the genes. First, when the En1 coding sequences are replaced with those of an En2 cDNA using a gene-targeting approach, the mes/met phenotype of En1 mutants is rescued (Hanks et al 1995). It is interesting that En2 cannot fully rescue all the limb defects seen in En1 mutants, and it is significant that Drosophila en can rescue the En1 brain defects but none of the limb defects (Hanks et al 1998). Second, double mutants of the two En genes have a more severe early phenotype than do either of the single mutants (Liu & Joyner 2001). In such double mutants, Wnt1, Fgf8, and Pax5 expression is initiated by the 5-somite stage but fails to be maintained in the mes/met by the 11-somite stage. Taken together, these studies show that an EN protein must be present during early somite stages when En1, but not En2, is normally expressed. Without early expression of an EN protein, further mes/met development is greatly compromised.

Based on a correlation between the decreasing EN protein gradient from posterior to anterior midbrain and the A/P polarity of the midbrain before and after 180° rotation of the anterior midbrain in chicks, it was suggested that the En genes
could be involved in setting up the topographic axon projection map of the midbrain (Itasaki et al 1991). This suggestion was addressed by expressing En genes ectopically in the anterior midbrain in both chicken and mouse embryos. In chicks, ectopic expression of En1 or En2 in the rostral midbrain induces EphrinA2 and EphrinA5, two genes involved in repulsing retinal temporal axons in the posterior midbrain and ectopic projections of retinal nasal axons in the rostral tectal tissue (Friedman & O’Leary 1996, Itasaki & Nakamura 1996, Logan et al 1996). In mice, ectopic expression of En1 driven by a Wnt1 enhancer causes ectopic EphrinA2 expression in the dorsal midline of the rostral midbrain (Lee et al 1997). Thus, the expression level of En genes can directly, or indirectly, determine the A/P positional cues of midbrain cells.

In chicks, ectopic expression of En2 in the diencephalic region results in expansion of tectal tissue at the expense of caudal diencephalic tissue (Araki & Nakamura 1999). It is striking that this phenotype is accompanied by repression of the diencephalon gene Pax6 prior to induction of the mes/met genes Pax5, Wnt1, and Fgf8. Because it has been shown that the EN family of proteins are transcriptional repressors (Jaynes & O’Farrell 1991, Smith & Jaynes 1996, Hanks et al 1998), it is possible that EN is normally involved in setting up the mid/forebrain boundary by repressing forebrain genes. Consistent with this hypothesis, a mutant form of EN2 in which a single amino acid substitution disrupts its interaction with the corepressor, GROUCHO, cannot transform the forebrain or induce mid/hindbrain gene expression. Furthermore, when a chimeric EN protein in which the repression domain of EN2 was replaced by the transactivation domain of VP16 was expressed in chick brain, the midbrain was greatly reduced and Pax6 expression expanded into the midbrain region (Araki & Nakamura 1999).

It has also been shown in medaka fish and frog embryos that ubiquitous expression of the fish En2 gene, Ol-eng2, can induce ectopic midbrain development and repress formation of diencephalic tissue, whereas expression of an Ol-eng2 gene with a mutation in the homeodomain does not produce such an effect (Ristoratore et al 1999). It is interesting to note that despite widespread expression of injected Ol-eng2, ectopic expression of fgf8, pax2, and endogenous eng2 is restricted only to regions of the posterior forebrain. Furthermore, the ectopic midbrain that is induced always has a reversed A/P polarity. Induction of an ectopic isthmic organizer by Ol-eng2 therefore seems to require cofactors that are expressed locally in the forebrain.

The Pax2/5 Genes have Overlapping Functions in Development of the Mes/Met

The Pax2 and Pax5 genes have been shown to be required for mouse mes/met patterning using loss-of-function mutants. Several mouse Pax2 mutant alleles have been generated and surprisingly they have different phenotypes. One targeted deletion allele that removes most of the coding sequences, referred to as Pax2<sup>ko</sup>, appears to show no mes/met A/P patterning defects, just a varied degree
of exencephaly, depending on the genetic background (Torres et al 1996, Schwarz et al 1997). In contrast, homozygotes for the spontaneous Pax2\textsuperscript{1Neu} allele, which contains a frame-shift mutation that truncates the protein, have a brain deletion that includes most of the mes/met region (Favor et al 1996). More recently, another mouse Pax2 allele was generated by gene targeting, which is thought to be a null allele, and homozygous embryos for this allele have an early deletion of the mes/met, similar to Pax2\textsuperscript{1Neu} mutants (Bouchard et al 2000). Pax5\textsuperscript{−/−} mutant mice, in contrast, have only a partial deletion of the inferior colliculi (posterior midbrain) and a slightly enlarged third lobe of the cerebellum (Urbanek et al 1994).

Overlapping functions of Pax2 and Pax5 have been demonstrated using double mutants. For example, Pax2\textsuperscript{ko/+}; Pax5\textsuperscript{−/+} and Pax2\textsuperscript{ko/ko}; Pax5\textsuperscript{−/−} mutants lack most of the midbrain and cerebellum (Schwarz et al 1997). Although heterozygous Krd mutants, which contain a 7-cM deletion that includes the Pax2 gene (Keller et al 1994), and Pax5\textsuperscript{+/-} mice are phenotypically normal, compound mutants Krd\textsuperscript{+/-}; Pax5\textsuperscript{+/-} and Krd\textsuperscript{+/-}; Pax5\textsuperscript{−/-} have deletions of the midbrain and cerebellum, similar to Pax2 null mutants (Urbanek et al 1997). Furthermore, Pax5 is able to rescue the brain phenotype of the Pax2 null allele by expressing it from the Pax2 locus. Therefore, like the EN proteins, a PAX2/5 protein must be expressed at early somite stages to sustain mes/met development. In addition, Pax6 expression, as well as the posterior commissure, a morphological landmark of the posterior diencephalon, are expanded posteriorly in Pax2/5 double mutants, which suggests that Pax2/5 might be involved in maintaining mes/met development by repressing forebrain development (Schwarz et al 1999).

The function of the PAX2/5/8 subfamily of Pax genes in brain patterning has also been studied in zebrafish. There are four genes in the Pax2/5/8 family in zebrafish, pax2.1, pax2.2, pax5, and pax8 (Lun & Brand 1998). Injection of a neutralizing antibody against PAX2.1 into zebrafish early embryos resulted in a deletion of most parts of the midbrain and cerebellum (Krauss et al 1992). Noi (no isthmus) mutants in which the zebrafish pax2.1 gene is mutated have a similar deletion of the caudal midbrain and cerebellum (Brand et al 1996). Expression of pax2.2, pax5, and pax8 is never detected in noi mutants, indicating that PAX2.1 regulates the other pax genes and that the noi mutant is actually equivalent to a pax2.1/2.2/5/8 quadruple mutant (Lun & Brand 1998). Similarly, Pax2 in mice has been shown to be involved in regulating Pax5 mes/met expression (Pfeffer et al 2000).

In noi mutants, eng2 and eng3 expression is not initiated normally, whereas fgf8 and wnt1 expression initiates normally but is lost before the mes/met tissue undergoes apoptosis (Pfeffer et al 1998). These changes in mes/met gene expression indicate that pax2.1 is necessary for the normal pattern of mes/met gene expression, as well as for the survival of mes/met tissue. The Pax genes also have been implicated in regulating expression of En2 in mice. Pax2/5/8 proteins were shown to bind to two DNA sequences in an En2 DNA enhancer fragment that is sufficient to direct expression of a lacZ reporter gene in the isthmus (Logan et al 1993, Song et al 1996). Furthermore, mutation of the Pax2/5/8 binding sites abolishes the reporter gene expression. Deletion of the two Pax2/5/8-binding sites
in the endogenous En2 gene by gene targeting, however, showed that the binding sites are required only for normal initiation, not maintenance, of endogenous En2 expression (Song & Joyner 2000).

Gain-of-function studies in chicks have shown that the Pax genes, like En1/2 and Fgf8, can induce midbrain development. Ectopic expression of either Pax2 or Pax5 in the diencephalon is sufficient to induce ectopic expression of mes/met genes, such as Fgf8 and En, and to transform diencephalic tissue into midbrain structures (Funahashi et al 1999, Okafuji et al 1999). Furthermore, Pax5, but not Pax2, misexpression in the anterior midbrain can induce Fgf8 and En2 expression, indicating that the two PAX proteins have developed some different functions during evolution.

CROSS-REGULATION BETWEEN THE MES/MET GENES UNDERLIES ORGANIZER FUNCTION AND MES/MET DEVELOPMENT

The expression studies and functional analyses of mes/met genes have begun to elucidate a set of complex cross-regulatory interactions between these genes during midbrain and cerebellum development. Some of these same gene families are also involved in regulatory networks in Drosophila. For example, the Pax homologue, prd, is required for initiation of en and wg expression in ectoderm segments; then en expression and wg expression become dependent on each other, and finally en is autoregulated (for a review, see Hooper & Scott 1992, Perrimon 1994). Some similar, but not identical, regulatory interactions are found during vertebrate mes/met development. As mentioned above, Pax2 expression precedes and overlaps with En1 and Wnt1 in mice, and genetic studies implicate Pax2/5 in regulating En1/2 expression in zebrafish and mice. In contrast to the situation in Drosophila, aspects of Wnt1 expression do not seem to require PAX2 function (Lun & Brand 1998, Schwarz et al 1999). There is also no evidence for autoregulation of the En genes (Logan 1993, Liu & Joyner 2001).

Several lines of evidence indicate that En and Wnt1 are involved in regulating each other’s expression, although it is not clear whether this is direct. As described above, in Wnt1 null mutant mice, En1 expression is initiated normally but lost, and in En1/2 double mutant embryos, Wnt1 expression is rapidly lost. Furthermore, when En1 expression is maintained in Wnt1 mutants, a nearly complete rescue of the Wnt1 brain phenotype is observed. In addition, ectopic expression of En can induce ectopic Wnt1 expression (Araki & Nakamura 1999, Ristoratore et al 1999), although the reverse does not seem to be true (Adams et al 2000).

Many gain- and loss-of-function studies have shown that Otx2 and Gbx2 are involved in a negative feedback loop (see above). We recently explored whether Gbx2 acts directly or indirectly to repress Otx2 using a midbrain explant system and FGF8-soaked beads (Liu & Joyner 2001). It is surprising that although Gbx2 was required for repressing Wnt1 expression, it was not required for repressing Otx2.
Therefore, FGF8 can regulate Otx2 expression through a Gbx2-independent pathway, and it is possible that Gbx2 can repress Otx2 through up-regulation of Fgf8.

Finally, as discussed above, FGF8 has been shown to be able to induce Pax2, Pax5, En1, En2, Wnt1, Lmx1b, and Gbx2 in midbrain and caudal forebrain tissue, as well as to repress Otx2. Nevertheless, the normal timing of gene expression shows that Fgf8 expressed in the metencephalon is unlikely to be responsible for inducing initial expression of these mes/met genes. Whether another FGF, possibly one expressed outside the neural tube, initiates mes/met gene expression is not clear. Fgf8 is, however, likely to be critical for modulating and maintaining expression of many mes/met genes after the 5-somite stage in mice.

PROSPECTS AND FUTURE DIRECTION

The many genetic studies we have described have revealed that multiple genetic pathways regulate early patterning of the vertebrate midbrain and cerebellum. An early step in patterning the mes/met seems to be a genetic interaction at the border of Otx2- and Gbx2-expressing cells, which determines where the midbrain and cerebellum will form. It is also clear that by the 10-somite stage, a self-sustaining genetic network is set up within the mes/met that is controlled centrally by an isthmic organizer. Furthermore, FGF8-like molecules alone can appropriately regulate the expression of most other mes/met genes and induce midbrain and cerebellum development. Thus, FGF8 and related factors are likely central to this self-sustaining network. It must, however, be more complicated because loss of En, Wnt1, or Pax2/5 function alone leads to a collapse of mes/met development.

A number of key questions nevertheless remain to be addressed. For example, none of the loss-of-function mutants described seems to lack initial expression of all other genes in the mes/met region, raising the question of what signal triggers the mes/met molecular pathway before the early somite stages. Also, the epistatic relationships among the known genes need to be established using new approaches because they have been hard to explore in null mutants owing to simultaneous early loss of expression of multiple genes. Another important and long-standing question is how the signals from the mid/hindbrain organizer are transmitted across the entire length of the midbrain and r1. Does a relay mechanism exist or are signals transmitted from the isthmus directly to cells at a distance? Finally, it has been shown that isthmic tissue cannot induce ectopic mes/met development in either the anterior forebrain (Martinez et al 1991) or the spinal cord (Grapin-Botton et al 1999), raising the question of what regulates the competence of cells to respond to isthmic organizing signals.

ACKNOWLEDGMENTS

We thank Sandrine Millet and Yuanhao Li for insightful discussions and helpful comments on the manuscript. The work described from our laboratory was
supported by grants from the NINDS to ALJ. ALJ is an investigator of the Howard Hughes Medical Institute.

Visit the Annual Reviews home page at www.AnnualReviews.org

LITERATURE CITED


MID/HINDBRAIN EARLY PATTERNING

between midbrain and hindbrain. Development 123:179–90
Crossley PH, Martin GR. 1995. The mouse Fgf8 gene encodes a family of polypeptides and is expressed in regions that direct outgrowth and patterning in the developing embryo. Development 121:439–51
cord override the organizing activity of the isthmus. Mech. Dev. 84:41–53
Irving C, Mason I. 1999. Regeneration of isthmic tissue is the result of a specific and direct interaction between rhombomere 1 and midbrain. Development 126:3981–89
Liu A, Losos K, Joyner AL. 1999. FGF8 can activate Gbx2 and transform regions of the rostral mouse brain into a hindbrain fate. Development 126:4827–38
Min H, Danilenko DM, Scully SA, Bolon B,
Shamim H, Mahmood R, Logan C, Doherty P,
MID/HINDBRAIN EARLY PATTERNING

Walther C, Gruss P. 1991. Pax-6, a murine paired box gene, is expressed in the developing CNS. Development 113:1435–49
Wasef M, Joyner AL. 1997. Early mesencephalon/metencephalon patterning and
development of the cerebellum. Perspect. Dev. Neurobiol. 5:3–16
Figure 1  Expression patterns of mes/met genes in the mouse at E8.5 and E9.5. (A) At the 3-5 somite stage (E8.5), Otx2 and Wnt1 are expressed broadly in the midbrain with Otx2 also in the forebrain. Fgf8 and Gbx2 are expressed broadly in the anterior hindbrain, and En1, En2, Pax2 and Pax5 are expressed in the entire mes/met region. (B) At E9.5, Wnt1 expression is restricted to a narrow ring anterior to the mid/hindbrain junction. Pax2, Fgf8 and Gbx2 are expressed in narrow rings caudal to the mid/hindbrain junction. En1, En2 and Pax5 are expressed in regions of the midbrain and anterior hindbrain. The thick lines indicate that expression is along the entire D-V axis and the thin lines indicate that expression is only in the dorsal or ventral midline of the neural tube.