Role of hindbrain in inner ear morphogenesis:
Analysis of Noggin knockout mice

Jinwoong Bok a, Lisa J. Brunet b, Omar Howard a, Quianna Burton a, Doris K. Wu a,⁎

a National Institute on Deafness and Other Communication Disorders, 5 Research Ct., Rm 2B34, Rockville, MD 20850, USA
b Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720, USA

Received for publication 15 August 2006; revised 2 August 2007; accepted 6 August 2007
Available online 16 August 2007

Abstract

Signaling from rhombomeres 5 and 6 of the hindbrain is thought to be important for inner ear patterning. In Noggin −/− embryos, the gross anatomy of the inner ear is distorted and malformed, with cochlear duct outgrowth and coiling most affected. We attributed these defects to a caudal shift of the rhombomeres caused by the shortened body axis and the kink in the neural tube. To test the hypothesis that a caudal shift of the rhombomeres affects inner ear development, we surgically generated chicken embryos in which rhombomeres 5 and 6 were similarly shifted relative to the position of the inner ears, as in Noggin mutants. All chicken embryos with shifted rhombomeres showed defects in cochlear duct formation indicating that signaling from rhombomeres 5 and 6 is important for cochlear duct patterning in both chicken and mice. In addition, the size of the otic capsule is increased in Noggin −/− mutants, which most likely is due to unopposed BMP signaling for chondrogenesis in the peri-otic mesenchyme.

Published by Elsevier Inc.

Keywords: Noggin; BMP; Inner ear; Rhombomere; Hindbrain; Chondrogenesis

Introduction

The formation of the structurally complex vertebrate inner ear requires instructive signaling from surrounding tissues such as the hindbrain, mesenchyme, neural crest, and endoderm. Among these tissues, the hindbrain, in particular, has received the most attention because of existing hindbrain mutants with inner ear defects such as the Kreisler and Hoxa1 knockout mice (for review, see Kiernan et al., 2002). More detailed analyses of these and other mutants indicate that rhombomeres 5 and 6 (r5 and r6) of the hindbrain, which are located immediately adjacent to the developing inner ear, are important for its patterning.

Noggin is a secreted polypeptide first isolated from the Spemann’s organizer in Xenopus (Smith and Harland, 1992). It functions as an antagonist of BMP pathways by binding to BMPs with high affinity, in particular BMP2 and BMP4 and preventing them from activating their receptors (Holley et al., 1996; Zimmerman et al., 1996). Gain of function studies in frogs and fish implicated a role for Noggin in dorsalizing the embryo with respect to specification of neural and mesodermal fates (for review, see De Robertis and Kuroda, 2004). However, analyses of Noggin knockout mouse embryos indicate that Noggin is not required for neural specification or head formation in mammals but is required for proper differentiation of the caudal neural tube and formation of somites and joints (Brunet et al., 1998; McMahon et al., 1998). In Noggin mutants, the neural tube forms normally but becomes kinked by 9.5 days post coitum (dpc), and the body axis is shortened. Some mutant embryos also display exencephaly. Beside the kinks and exencephaly, gene expression analyses indicate that the part of the neural tube rostral to the forelimbs is fairly normal, whereas the differentiation of caudal neural tube and somites is severely affected in Noggin mutants (McMahon et al., 1998).

Mutations of the NOGGIN gene in humans are associated with several autosomal dominant disorders that are characterized
by skeletal and joint defects such as proximal symphalangism (SYM1) and multiple-synosostoses syndrome (SYNS1) (Gong et al., 1999; Takahashi et al., 2001). Conductive hearing loss due to fixation of the stapes to the temporal bone is also a manifestation of NOGGIN mutations (Brown et al., 2002; Gong et al., 1999; Weekamp et al., 2005).

In Noggin −/− mouse mutants, the eyes and ears are also malformed (McMahon et al., 1998). Since BMPs have been implicated in inner ear development (Chang et al., 1999, 2004; Gerlach et al., 2000), we analyzed the inner ears of Noggin knockout mice as a potential animal model for gain of BMP functions. Our results show that the gross anatomical defects observed in the inner ears of Noggin mutants are largely due to misalignment of the otocysts with the hindbrain during early development rather than a mis-regulation of BMP levels within the inner ear. However, the increase in otic capsule size observed in the Noggin mutants is most likely due to a net gain of BMP functions.

Materials and methods

Mice, paint-fills, in situ hybridization and β-gal histochemistry

Noggin homozygous mice in C57BL/6 or a mixed C57BL/6 and FVB background where indicated were generated and genotyped as described (Brunet et al., 1998). Gross anatomical analyses by injecting 0.1% alkyd white paint solution in methyl salicylate to the cavity of the inner ear and in situ hybridization experiments were performed as described (Morsli et al., 1998). A total of 8 embryos between 12 and 13.5 dpc and 15 embryos between 15.5 and 17.5 dpc were processed for paint-fill analyses. A total of 32 Noggin homozygous embryos were used for gene expression analyses: 26 between 8.5 and 10.5 dpc, 5 between 11.5 and 12.5 dpc, and 9 between 14.5 and 16.5 dpc. RNA probes for Aggrecan (Watanabe et al., 1995), Bmp2 (Lyons et al., 1989), and Bmp4 (Morsli et al., 1998) were generated as described. A Pst1–Apal fragment from nucleotides 1142 to 1895 of Krox20 cDNA (NM_0101118) including the zinc-finger region was used to generate RNA probes for Krox20 (gift of David Wilkinson). lacZ plasmid for generation of RNA probes was a gift from David Lin at Cornell University. RNA probes for Hoxb1 were generated from nucleotides 71 to 1017 of the Hoxb1 cDNA (NM_0008266). For the detection of Pax6 expression, RNA probes were generated from two cDNA regions, a 184 base pair fragment described by Planchov et al. (1990) and a 674 base pair fragment from nucleotides 595 to 1269 (NM_011040). β-Galactosidase (β-gal) histochemistry on Noggin +/− embryos at 9.5 dpc was performed by fixing embryos with 1% formaldehyde/0.2% glutaraldehyde for 30 min, before processing for β-gal staining as described (Epstein et al., 2000). Embryos at 11.5 to 16.5 dpc were first fixed with 2% paraformaldehyde overnight and then processed for cryosectioning and β-gal histochemistry.

Chicken, surgical procedures

Fertilized eggs (CBT farms, MD) were incubated at 37 °C in a humidified chamber. For rhombomere transplantation procedures, embryos at embryonic day 1.5 (E1.5) equivalent to 12–15 somite stages (ss) or Hamburger Hamilton stage 10–12 (HH 10–12) were used (Hamburger and Hamilton, 1951). A number of surgical permutations were performed (see Results section) before deciding on the optimal condition, which was performed by removing a segment of the hindbrain between r3 and r6 using a micro-surgical blade. Then, the isolated hindbrain segment was transplanted to an age-matched host embryo with r5 to r7 removed for controls. The operated embryos were further incubated and subsequently harvested at E2.5 for gene expression analyses using whole mount in situ hybridization, or harvested at E9 for anatomical analyses using the paint fill technique (Bissonnette and Fekete, 1996).

Results

Inner ear phenotype of Noggin knockout mice

The anatomy of Noggin mutant inner ears was visualized by injecting 0.1% alkyd white paint solution to the cavity of the inner ear (Fig. 1). A total of 15 homozygous mutant embryos
were analyzed between 15.5 and 17.5 dpc. Homozygous inner
ears in a mixed C57BL/6/FVB background (Fig. 1B; n = 4) have
a milder phenotype than those in C57BL/6 background (Fig. 1D;
n = 11). Although all inner ear components are present in most
Noggin mutants, the overall patterning of the inner ear appears
distorted compared to heterozygotes. For example, the shape of
the endolymphatic duct is abnormal (Fig. 1, asterisk; Table 1),
the common crus is slightly widened and appears slanted (Fig. 1,
double asterisks), the anterior canal is extended (Fig. 1, double-
headed arrow), and the saccule is often malformed (Fig. 1D,
arrow; Table 1). More importantly, the cochlear duct is
invariably shortened and shows aberrant coiling. The outgrowth
of a normal cochlear duct extends ventrally in an antero-medial
direction, and then coils laterally. Fig. 1B shows the cochlear
duct of a Noggin mutant that displays abnormal protrusions
(arrowheads) and terminates prematurely in an upward direction
(arrow). In more severe cases, the cochlear duct coils medially
instead of laterally (Fig. 1D, red arrowheads). In addition, these

Fig. 2. Gene expression patterns in Noggin +/- and -/- inner ears. (A–D, G) Noggin expression domains identified by β-gal histochemistry in sections of Noggin +/- inner ears at 9.5, 12, 14, and 16.5 dpc. (E–F, H, I) Gene expression patterns of Bmp4 (E, H, I) and Bmp2 (F) in Noggin +/- (E, F, H) and -/- (I) inner ears. (A) At 9.5 dpc, Noggin expression is detected in the dorsal periotic mesenchyme (arrowheads). (B) At 12 dpc, Noggin expression is only detected in the hindbrain, notochord and some mesenchymal cells by the pharyngeal pouch (PP). (C) At 14 dpc, Noggin is expressed in the developing otic capsule, as well as the semicircular canals (arrowheads) and cochlear duct (arrows). (D) At 16.5 dpc, β-gal staining is also present in the lateral crista, as shown in the insert. β-gal-positive regions in the lateral cristá and semicircular canals (arrowheads) are associated with Bmp4 (E) and Bmp2 (F) positive regions, respectively. The β-gal-positive region in the cochlear duct (G) also includes the Bmp4-positive region (E, H). Weak Bmp4 expression (E, arrowsheads) is also detected adjacent to the Noggin-positive otic capsule (D). This Bmp4 expression appears slightly upregulated in Noggin -/- mutants (I, arrowheads). Abbreviations: CD, cochlear duct; HB, hindbrain; LC, lateral crista; MU, macula utriculi; OC, otic capsule; PP, pharyngeal pouch. Orientations: D, dorsal; M, medial; A, anterior; L, lateral. Orientation in panel B applies to panels C–I. Scale bar=100 μm. Scale bar in panel D applies to panels E, F, and I; panel G applies to panel H.
inner ears are smaller in size, and the posterior semicircular canal is truncated or not resorbed (white arrowheads) (Fig. 1D, arrow).

Normal expression of Noggin in the developing inner ear

In an attempt to understand the observed phenotype in Noggin knockout mice, we investigated Noggin expression in the developing inner ear using RNA probes against Noggin in wild type embryos, or performing β-gal histochemistry or detecting lacZ mRNA using in situ hybridization in Noggin heterozygous embryos. Among the three techniques used, β-gal histochemistry appears to be the most sensitive in identifying Noggin expression domains. Noggin is transiently expressed in the dorsal periotic mesenchyme at 9.5 dpc (Fig. 2A, arrowheads). This mesenchymal expression is not detectable at 11.5 and 12 dpc (Fig. 2B and data not shown). At 14 dpc, Noggin expression is expressed in the cochlear duct (Fig. 2C, arrows) and semicircular canals of the inner ear (Fig. 2C, arrowheads), as well as the developing otic capsule surrounding the labyrinth (Fig. 2C). By 16.5 dpc, in addition to the expression in the cochlear duct (Fig. 2G), semicircular canals (Fig. 2D, arrowheads) and otic capsule (Figs. 2D, G), Noggin is also present in the center region of the crista ampullaris (Fig. 2D, insert).

In other parts of the body, Noggin expression domains are often found intimately associated with expression domains of Bmps (Marcelle et al., 1997; Tonegawa and Takahashi, 1998). Similarly, Noggin expression domains in the cristae (Fig. 2D) and cochlear duct (Fig. 2G) are associated with that of Bmp4 (Figs. 2E, H), whereas its expression domain in the canals is associated with that of Bmp2 (Fig. 2F, arrowheads). A faint Bmp4 hybridization signal is sometimes detectable in wild type otic capsule (Fig. 2E, arrowheads), and this expression appears upregulated in the Noggin mutants (Fig. 2I, arrowheads). No upregulation of Bmps is evident in the membranous labyrinth of the mutant inner ear (data not shown).

Fig. 3. Inner ear phenotypes of Noggin mutants at 12 and 13.5 dpc. Lateral views of paint-filled right inner ears from Noggin +/- (A, D) and +/- (B, C, E) embryos at 12 (A–C) and 13.5 (D, E) dpc. (A) A 12 dpc heterozygous inner ear showing small resorption domains (rd) in the vertical canal pouch (cp) that later develops into the anterior and posterior canals. The cochlear duct outgrowth is extending in a ventromedial and anterior orientation. (B, C) Mutant inner ears show a canal pouch that is extended anteriorly (arrowheads), a smaller endolymphatic duct (asterisk), and a shorter cochlear duct than heterozygotes (A). (D) At 13.5 dpc, the gross development of the vestibular apparatus in the heterozygous inner ear is complete, but the cochlear duct is less than one coil. (E) The mutant inner ear shows an extended anterior canal (double-headed arrow), a malformed endolymphatic duct (asterisk), a slanted common crus (double asterisks), and a shortened cochlear duct. Refer to Fig. 1 for abbreviations and orientations. Scale bars=200 μm. Scale bar in panel C applies to panels A, B; panel D applies to panel E.
Early appearance of inner ear defects in Noggin mutants

Since Noggin expression is not robustly detected in the otic epithelium or periotic mesenchyme until late-gestation, we investigated the onset of the gross anatomical defects in the mutants at earlier stages. All the inner ear defects observed at 16.5 dpc are already apparent at 12 and 13.5 dpc (n=8), including defects in the cochlear duct outgrowth (Figs. 3B, C, E), endolymphatic duct (Figs. 3C, E, asterisk), and common crus (Fig. 3E, double asterisks). The extended anterior canal phenotype is already evident at 12 dpc when the prospective canal is a pouch (Figs. 3B, C, arrowheads). Since the morphological defects in the mutants are evident earlier than any robust Noggin expression domains associated with the developing inner ear, it suggests that the inner ear defects caused by the lack of Noggin may be indirect. The shortened neural axis and the kinky notochord reported in Noggin mutants prompted us to investigate the location of the otocysts in relationship to other body parts, in particular r5 and r6, which are thought to provide important signaling molecules for inner ear patterning.

Abnormal alignment of otic vesicles with the hindbrain in Noggin mutants

At 8.5 dpc, the otic placode region, as indicated by the expression domain of Pax8, is broad and its anterior half corresponds to the position of Hoxb1-positive, rhombomere 4 (Figs. 4A, B). At 9.5 dpc, the location of the newly formed otocysts corresponds to the same axial positions of rhombomeres 5 and 6 in the hindbrain (Kiernan et al., 2002). Figs. 4D and F illustrate the anterior limit of normal otocysts at 9.5 dpc corresponding to the junction of r4 and r5 based on the expression domains of Hoxb1 and Krox20. In Noggin mutants, the relative relationship between the otic placode and r4 appears normal at 8.5 dpc (Fig. 4C; n=3). By 9.5 dpc, the size of the homozygous otocysts is generally slightly smaller than heterozygotes (Figs. 4E, G, black brackets), and the alignment of otocysts with the rhombomeres in the hindbrain is abnormal (n=8). All specimens show either an alignment with r4 and r5 (Figs. 4E, G) or with r4 alone (data not shown) instead of the normal alignment with r5 and r6. However, the relative position between the otocysts and the second branchial arch remains unchanged (data not shown). This caudal shift of the hindbrain alignment with the otocysts could affect the ability of normal signaling molecules emanating from r5 and r6 to reach the otic epithelium and may be the cause of Noggin −/− malformed inner ears.

Caudal shift of rhombomeres in chicken embryos affects inner ear morphogenesis

To test the hypothesis that this abnormal alignment between otocysts and hindbrain could indeed result in dysmorphogenesis...
of the inner ear, we generated an experimental paradigm in developing chicken embryos similar to the Noggin mutants, in which the hindbrain is shifted caudally by one rhombomere in relation to the otocyst. We tried to accomplish this by duplicating some rhombomeres rostral to the inner ear position using transplantation techniques when the inner ear is a shallow cup. For reasons that are not clear, duplicating r2 or r3 by transplanting r3–6 into the space of r4–7 or transplanting r2–6 into the space of r3–7, respectively, did not result in a caudal shift of r5 and r6 in relationship with the otocysts, 24 h after surgery. On the other hand, duplicating both r2 and r3 by replacing r4–7 with r2–6, or duplicating both r1 and r2 by replacing r3–7 with r1–6 resulted in too big a shift. The otocysts were aligned with r3 and r4, rather than r4 and r5 as we had intended (data not shown). The only permutation that resulted in a stable caudal shift of rhombomere by one position in relationship to the inner ear was replacing r5–7 with r3–6 (Fig. 5A). Fig. 5 illustrates an operated embryo where the otocysts are aligned with r4 and r5 instead of the normal r5 and r6, based on the expression patterns of EphA4 (n = 18) and Hoxb1 (n = 4). Ectopic otocysts formed as a result of transplantation were surgically removed 24 h after transplantation. Then, embryos were further incubated and harvested at E9 and processed for paint-fill analyses. Our results show that inner ears in rhombomere-shifted embryos display relatively normal dorsal vestibular structures that are not extended like those in Noggin −/− mutants (Figs. 6B, C). However, both the saccule and cochlear duct are malformed in the rhombomere-shifted embryos. The saccule is usually under-developed and not clearly separated from the utricle and cochlear duct (Figs. 6B, C; n = 8/8). The cochlear development is severely affected, either widened or shortened (Figs. 6B, C; n = 8/8). Inner ears of control embryos in which r3–6 were replaced by r3–6 from an age-matched donor did not show any obvious morphological defects (Fig. 6A; n = 6). These results indicate that the normal alignment between otocysts and hindbrain is important for normal morphogenesis of the chicken inner ear, especially the saccule and cochlear duct, and further suggest that these defects in Noggin mutants are likely due to the abnormal signaling from the hindbrain caused by the changes in the spatial relationship between otocysts and hindbrain.

Increase in otic capsule size in Noggin mutants

Since Bmp4 expression appears to be upregulated in the otic mesenchyme of Noggin mutants, we examined the consequence of this effect on otic capsule formation. Our results show that the chondrogenic region in the otic capsule is expanded in Noggin mutants based on the expression patterns of lacZ and Aggrecan (Figs. 7A–D). Noggin and Aggrecan are normally expressed in the chondrocytes only and their expression is not detected in the perichondrium (Figs. 7E, G, arrowheads). However, in the Noggin mutants, Aggrecan expression is upregulated in the perichondrium (Fig. 7H; arrowheads), whereas Noggin expression was limited to the chondrocytes (Fig. 7F). In addition, Bmp4 expression in the mesenchymal cells along the membranous side of the perichondrium is quite apparent (Fig. 7I). The increase in chondrogenesis in Noggin mutants suggests that BMP4 and Noggin normally regulate each other’s activity in mediating the chondrogenic process in the otic capsule.
Discussion

Misalignment of the otocysts with the hindbrain affects inner ear morphogenesis

In Noggin mutants, the inner ear defects are evident before Noggin expression is evident in the otic epithelium, suggesting that sources outside the inner ear are at play. The shortened body axis and/or the kink in the neural tube causes a misalignment of the otocysts with r5 and r6. The relative position of the otocysts in relationship to the branchial arches, however, remains unchanged (data not shown). Based on existing hindbrain mutants with inner ear defects, we postulate that the inner ear malformations observed in Noggin mutants are largely due to an inner ear–rhombomere misalignment at early stages. This hypothesis is strongly supported by the inner ear phenotypes of chicken embryos in which the rhombomeres were surgically shifted caudally relative to the otocysts. While the semicircular canals in the rhombomere-shifted chicken embryos are not perfectly normal, they are not extended like those observed in Noggin −/− embryos, suggesting the two types of vestibular defects are not related. However, the caudal shift of rhombomeres appears to affect saccule and cochlear duct formations in a similar manner between chicken and mice. Within the malformed cochlear duct of Noggin −/− mutants, the relative expression domains of a number of genes including Bmp4, Bmp7, lacZ (Noggin), Lunatic fringe, Otx2 and EphB2 did not change (data not shown). This is in contrast to the cochlear duct of Pax2 −/− mutants, in which the normal relationships among various gene expression domains are perturbed (Burton et al., 2004).

Our rhombomere-shift paradigm in chicken introduces a duplication of r3 and r4, which in itself could contribute to the observed cochlear phenotype. However, our preliminary results on individual rhombomere removal suggest that this duplication most likely does not have much effect on inner ear morphogenesis (Liang and Wu, unpublished observation). We interpreted the similarity in saccule and cochlear malformations between Noggin mutants and rhombomere-shifted chicken embryos to be due to abnormal hindbrain signaling resulting from an increase in distance between r5 and r6 and the otic epithelium. Even though FGF3 expressed in r5 and r6 has been implicated in inner ear development, the signaling mechanism that is disrupted by the caudal shift is not necessarily limited to secreted ligands and could include adhesion molecules as well. In addition, the patterning defects could be a result of discrepancy among instructive signals from rhombomeres and underlying mesoderm. In vitamin A deficient quail embryos where there appears to be a disparity in the locations of the hindbrain and mesodermal signals, inner ear patterning is also severely disrupted (Kil et al., 2005). The inner ear phenotypes observed in Noggin mutants or rhombomere-shifted chicken embryos, however, are unlikely to be caused by a major change in the anteroposterior (A/P) axis of the otocysts. This assumption is based on our previous study showing that the reversal of the A/P polarity of rhombomeres 4 to 7 did not affect the A/P axis of otocysts with respect to ganglion delamination and expression of genes that are asymmetrically expressed along the A/P axis (Bok et al., 2005).

Interaction of Bmps with Noggin during inner ear development

Noggin is expressed in regions of the developing inner ear that are closely associated with Bmp-positive domains, and as it does in other organs, Noggin most likely functions to modulate BMP levels in the inner ear. At the otic cup and early otocyst

---

**Fig. 6.** Inner ear phenotypes of rhombomere-shifted chicken embryos. Paint-filled inner ears of a control (A) and two rhombomere-shifted (B, C) chicken embryos at E9. (A) A normal inner ear, in which a segment of hindbrain between rhombomeres 3–6 was replaced by a comparable hindbrain segment from an age-matched donor at E1.5 (n=6). (B, C) Inner ears from rhombomere-shifted embryos show relatively normal semicircular canals and ampullae. On the other hand, the saccule and the cochlear duct are always malformed (n=8/8). Five out of 8 embryos show widened cochlear ducts (B, arrowheads), whereas 3 out of 8 embryos show shortened and pointed cochlear ducts (C, arrow). The saccule is usually under-developed and not clearly separated from the utricle and cochlear duct (n=8/8). The endolymphatic duct is present in all three ears but failed to fill with paint. Refer to Fig. 1 for abbreviations and orientations. Scale = 500 μm.
stages, Noggin expression is transiently detected in the mesenchyme surrounding the developing inner ear, whereas Bmp4 is expressed at the rim of the otic cup (Morsli et al., 1998). In Noggin mutants, the size of the otocysts is usually slightly smaller. It is not clear if Noggin and BMP4 interact to dictate the size of the otocyst at this stage, or the observed size reduction is solely due to a change in signals from misalignment with the rhombomeres.

At the otocyst stage, BMPs in the presumptive cristae and possibly canal pouch has been implicated in crista and canal formation (Chang et al., 1999, 2004; Gerlach et al., 2000). Noggin expression is not detected during these stages of development. Consistent with these observations, the cristae and canals do form in most Noggin mutants. At later stages of development, Noggin expression in the canals is closely associated with that of Bmp2, and its expression in the cristae and cochlear duct with that of Bmp4. The lack of Noggin in these regions could disrupt local cellular patterning. However, any phenotypic analysis of these specific regions will most likely be confounded by the early morphogenetic defects observed in the Noggin mutants. A mouse model in which Noggin can be knocked out temporally may be more suitable for investigating any potential phenotypes in later developmental stages. Nevertheless, the normal balance of BMPs and Noggin that mediates chondrogenesis is clearly disrupted in the Noggin knockout mice.

Role of BMP antagonists in inner ear development

Both Noggin and Chordin are BMP antagonists expressed in the Spemann’s organizer that mediate dorsal patterning of the Xenopus embryo, including specification of neural and mesodermal fates (for review, see De Robertis and Kuroda, 2004). In mice, both of these antagonists are expressed in the node, the equivalent structure of the Spemann’s organizer in Xenopus. Single knockout of Noggin or Chordin in mice does not display
strong defects in gastrulation or neural specification. Mutant embryos with knockouts of both Noggin and Chordin show that these antagonists share redundant functions in forebrain and mesoderm development that reflects defects in body axial specification (Bachiller et al., 2000). Interestingly, double knockouts of Noggin and Chordin embryos have no inner ears (Bachiller et al., 2000). Single knockout of Chordin embryos have otocysts that are half the normal size (Bachiller et al., 2003). Even though the cause of the otic phenotype in Chordin mutants has not been investigated in detail, the poorly developed endoderm and mesoderm in these mutants probably led to the inner ear defects. Therefore, defects of gross inner ear morphogenesis in both Noggin and Chordin mutants are most likely due to failure of proper patterning of the body axis.

Chondrogenesis of the inner ear

The vertebrate inner ear is comprised of two parts, a membranous labyrinth that is derived from the otocyst and a bony labyrinth that is derived from the mesenchymal cells surrounding the vesicle. A failure to coordinate the formation of the two labyrinths during development, could cause deleterious results since the overall shape of the bony labyrinth largely follows the intricate contour of the membranous labyrinth.

Here, we show that Noggin and Bmp4 expression domains in the developing bony labyrinth are closely associated with each other: Noggin in the chondrocytes of the otic capsule and Bmp4 in cells around the perichondrium of the otic capsule. The increase in chondrogenesis in Noggin mutants suggest that normal chondrogenesis is regulated by the activities of both BMP4 and Noggin. We postulate that chondrogenesis in the otic capsule is induced by BMPs and that Noggin expressed by the chondrocytes serves to regulate the extent of chondrogenesis. Therefore, the lack of Noggin causes an increase in chondrogenesis. Similar phenotypes have been reported in other skeletal structures of this mutant, including the middle ear ossicles (Brunet et al., 1998; Hwang and Wu, unpublished observation). The increase in the number of chondrocytes in the otic capsule of mutants is not due to a premature initiation of chondrogenesis based on the expression patterns of several chondrogenic markers such as Fgfr3, Aggrecan, and Sox9 at 12.5 dpc (data not shown). The chondrogenic induction of the otic capsule by BMPs is also supported by our earlier study in chicken showing that ectopic expression of constitutively activated Bmpr1b receptors increases cartilage formation whereas ectopic dominant negative Bmpr1b receptors causes a reduction in cartilage formation (Chang et al., 2002). However, BMP2 rather than BMP4 may be the predominant BMP responsible for inner ear chondrogenesis in chicken, since Bmp2 expression is observed in similar locations as what is shown here for Bmp4 in mice, in cells surrounding the perichondrium (Chang et al., 2002).

In heterozygous mutants, there is no obvious defect in the membranous or bony labyrinth. However, some of these mice are deaf presumably due to conductive hearing loss (Hwang and Wu, unpublished observation). In human patients with NOGIN mutations, there have been reports of poor sustainable hearing improvement in patients after otologic surgery, suggestive of recurrent bone defects or other defects on unknown etiology (Brown et al., 2003; Ensink et al., 1999). In summary, our mouse study here shows multiple roles of Noggin in ear development, and these mutant mice will continue to serve as a useful animal model for the study of hearing disorders associated with NOGGIN mutations.

Acknowledgments

We are grateful to Dr. Weise Chang for discussion and intellectual contributions throughout this project, and to Dr. Daniel Choo for paint-filling our first Noggin mutant. In addition, we thank Drs. Susan Sullivan and Konrad Noben-Trauth for critical reading of the manuscript. This project is funded by NIDCD Intramural program and GM 49346 (L.J.B.).

References


