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BMP signaling is required for development of the ciliary body

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SUMMARY

The ciliary body in the eye secretes aqueous humor and glycoproteins of the vitreous body and maintains the intraocular pressure. The ciliary muscle controls the shape of the lens through the ciliary zonules to focus the image onto the retina. During embryonic development, the ciliary epithelium is derived from the optic vesicle, but the molecular signals that control morphogenesis of the ciliary body are unknown. We report that lens-specific expression of a transgenic protein, Noggin, can block BMP signaling in the mouse eye and result in failure in formation of the ciliary processes. Co-expression of transgenic BMP7 restores normal development of the ciliary epithelium. Ectopic expression of Noggin also promotes differentiation of retinal ganglion cells. These results indicate that BMP signaling is required for development of the ciliary body and may also play a role in regulation of neuronal differentiation in the developing eye.

Key words: Ciliary body, Eye, BMP4, BMP7, Noggin, Smad1, Msx1, Otx1, Mouse

INTRODUCTION

During vertebrate eye development, the optic vesicle grows out from the diencephalon and invaginates to form the optic cup. The optic cup gives rise to the neural retina, the retinal pigment epithelium (RPE), and the epithelia of the iris and the ciliary body. The ciliary epithelium differentiates from the two layers of neuroepithelial cells at the rim of the optic cup. The unpigmented inner layer is continuous with the neural retina and the iris while the pigmented outer layer lies between the RPE and the outer iris (Beebe, 1986). During eye development, the ciliary epithelium folds to form the ciliary processes, while the mesenchymal cells of neural crest origin differentiate into the connective tissue of the ciliary body and part of the ciliary muscle (Johnston et al., 1979). The non-pigmented ciliary epithelium secretes fibrillins that are the primary component of the ciliary zonules, the suspensory ligaments of the lens (Hanssen et al., 2001). Primary functions of the ciliary body include: (1) secretion of aqueous humor and glycoproteins of the vitreous body, (2) maintenance of the intraocular pressure, and (3) controlling the shape of the lens through the ciliary muscle and the ciliary zonules.

Earlier transplantation and mutant animal studies show that the lens induces formation of the ciliary body (for a review, see Beebe, 1986) and that intraocular pressure is required for growth of the eye and formation of the ciliary folds (Coulombre, 1965). Genetic ablation of the lens using diphtheria toxin retards development of the ciliary body (Harrington et al., 1991). A recent study shows that the lens can induce expression of genes specific to the prospective ciliary body and iris (Thut et al., 2001). Increases in cell number and cell volume in the ciliary epithelium are associated with formation of the ciliary folds (Bard and Ross, 1982a; Bard and Ross, 1982b; Reichman and Beebe, 1992). Radial capillaries appear in the mesenchyme on the outer surface of the ciliary epithelium prior to fold formation. These blood vessels are believed to play a role in arranging the regularity of the folds (Beebe, 1986). These studies indicate that tissue interactions are essential for development of the ciliary body. However, the molecular signals for morphogenesis of the ciliary processes remain unknown.

Members of the bone morphogenetic protein (BMP) family have been shown to be essential for cell differentiation and morphogenesis of several tissues during embryonic development, including dorsoventral patterning (Graff, 1997; Dale and Jones, 1999), development of the limb (Hofmann et al., 1996; Zou et al., 1997), tooth and bone (Reddi, 1994; Luo et al., 1995; Katagiri, et al., 1998; Cheifetz, 1999), kidney (Godin et al., 1999), lung (Weaver et al., 1999), heart (Kim et al., 2001), and liver (Duncan and Watt, 2001). Several members of the BMP family are expressed in developing mouse eyes (Luo et al., 1995; Dudley et al., 1995; Dudley and Robertson, 1997; Furuta and Hogan, 1998; Hung et al., 2002). Knockout studies have shown that BMP4 and BMP7 are essential for early morphogenesis of the eye (Furuta and Hogan, 1998; Luo et al., 1995; Dudley et al., 1995; Jena et al., 1997; Wawersik et al., 1999). Unfortunately, owing to early lethality of the homozygous embryos, these knockout mice cannot provide information regarding the roles of BMP4 and BMP7 at later stages of eye development.

To investigate functions of BMP proteins in ocular development from mid-gestation to postnatal stages, we

generated transgenic mice that ectopically express a BMP antagonist, Noggin, in the eye. Noggin can bind to BMP proteins and prevent their interactions with BMP receptors (Zimmerman et al, 1996; Holley et al., 1996; McMahon et al., 1998; Lim et al., 2000). One of the striking abnormalities in these transgenic mice is the absence of the ciliary body. We show that endogenous *Bmp4* and *Bmp7* are highly expressed in the presumptive ciliary epithelium in late-stage embryos and postnatal mice. Lens-specific expression of Noggin inhibited BMP signaling and also suppressed expression of endogenous *Bmp4*, *Bmp7* and genes encoding transcription factors Msx1 and Otx1 in the presumptive ciliary epithelium. Co-expression of BMP7 rescued the defects in the ciliary epithelium caused by ectopic Noggin expression. These results indicate that BMP signaling is essential for morphogenesis of the ciliary body.

MATERIALS AND METHODS

Transgenic mice

To express Noggin in the developing eye, a *Xenopus* Noggin cDNA (Smith and Harland, 1992) was inserted downstream of a 0.3 kb αA-crystallin promoter (CPV2) (Lovicu and Overbeek, 1998) and upstream of a 0.8 kb fragment containing the SV40 small t intron and early region polyadenylation sequences (SV40 pA). The DNA construct was injected into one-cell stage embryos from FVB/N female mice and the embryos were subsequently transferred to pseudopregnant ICR females to produce transgenic mice (Hogan et al., 1994; Taketo et al., 1991). Transgenic mice or embryos were identified by polymerase chain reaction (PCR) using genomic DNA extracted from mouse tails. The primer pair used for PCR are specific to the SV40 pA sequences (primer A, 5′-GTG AAG GAA CCT TAC TTC TGT GGT G-3′; primer B, 5′-GTC CTT GGG GTC TTC TAC CTT TCT C-3′), amplifying a 0.3 kb fragment (Zhao and Overbeek, 1999).

Generation and phenotypic analysis of transgenic mice expressing BMP7 under the control of the same CPV2 promoter have been reported elsewhere (Hung et al., 2002). CPV2-BMP7 mice were mated with CPV2-Noggin mice to determine whether the Noggin-induced abnormalities could be corrected by elevated expression of BMP7. Mice derived from the cross-mating were genotyped by PCR using two pairs of primers. The CPV2-Noggin transgene was identified using primer B and a Noggin-specific primer (5′-GCATGAGCATTTGCACTCGG-3′). The CPV2-BMP7 transgene was identified using primer B and a BMP7-specific primer (5′-CGGGCCTGTGGCTGCCACTAG-3′) (Hung et al., 2002).

In situ hybridization

Mouse embryonic heads or postnatal eyes were fixed in 10% formalin and embedded in paraffin wax. Tissue sections (5 μm) were cut and used for in situ hybridization using antisense ^{35}S-UTP-labeled RNA probes specific to SV40 pA and mouse *Noggin*, *Brn3b* (*Pou4f2* – Mouse Genome Informatics), *Bmp4*, *Bmp7*, *Msx1* and *Otx1* as previously described (Zhao et al., 2001). Hybridized tissue sections were dehydrated and coated with Kodak NTB-2 emulsion for autoradiography. The slides were developed, counterstained with Hematoxylin, and mounted with a coverslip for examination of silver grains under dark-field illumination. Dark-field images were superimposed onto the corresponding bright-field images using Photoshop software (Adobe; San Jose, CA). Light-scattering silver grains in the dark-field images were pseudocolored red to improve the contrast.

Immunohistochemistry

To examine BMP signaling in developing eyes, rabbit antiserum against phosphorylated Smad1 (Calbiochem, Cat. No.566411) was used for immunolabeling of tissue sections. An antigen retrieval procedure was carried out by boiling tissue sections in 10 mM sodium citrate buffer (pH 6.0) in a 800 W microwave oven at full power for 15 minutes before incubation with the primary antibodies. The secondary antibody was biotinylated anti-rabbit IgG which was subsequently labeled with the ExtrAvidin conjugated with FITC (Vector Laboratory). Slides were covered with mounting solution mixed with 4,6-diamidino-2-phenylindole (DAPI) (Vector Laboratories) for visualization of cell nuclei. All tissue sections were processed and immunolabeled together and photographed under the same conditions. To examine iris differentiation, tissue sections were stained with antibodies against α-smooth muscle actin (αSMA). The slides were first treated with 10% methanol and 3% hydrogen peroxide to quench endogenous peroxidase activity before incubation with mouse αSMA antiserum (Sigma). Biotinylated anti-mouse IgG was used as the secondary antibody, followed by streptavidin conjugated horse radish peroxidase. Peroxidase activity was visualized by incubation with diaminobenzidine (DAB)-H_2O_2 (Vector Laboratories, Kit SK-4100). The tissue sections were subsequently counter-stained with Hematoxylin.

RESULTS

Transgenic mice

Seven transgenic mouse families (designated as OVE1194, 1195, 1196, 1197, 1198, 1200, 1201) were generated by microinjection of the CPV2-Noggin construct. Transgene expression at embryonic day 15 (E15) was examined by in situ hybridization using a riboprobe specific for SV40 pA sequences. As expected, the transgene was specifically expressed in the fiber cells of the lens (Fig. 1A-C). Different transgenic families exhibited different levels of transgene expression. The order of transgene expression (from low to high) is OVE1198, 1196, 1195, 1201 and 1194. Embryonic eyes appeared grossly normal in these transgenic families. Postnatally, mice from families OVE1196, 1195, 1201 and 1194 developed small eyes and cataracts. Neither ocular abnormalities nor transgenic transcripts were detected in OVE1197 and OVE1200. Therefore, these two families were discarded.

To examine whether endogenous Noggin might play a role during normal eye development, in situ hybridization was carried out using a ^{35}S-labeled mouse Noggin riboprobe. At E13, Noggin transcripts were not detected in the eye (data not shown). By E18, Noggin mRNA was detected in the majority of the RPE layer except for the most anterior region (Fig. 1D). This pattern of expression persisted in postnatal eyes (Fig. 1E,F). No Noggin transcripts were detected in the prospective ciliary body or iris (Fig. 1).

The absence of the ciliary body

The five transgenic families with detectable transgene expression showed defects in development of the ciliary body (Fig. 2). In wild-type eyes at postnatal day 1 (P1), the presumptive inner ciliary epithelium had become a thin layer of cells and a distinct boundary between the neural retina and the developing ciliary body had formed (Fig. 2A). In CPV2-Noggin transgenic mice, development of the ciliary body was either partially (Fig. 2B) or totally disrupted (Fig. 2C). At P7, the ciliary epithelium in wild-type mice had started to fold

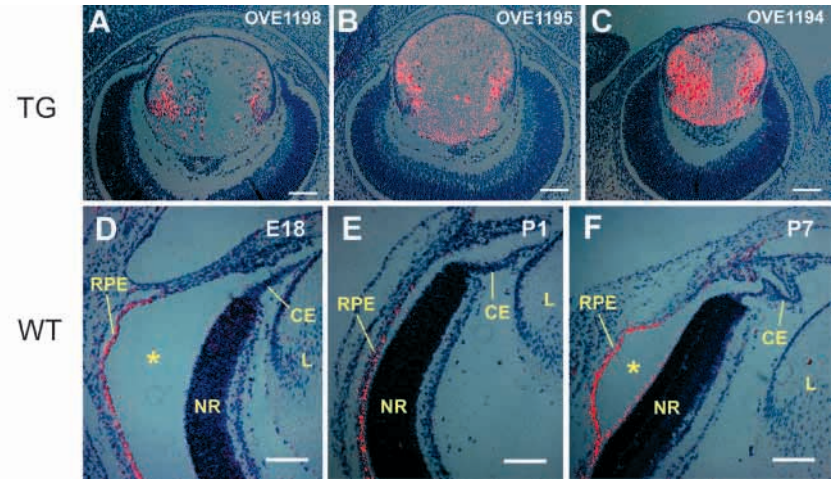

Fig. 1. Expression of transgenic (TG) (A-C) and endogenous (D-F) Noggin in developing eyes. In situ hybridization using a ^{35}S-labeled SV40 riboprobe show that Noggin transgene was specifically expressed in the lens but at different levels in different families (A-C). Three representative families, OVE1198 (A), OVE1195 (B) and OVE1194 (C) are shown here. In wild-type (WT) developing eyes, endogenous Noggin transcripts were not detected in E13 eyes by in situ hybridization (data not shown). In E18 (D), P1 (E) and P7 (F) eyes, endogenous Noggin transcripts were present in most of the RPE except for the anterior region. The gaps between the neural retina (NR) and the RPE indicated by asterisks (D,F) are processing artifacts. Abbreviations: CE, ciliary epithelium; L, lens. Scale bars: 100 μm.

(Fig. 2D). The transgenic eyes at this age still exhibited defects in the ciliary epithelium similar to those at P1 (Fig. 2E,F). In general, the severity of the defect at the anterior margin of the retina appeared to correlate with the level of transgene expression. In the low expressing line OVE1198 (Fig. 1A), the vast majority of the anterior retinae examined exhibited retarded ciliary epithelium differentiation as shown in Fig. 2B,E. In all other transgenic lines, both partially (Fig. 2B,E) and totally (Fig. 2C,F) disrupted ciliary epithelia were observed, even in the same eyes.

In transgenic eyes, the disrupted ciliary epithelium appeared to have become an extension of the ganglion cell layer of the neural retina (Fig. 2C,F). To determine whether these cells expressed markers for retinal ganglion cells, in situ hybridization was performed using a ^{35}S-labeled riboprobe specific to mouse *Brn3b* (Xiang et al., 1993; Erkman et al., 1996; Gan et al., 1999). In CPV2-Noggin transgenic mice, *Brn3b* was expressed in the region where the ciliary body was supposed to form (Fig. 3B), indicating that many of the presumptive ciliary epithelial cells had begun to differentiate as retinal ganglion cells.

During eye development, some of the epithelial cells of the developing iris invaginate into the mesenchymal cells of iris stroma and differentiate into smooth muscle cells (Ferrari and Koch, 1984a; Ferrari and Koch, 1984b; Link and Nishi, 1998a; Link and Nishi, 1998b). To examine whether iris development was affected in the CPV2-Noggin transgenic mice, immunohistochemistry was carried out using antibodies against α-smooth muscle actin (αSMA). In P1 wild-type eyes (Fig. 3C), intense staining was found in the stroma near the anterior margin of the developing iris. In P1 transgenic eyes (Fig. 3D), αSMA was detected at the same region but at a reduced level. In transgenic mice, the inner layer of iris epithelial cells was thinner while the outer layer appeared disorganized (Fig. 3D) compared with the wild-type controls (Fig. 3C). Similar but more severe biochemical and morphological abnormalities were found in P7 and P14 transgenic eyes (data not shown). These results indicate that iris differentiation was altered but not eliminated in the transgenic mice.

BMP expression and signaling

Expression patterns of endogenous *Bmp4* and *Bmp7* in wild-type and transgenic eyes were examined by in situ hybridization. In wild-type eyes, *Bmp4* was highly expressed in the developing inner ciliary epithelium (Fig. 4A,C,E). No

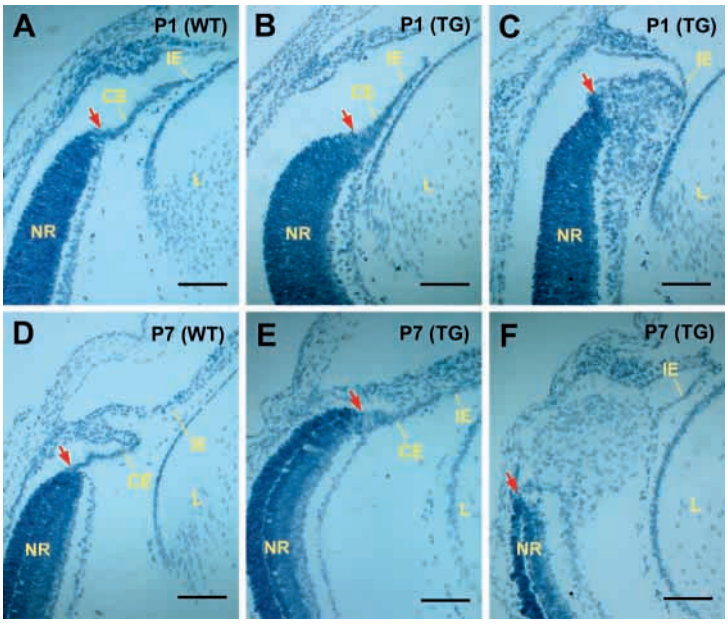

Fig. 2. Altered morphogenesis of the ciliary epithelium in CPV2-Noggin transgenic mice. In P1 wild-type (WT) eyes (A), the ciliary epithelium (CE) had become a thin monolayer of cells distinct from the neural retina (NR). A clear boundary had formed between these two tissues (arrow in A). In CPV2-Noggin transgenic (TG) mice at P1, the CE had either become thickened (B) or was completed disrupted (C). In the latter case, a lump of cells resembling retinal ganglion cells replaced the presumptive CE (C). The boundary between the retinal cells and those of the presumptive ciliary epithelium became less distinct (arrows in B,C). In P7 wild-type eyes (D), the CE became thinner and had started to fold. The presumptive CE in P7 transgenic mice had defects similar to those in the P1 eyes, either thickened (E) or totally altered (F). Abbreviations: L, lens; IE, iris epithelium. Scale bars: 100 μm.

Bmp4 transcripts were detected in the developing iris (Fig. 4C,E). In CPV2-Noggin transgenic embryos, no significant alteration in *Bmp4* expression was detected at E18 (Fig. 4B).

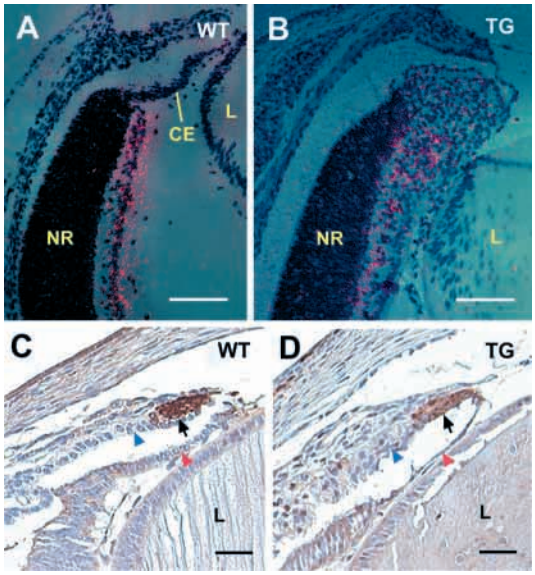

Fig. 3. Expression of *Brn3b* (A,B) and αSMA (C,D) in developing eyes. In situ hybridization using a ^{35}S-labeled riboprobe shows that *Brn3b* was specifically expressed in retinal ganglion cells in P1 wild type eyes (A). In P1 transgenic eyes, *Brn3b* transcripts were also detected in the region of the presumptive ciliary epithelium (B). Immunohistochemistry (C,D) shows that αSMA was present in the wild-type developing iris (arrow in C) but its level was reduced in transgenic eyes (arrow in D). Morphologies of the inner (red arrowheads) and outer (blue arrowheads) layers of iris epithelia were altered in transgenic mice (D) when compared with the wild-type controls (C). Abbreviation: L, lens. Scale bars: 100 μm in A,B; 50 μm in C,D.

However, in postnatal transgenic mice, *Bmp4* expression was downregulated in the presumptive ciliary epithelium while its expression appeared to be activated in the iris (Fig. 4D,F). *Bmp7* was expressed in both the inner and outer layers of the developing ciliary and iris epithelia in wild-type mice (Fig. 4G). No apparent changes in *Bmp7* expression were found in E18 transgenic embryos compared with their wild-type littermates (data not shown). Downregulation in *Bmp7* expression was found in the ciliary and iris epithelia of postnatal transgenic mice (Fig. 4G).

Smad1 is a transcription factor that responds to BMP signaling. Smad1 is phosphorylated and activated by the type I BMP receptors (Hoodless et al., 1996; Kretzschmar et al., 1997). Upon activation, Smad1 partners with Smad4 and translocates into the nucleus to regulate gene expression (Hoodless et al., 1996; Liu et al., 1996; Attisano and Wrana, 2000). To assay for inhibition of BMP signaling by transgenic Noggin in developing eyes, immunohistochemistry was performed using an antibody that recognizes only the phosphorylated form of Smad1 (pSmad1). At E12, pSmad1 was detected in most of cells in both wild-type (Fig. 5B) and transgenic eyes (Fig. 5D). pSmad1 was detected in both the cytoplasm and the nucleus. In E15 wild-type eyes, pSmad1 was still present in most of cells, but was reduced in corneal stroma cells (Fig. 5F,H). In postnatal wild-type eyes, intense nuclear labeling was detected in the lens epithelium, the neural retina, outer layer of the infolding ciliary epithelium, the corneal endothelium and epithelium, and mesenchymal cells between the cornea and the iridial/ciliary epithelium (Fig. 5J,N). In the postnatal transgenic eyes, pSmad1 levels were reduced in most of these tissues (Fig. 5L,P) except in the corneal epithelial cells (Fig. 5P). pSamd1 levels in cells outside the eye were not affected by transgenic Noggin (data not shown). These results suggest that transgenic Noggin strongly inhibited BMP-induced phosphorylation (or expression) of Smad1 in postnatal eyes.

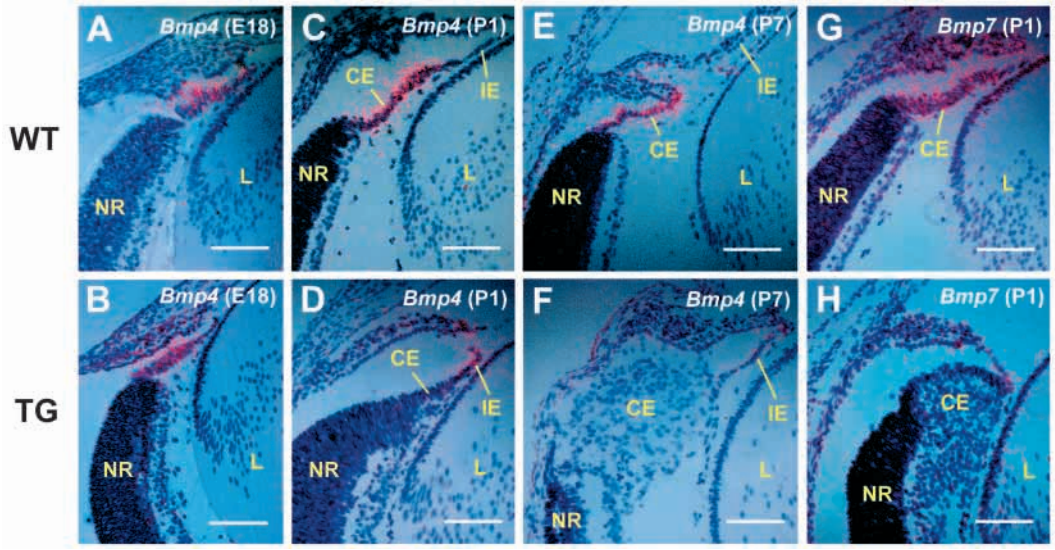

Fig. 4. Downregulation of BMP expression by transgenic Noggin. In wild-type eyes, *Bmp4* was highly expressed in E18 (A), P1 (C) and P7 (E) ciliary epithelia (CE). No apparent change in *Bmp4* expression was detected in the CE of E18 transgenic eyes (B) but its transcripts were significantly downregulated in P1 (D) and P7 (F) eyes. Similarly, transgenic Noggin had no apparent effect on *Bmp7* expression in the CE of E18 embryos (data not shown) but drastically downregulated its expression in the postnatal presumptive CE (compare H with G). Abbreviations: IE, iris epithelium; L, lens; NR, neural retina. Scale bars: 100 μm.

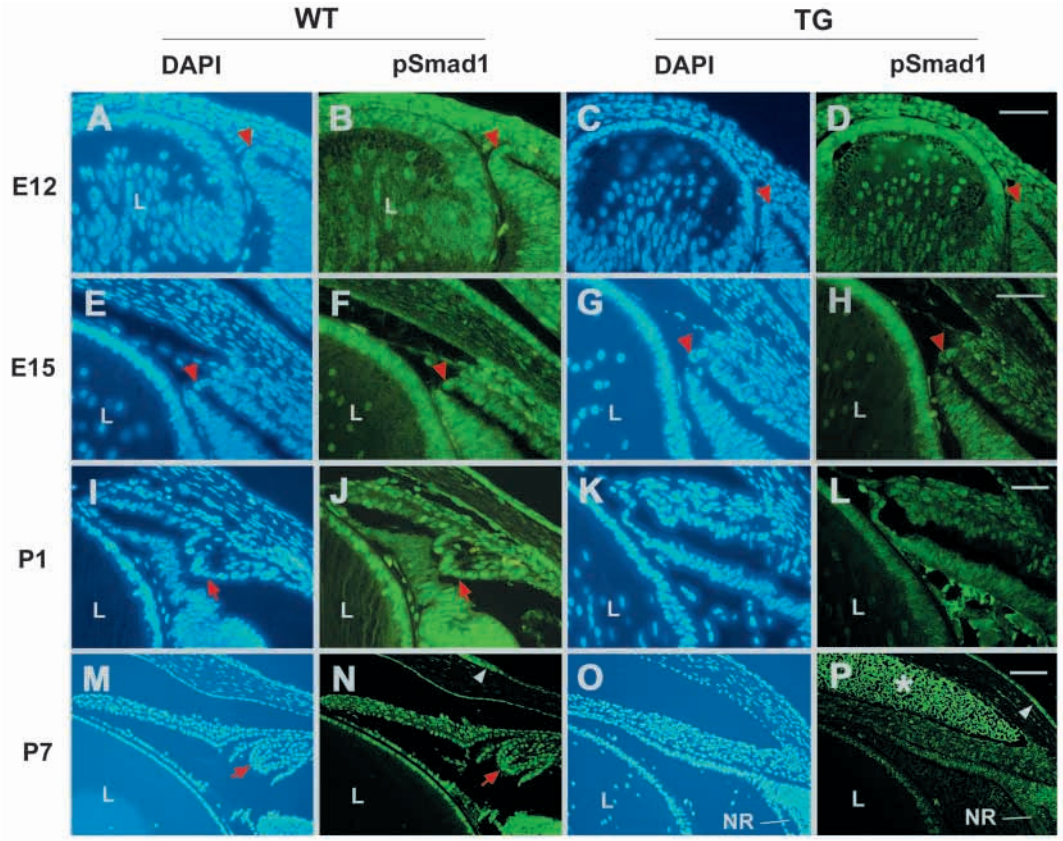

Fig. 5. Phosphorylation of Smad1 protein in the developing eyes. Nuclei were visualized by blue labeling with DAPI (A,C,E,G,I,K,M,O). Phosphorylated Smad1 (pSmad1) was detected by immunohistochemistry (green fluorescence) (B,D,F,H,J,L,N,P). At E12, pSmad1 was detected in the cytoplasm and the nucleus of most of the cells in wild-type (B) and transgenic eyes (D). At E15, pSmad1 levels decreased in corneal stroma cells in both wild type (F) and transgenic eyes (H). In transgenic P1 and P7 eyes (L,P), pSmad1 levels were significantly reduced in the lens epithelium, the iris, the ciliary body and the corneal endothelium compared with the wild-type eyes (J,N). However, pSmad1 labeling in the corneal epithelial cells was not significantly affected in transgenic eyes (compare white arrowheads in N and P). Arrowheads in A-H indicate the anterior margin of the optic cup. Arrows in I,J,M,N indicate the infolding ciliary epithelium, which was absent in the transgenic eyes. The asterisk in P indicates nonspecific fluorescent labeling. Abbreviations: L, lens; NR, neural retina. Scale bars: 100 μm.

Msx1 and *Otx1* expression

Homeobox transcription factors Msx1 and Msx2 have been implicated in eye development (Monaghan, 1991; Foerst-Potts and Sadler, 1997). Msx1 expression can be activated by BMP signaling (Suzuki et al., 1997; Furuta et al., 1997; Chen et al., 1996; Bei and Maas, 1998; Kim et al., 1998). In E18 and P1 wild-type eyes, *Msx1* was highly expressed in the inner layer of the developing ciliary epithelium (Fig. 6A,B). In P7 wild-type eyes, its expression was still present in the ciliary epithelium but at a reduced level (Fig. 6C). Like *Bmp4*, *Msx1* was not expressed in the developing iris. In CPV2-Noggin transgenic mice, *Msx1* expression was significantly downregulated at E18 and postnatally (Fig. 6D-F).

Transcription factor Otx1 is necessary for ciliary body development. Mice that lack Otx1 do not develop the ciliary body (Acampora et al., 1996). In wild-type eyes, *Otx1* was highly expressed in the developing ciliary and iris epithelia (Fig. 7A-C). In CPV2-Noggin transgenic eyes at E18 (Fig.7D), there was no dramatic change in *Otx1* mRNA levels compared with levels in wild-type embryos (Fig. 7A). In postnatal transgenic eyes, *Otx1* remained expressed in the developing iris but no transcripts were detected in the presumptive ciliary epithelium (Fig. 7E,F).

Rescue of defective ciliary epithelium by ectopic BMP7

To confirm that the Noggin-induced defect in the ciliary epithelium was indeed caused by inhibition of BMP signaling, we mated CPV2-Noggin transgenic mice (OVE1195) to two families of CPV2-BMP7 mice, OVE1340A and OVE1342B (Hung et al., 2002). Transgenic mice from the OVE1340A family express high levels of BMP7 transgene and exhibit complete retinal degeneration and retarded lens development by late embryonic stages (Hung et al., 2002), resulting in severe microphthalmia. No apparent apoptosis was detected in the presumptive ciliary and iris epithelia in these mice. Mice from the OVE1342B family express modest levels of BMP7 transgene and exhibit no apparent ocular defects (Hung et al., 2002). Double transgenics obtained from mating between CPV2-Noggin (OVE1195) and OVE1340A showed an ocular phenotype essentially identical to that of OVE1340A (data not shown), indicating a predominant effect of high-level BMP7. However, mating between OVE1195 and OVE1342B produced double transgenics that had essentially normal-looking ciliary epithelium (Fig. 8D). This experiment demonstrates that elevated BMP7 expression can overcome the inhibition by transgenic Noggin and restore development of the ciliary body.

DISCUSSION

Several members of the BMP family are widely expressed in early embryonic mouse eyes (Dudley and Robertson, 1997; Furuta and Hogan, 1998) but become restricted to the RPE and the ciliary and iris epithelia from midgestation to postnatal stages (Fig. 4) (Hung et al., 2002). In the present study, we demonstrate that lens-specific expression of transgenic Noggin disrupts morphogenesis of the ciliary body. Morphological abnormalities were not observed in these transgenic mice until around birth. An immunohistochemical assay for phosphorylation and nuclear translocation of Smad1 suggests that BMP signaling may be inhibited in late embryonic and postnatal stages (Fig. 5). The αA-crystallin promoter used in this study normally becomes active by E12 (Lovicu and Overbeek, 1998; Hung et al., 2002). Its activity level increases gradually and plateaus after E15 (data not shown). These observations suggest that transgenic expression of Noggin driven by this promoter would have no effects on cell-type specification of the ciliary epithelium in the optic vesicle and optic cup stages (E9-E11) and that transgenic Noggin inhibit the folding and morphogenesis of the ciliary epithelium at late stages of eye development. Blocking BMP signaling also changed the cell type of the presumptive ciliary epithelium to cells expressing the retinal ganglion cell marker *Brn3b* (Fig. 3), suggesting that BMP signaling may be required to maintain the differentiated state of the ciliary epithelium in the developing eye.

Previous studies have shown that the lens plays a role in induction of the ciliary epithelium (Thut et al., 2001) (for a review, see Beebe, 1986). Mice from several of our transgenic lines developed cataracts. However, mice from transgenic family OVE1198 did not have lens abnormalities but still failed to form the ciliary body, indicating that failure in ciliary body formation was not caused by lens defect. Rescue of the defective ciliary epithelium by co-expression of transgenic BMP7 further confirms the role of BMP signaling in ciliary body development.

Our in situ hybridization results show that endogenous Noggin is expressed in most of the RPE (except for the anterior region) in late embryonic and postnatal eyes (Fig. 1D-F). *Bmp4* and *Bmp7* are also expressed in the developing RPE (Hung et al., 2002) (data not shown). The co-localization of BMPs and Noggin expression in the RPE raises the issue of what a role Noggin plays in the developing eye. A recent study shows BMPs and Noggin are co-expressed by ependymal cells adjacent to the subventricular zone (SVZ) in the brain (Lim et al., 2000). BMP signaling blocked production of neurons from SVZ precursors by promoting glial differentiation, while exogenous Noggin protein enhanced neurogenesis and inhibited glial cell differentiation in the brain (Lim et al., 2000). We observed increase in thickness of the retinal ganglion cell layer in mouse families with high levels of transgenic Noggin expression. Whether this resulted from inhibition of differentiation of other cell types in the retina remains to be determined.

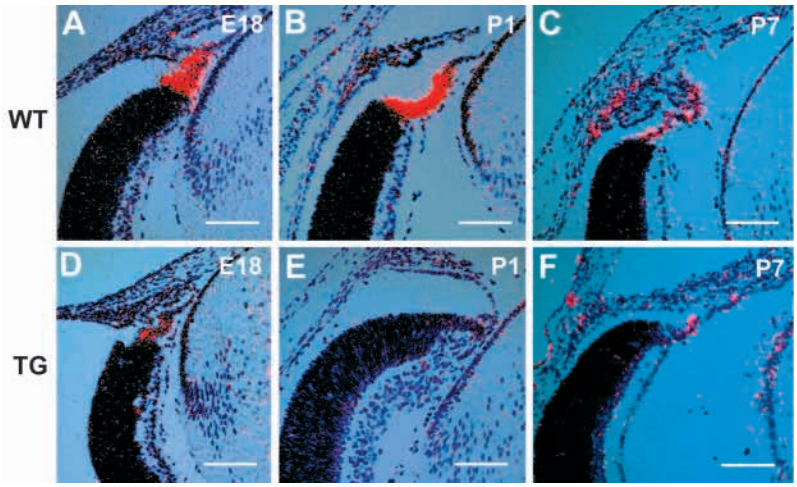

Fig. 6. Downregulation of *Msx1* expression. In situ hybridization shows that *Msx1* was expressed in the developing ciliary epithelium (CE) at E18 (A) and P1 (B), but at a reduced level by P7 (C). In CPV2-Noggin transgenic mice, *Msx1* expression was significantly downregulated in E18 (D), P1 (E) and P7 (F) eyes. Scale bars: 100 μm.

The observed downregulation of *Bmp4* and *Bmp7* expression in the ciliary epithelium in CPV2-Noggin transgenic mice (Fig. 4) raises the possibility that BMPs regulate their own expression in the developing ciliary epithelium. It has been shown that BMPs can regulate their own expression in dental mesenchyme (Chen et al., 1996) and can regulate its own promoter activity in cultured cells (Ghosh-Choudhury et al., 2001). Another possibility is that blocking BMP signaling by Noggin alters the cell type of the presumptive ciliary epithelium to neuronal cells that do not express BMPs.

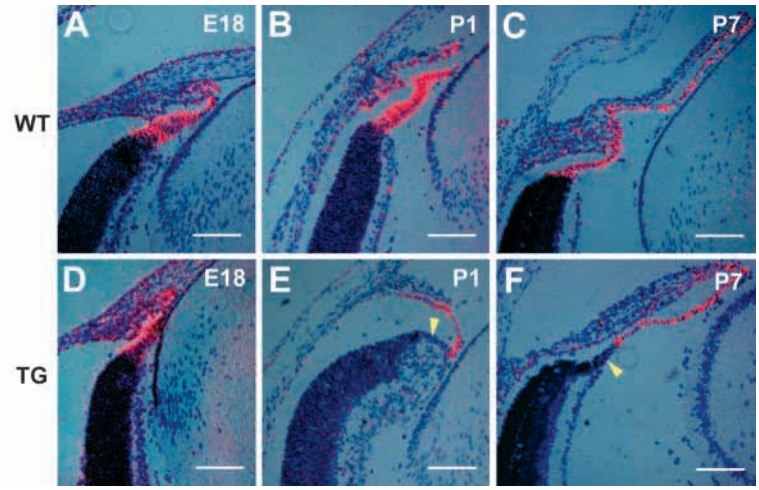

Fig. 7. Downregulation of *Otx1* expression. In wild-type mice, *Otx1* transcripts were detected by in situ hybridization in the developing ciliary and iris epithelia at E18 (A), P1 (B) and P7 (C). In CPV2-Noggin transgenic mice, no significant change in expression was observed in E18 eyes (D), but *Otx1* appeared to be switched off (arrowheads) in the presumptive ciliary epithelium of P1 (E) and P7 (F) eyes. Levels of *Otx1* transcripts remained essentially unchanged in the iris of transgenic mice. Scale bars: 100 μm.

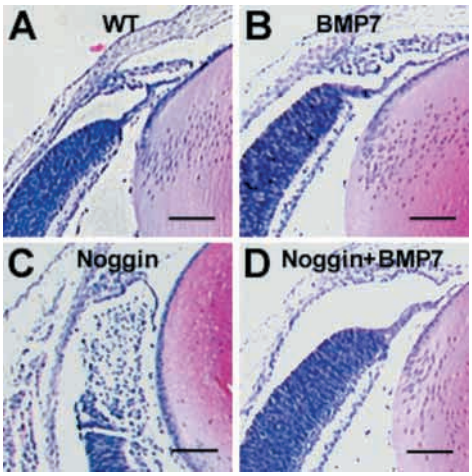

Fig. 8. Rescue of the ciliary defects by co-expression of BMP7. In CPV2-BMP7 transgenic mice (OVE1342B), the ciliary epithelium appeared normal (B) compared with the wild-type control (A). In CPV-Noggin transgenic mice (OVE1195), ciliary body development was disrupted (C). Co-expression of BMP7 and Noggin (OVE1342B × OVE1195) rescued the Noggin-induced defects in the developing ciliary epithelium (D). Tissue sections were obtained from mice at P1. Scale bars: 100 μm.

The expression pattern of homeobox gene *Msx1* suggests a role in tissue patterning in the anterior region of the developing mouse eye (Monaghan et al., 1991). *Msx1* is an immediate early-response gene of BMP signaling in epidermal induction in *Xenopus* (Suzuki et al., 1997) and in the brain (Furuta et al., 1997), the tooth (Chen et al., 1996; Bei and Maas, 1998) and the cranial suture (Kim et al., 1998) during mouse development. The high-level expression of *Msx1* correlates well with BMP4 and BMP7 expression in the developing ciliary epithelium (see Figs 4, 6). Therefore, *Msx1* expression in this region is presumed to be regulated by BMPs. This is further supported by our finding that blocking BMP signaling by Noggin dramatically downregulates *Msx1* expression in the presumptive ciliary epithelium (Fig. 6). It is still not known whether *Msx1* is required for morphogenesis of the ciliary processes, because mice lacking *Msx1* die at birth (Satokata and Maas, 1994).

Transcription factors Otx1 and Otx2 have been shown to be essential in development of the brain and sensory organs, including eyes in the mouse (Acampora and Simeone, 1999; Martinez-Morales et al., 2001). In mice that lack Otx1, the ciliary body does not form, indicating the requirement for Otx1 in morphogenesis of the ciliary processes. Our results suggest that *Otx1* expression in the developing ciliary body is regulated by BMP signaling. Whether expression of *Otx1* is directly targeted by BMP signaling or indirectly regulated by other transcription factors such as Msx1 has yet to be investigated.

During eye development, the margin between the neural retina and the RPE gives rise to two different tissues, the iris epithelium and the ciliary epithelium. Differences between these two tissues in the developing eye are indicated by distinct sets of genes expressed by them. For example, *Bmp4* and *Msx1* are expressed in the prospective inner ciliary epithelium but not in the iris epithelium (Figs 4, 6) while *Bmp7* and *Otx1* are expressed in both the ciliary and iris epithelia (Figs 4, 7). *Otx1* expression in the presumptive ciliary epithelium but not in the iris epithelium was inhibited by transgenic Noggin (Fig. 7F). It is interesting to note that in Otx1 knockout mice, the ciliary body failed to form while the iris was still present (Acampora et al., 1996). This is similar to the phenotype in our CPV2-Noggin transgenic mice. Immunohistochemistry using an antiserum against αSMA shows that iris differentiation proceeded but was retarded in our transgenic mice.

The choroid plexus in the brain and the ciliary body in the eye share remarkable similarities in structure, function, developmental processes and gene expression. The choroid plexus consists of a single continuous layer of epithelial cells overlying a vascular central core. These epithelial cells secretes cerebrospinal fluid into the ventricles of the brain. Like the ciliary body, the choroid plexus is derived from the neuroepithelium. A previous study showed overlapping expression of several members of the BMP family in the developing choroid plexus in the mouse brain (Furuta et al., 1997). The domains of BMP expression coincided with those of *Msx1* and were associated with limited growth of the neuroectoderm. Given the similarities between the choroid plexus and the ciliary body, it is conceivable that similar signaling mechanisms may be involved in cell differentiation and morphogenesis of both tissues. Whether blocking BMP signaling will prevent formation of the choroid plexus has yet to be confirmed.

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