BMP and Hedgehog signaling during the development of scleral ossicles

Kellie Duench, Tamara A. Franz-Odendaal *

Department of Biology, Mount Saint Vincent University, 166 Bedford Highway, Halifax, Nova Scotia, B3M 2J6, Canada

A R T I C L E   I N F O

Article history:
Received for publication 31 January 2011
Revised 9 February 2012
Accepted 10 February 2012
Available online 17 February 2012

Keywords:
Skeletal development
Intramembranous bone
Induction
Compensation
Galλus galλus
Scleral papillae
Ex ovo culturing
Condensations
Dermal bone conjunctival papillae

A B S T R A C T

Bone development is a complex process, involving multiple tissues and hierarchical inductive interactions. The study of skeletal development has largely focused on endochondral bones while intramembranous bones, such as the scleral ossicles within the avian eye, have received less attention. Our previous research directly demonstrated the involvement of sonic hedgehog and suggested the involvement of bmp2 and 4 during the development of scleral ossicles. The bones of the sclerotic ring are induced by overlying conjunctival papillae at HH 35 and 36. Here, we examine the spatial and temporal expression patterns of ptc1, ihh, bmp2, bmp4 and bmp7. We show that the cells of conjunctival papillae express ptc1, ihh and bmp2 at these stages; coincident with shh expression previously described. Interestingly, both ihh and ptc1 are also expressed in the mesenchyme underlying the papillae unlike shh and bmp2. Bmp4 and bmp7 are not expressed in these regions at any stages examined. Furthermore, using Noggin soaked beads implanted adjacent to papillae, we provide direct evidence that the BMP family of genes are important factors in the development of scleral ossicles. Localized inhibition of BMPs in this way causes a reduced expression of ihh in the surrounding tissue demonstrating that the BMP and Hedgehog pathways interact. Our data also demonstrates that the sclerotic ring has an intrinsic ability to compensate for missing elements. The scleral ossicle system provides a unique opportunity to investigate the epithelial-mesenchymal induction of intramembranous bones of the vertebrate skull.

© 2012 Elsevier Inc. All rights reserved.

Introduction

Skeletal development occurs via two main types of ossification; endochondral and intramembranous. Previous studies have largely focused on the development of endochondral bones (particularly in the limb) (reviewed in Johnson and Tabin, 1997). In comparison, far less is known regarding the development of intramembranous bones. The study of calvariae has produced the majority of the information regarding molecular pathways involved during intramembranous bone development (Abzhanov, et al., 2007; Cho et al., 2006; Elola et al., 2007; Hornik et al., 2004; Kang-Young et al., 2005; Kim et al., 1998; Opperman, 2000). However, the developmental pathways for other intramembranous bones, such as the scleral ossicles of the eye, remain unknown.

In birds, the scleral ossicles form a sclerotic ring, which plays a role during accommodation to achieve visual acuity and is responsible for maintaining eye shape (Franz-Odendaal and Vickaryous, 2006; Walls, 1942). These scleral ossicles are neural crest derived intramembranous bones that develop through interactions with conjunctival papillae (Couly et al., 2002; Franz-Odendaal and Vickaryous, 2006). These papillae are small, transient clusters of epithelial cells that form outgrowths of the conjunctival epithelium (Coulobre, 1962). Despite the fact that these papillae form from the conjunctiva, they are commonly referred to as 'scleral' papillae (Hamburger and Hamilton, 1951). These papillae can be found in direct correlation (1:1 ratio) with the number and pattern of scleral ossicles (Fig. 1) and there is a unique pattern to their development (first investigated and described by Coulobre and Coulobre, 1962). More recently this pattern was described in Galλus galλus (Franz-Odendaal, 2008) showing that the number of papillae present can range from 13 to 16 per eye. First a small group of papillae (3–4) will form over the ciliary artery, followed by a second group of papillae directly across from the first group (Fig. 1). Temporal then nasal groups of papillae form until there is a complete ring. Conjunctival papillae begin to develop at HH 30, by HH 37 the papillae have completely degenerated. The number of papillae (and therefore scleral ossicles) is often asymmetric from right to left eye in the same embryo, however there is rarely a difference of more than one papilla/ossicle per eye (Franz-Odendaal, 2008). Several studies demonstrate that the conjunctival papillae are inducing the underlying ectomesenchyme to form skeletal condensations. i) Removal of a single scleral (epithelial) papilla was shown to prevent the formation of the underlying ectomesenchymal scleral ossicle (Coulobre and Coulobre, 1962); ii) recombination experiments involving conjunctival epithelium and the mandibular or maxillary ectomesenchyme, induces bone in the mesenchyme (Hall, 1981); and iii) A diffusible factor signals between the scleral papillae and the underlying mesenchyme (Pinto and Hall, 1991). Despite this evidence, very little is
daughter members of the Hedgehog family, namely Indian and desert Hedgehog, are known about which signaling molecules are involved in the developmental pathway of scleral ossicles.

Recently, we identified the involvement of a Hedgehog family in scleral ossicle development (Franz-Odendaal, 2008). When exogenous cyclopamine, an inhibitor of Hedgehog proteins, was applied next to a papilla at stage HH 35 and 36, the formation of the underlying ossicle was inhibited. Furthermore, sonic hedgehog expression was found in the conjunctival papillae at these stages indicating that it was a possible candidate for the epithelial mesenchymal induction of scleral ossicles. This study suggested, for the first time, a role for the Hedgehog family in the development of scleral ossicles, however, other members of the Hedgehog family, namely Indian hedgehog and desert hedgehog, were not ruled out at the time. Desert hedgehog is not found in the genome of chicken, zebrafish or in the lizard genus, Anolis, and may be absent from all reptiles. Indian hedgehog however, is involved in osteoblast differentiation during endochondral ossification in the limb (e.g. Chung et al., 2001; Hu et al., 2005; Karp et al., 2000; Lai and Mitchell, 2005; Pathi et al., 1999) and more recently shown to be involved in the development of intramembranous neural crest derived bones of the avian and murine skull (Abzhanov et al., 2007). Ihh therefore could not be ruled out as a player in the induction of scleral ossicles and warrants further investigation.

The present study is a continuation of our investigation into scleral ossicle induction and development. First, we investigate the temporal and spatial expression pattern of the hedgehog receptor ptc1 during scleral ossicle development. Patched1 (ptc1) is a transmembrane ligand receptor for multiple Hh proteins and is associated with a G-protein coupled transmembrane receptor molecule smoothened (smo) (Carpenter et al., 1998). PtC1 normally inhibits the function of smo in the absence of any Hedgehog signal. However, when a Hedgehog protein binds to the ligand receptor ptc1, smo is no longer inhibited and a transmembrane transduction reaction occurs activating the downstream Hedgehog target. Ptc1 is also a downstream target of Hh signaling and therefore the location of the ptc1 receptor is often used as an indicator of Hh activity (Harfe et al., 2004; Marigo et al., 1999; Traiffort et al., 1998). Next, we investigated the expression pattern of Indian hedgehog during ossicle development for the reasons described earlier.

We also wanted to determine whether the BMP family was involved in ossicle induction and/or development. The Bone Morphogenetic Protein (BMP) family of genes are a family of secreted proteins known to be important in bone growth and development. The role of BMPs includes the recruitment of mesenchymal cells into skeletogenic condensations (Hall and Miyake, 2000), the commitment of neural crest derived mesenchymal cells to skeletogenic lineages (Abzhanov et al., 2007), and epithelial–mesenchymal interactions (e.g. tooth development) (Theisfeld, 2003). Our research previously demonstrated via real-time PCR that there is an increase in expression of the genes bmp2 and bmp4, at HH stage 36 (the induction stage) relative to HH stage 33 (prior to induction). Here, we investigate the spatial and temporal expression pattern of bmp2, bmp4, and bmp7 during scleral ossicle induction and demonstrate that only bmp2 is expressed between HH stages 34.5 and 36. Furthermore, we demonstrate that scleral ossicles can be locally inhibited by exogenous Noggin, a secreted protein which binds to BMP proteins (predominately bmp2, bmp4 and bmp7) rendering them inactive (Zimmerman et al., 1996).

Our findings suggest that ihh is likely an important signaling molecule during the development of scleral ossicles since its expression is most similar to the expression of its downstream target, ptc1. Shh is likely involved only in the maintenance and/or proliferation of the papillae. Furthermore, we show that BMPs play critical role in the development of scleral ossicles, and that bmp2 signaling interacts with ihh signaling in this system. Finally, our findings suggest that scleral ossicles have an intrinsic ability to compensate for missing elements within the sclerotic ring. Overall, these results provide a significant contribution to unravelling the molecular pathway underlying the development of scleral ossicles, and shed light on the development of other intramembranous bones within the vertebrate skull.

Materials and methods

Chicken embryos

Fertilized chicken eggs of the strain Gallus gallus were obtained from Cox Brothers Ltd, Truro, Nova Scotia, Canada. Eggs were incubated at 37 °C with approximately 40% humidity and turned once daily. Chicken embryos were staged using the Hamburger and Hamilton (1951) staging chart. Embryos were staged at HH stage 19 at the onset of ex ovo culturing and again prior to bead implantation at HH stage 35.

Ex ovo culturing

An ex ovo culturing method was used instead of windowing the eggs to ensure that there was no restriction in accessing the embryo during bead implantation at advanced stages of embryonic development. The ex ovo method was used as described in Franz-Odendaal.
Phosphate substrate solution for an hour at room temperature. After were then placed in the dark and transferred into napthol-AS-TR-Maleate buffer (pH 8.3) for 1 h at room temperature. The embryos washed several times in distilled water and then incubated in Tris when distinct condensations are visible. Brie fi and Franz-Odendaal (2010), on embryos at HH stage 38 and older, Alkaline phosphatase staining and condensation measurements series.

Bead preparation

Affi-gel beads (BioRad 153–7302) were used for all experiments. Beads were washed repeatedly in 1× Phosphate Buffer Solution (PBS), pH 7.4 and stored at 4 °C in PBS. Beads were absorbed with 1 μg/μl of recombinant mouse Noggin (R&D Systems, 1967–NG) and incubated at room temperature for 1 h. Control beads were rinsed 20 times and absorbed with 1× PBS.

Affi-gel bead implantation

An ex ovo cultured embryo at HH stage 35 was placed under a Nikon (SMZ1000) dissecting microscope. Access to the eye was gained through the membranes and a small hole was made in the conjunctiva-epithelium of the eye directly next to a scleral papilla. A bead was gently pushed into this hole and a 45 μl dose of penicillin/streptomycin (5000 U penicillin:5 mg streptomycin, Sigma P4458) was placed on the albumin of the embryo to prevent infections. The embryo was returned to the incubator and allowed to develop until HH stages 39–41 (13–15 days of incubation). The embryo was then fixed in 10% Neutral Buffered Formalin (Fischer Scientific-24568S) overnight at room temperature followed by processing through a graded ethanol series.

Alkaline phosphatase staining and condensation measurements

An alkaline phosphatase stain was performed according to Edsall and Franz-Odendaal (2010), on embryos at HH stage 38 and older, when distinct condensations are visible. Briefly, specimens were fixed in 4% paraformaldehyde overnight at 4 °C. Embryos were washed several times in distilled water and then incubated in Tris-Maleate buffer (pH8.3) for 1 h at room temperature. The embryos were then placed in the dark and transferred into naphthol-AS-TR-phosphate substrate solution for an hour at room temperature. After rinsing a few times in saturated sodium borate, the embryos were bleached overnight in a 3% H2O2/1% KOH solution. Embryos were stored in 80% glycerol. Once ossicles were stained with alkaline phosphatase, ossicle surface area was measured (maximum length multiplied by maximum width of each ossicle) using Nikon NIS-Elements software (3.0). Measurements were averaged and the standard deviation was calculated (Table 1). Lastly, the maximum internal diameter of the sclerotic ring was measured for each eye.

Results

Patch1 is expressed in the conjunctival papillae and underlying mesenchyme

Since we had previously determined the involvement of shh in os- sicle development, we wanted to determine which tissues it (and possibly ihh) act upon (via analysis of the expression of the Hh recep- tor patched1) bearing in mind that Hh proteins can act at both long and short ranges (Drossopoulou et al., 2000). No patched1 expression was present in HH stages 30 through 34. Patched1 expression is first detected in the papillae at HH stage 35. As the embryo develops and papillae degenerate, the expression declines in the papillae and appears within the underlying mesenchyme. At HH stage 36 the expression of patched1 is both in the papillae and in the mesenchyme below the papillae (Fig. 2A,B), however it is not detected in the epithelium of the interpalpebral region (Fig. 2A, arrows). To confirm the localization of this patched1 expression pattern, whole mount in situ hybridization tis- sue was sectioned (Fig. 2B). At HH stage 37 the papillae are in an advanced stage of degeneration and condensation formation begins. No patched1 expression was detected at this stage. Similarly patched1 expression was not found in HH stages 38 through 40. Additionally, no expression was detected in the negative controls. These results indicate that patched1 is expressed in a broader domain than shh, namely in both the papillae and in the underlying mesenchyme at HH 35 through 36. Further indicating that Hedgehog activity occurs in both tissues.

Indian hedgehog is expressed during scleral ossicle induction

Next, we wanted to determine whether ihh plays a role during the induction of scleral ossicles since shh expression was found localized to the papillae at stages HH 35 and 36 (Franz-Odendaal, 2008), while patched1 expression was found in the papillae as well as in the epithelium and mesenchyme surrounding the papillae at these stages. Indian hedgehog expression on the other hand, was found during a very nar- row window at late stage 35 and early stage 36 corresponding to within the timeframe for induction of the ossicles. Importantly Ihh was found in both the conjunctival papillae and within the underlying mesenchyme at both stages, unlike shh (Fig. 2CD); this expression pattern was confirmed with sectioning. Ihh transcripts were not found earlier or later when the papillae degenerate and were absent from the interpalpebral regions, similar to patched1 (Fig. 2C, arrows). Ihh was not detected in the negative controls. These results indicate

<table>
<thead>
<tr>
<th>HH stage</th>
<th># of ossicles, control eye</th>
<th>Surface area, control ossicles (mm²)</th>
<th>Diameter of sclerotic ring, control (mm)</th>
<th># of ossicles, experimental eye</th>
<th>Surface area, experimental eye (mm²)</th>
<th>Diameter of sclerotic ring, experimental eye (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>38</td>
<td>15</td>
<td>0.623</td>
<td>3.42</td>
<td>14</td>
<td>0.594</td>
<td>3.16</td>
</tr>
<tr>
<td>39</td>
<td>15</td>
<td>0.239 ± 0.11</td>
<td>3.53</td>
<td>13</td>
<td>0.689 ± 0.06</td>
<td>3.26</td>
</tr>
<tr>
<td>39</td>
<td>15</td>
<td>0.388 ± 0.04</td>
<td>4.06</td>
<td>15</td>
<td>0.827 ± 0.07</td>
<td>3.77</td>
</tr>
<tr>
<td>39</td>
<td>15</td>
<td>0.295 ± 0.06</td>
<td>3.72</td>
<td>14</td>
<td>0.793 ± 0.03</td>
<td>3.65</td>
</tr>
<tr>
<td>40</td>
<td>15</td>
<td>0.258 ± 0.017</td>
<td>3.07</td>
<td>14</td>
<td>0.416 ± 0.06</td>
<td>3.82</td>
</tr>
<tr>
<td>41</td>
<td>14</td>
<td>0.886</td>
<td>3.89</td>
<td>13</td>
<td>1.465</td>
<td>4.43</td>
</tr>
</tbody>
</table>
that ihh acts in both the conjunctival papillae and in the underlying mesenchyme.

### Inhibition of BMPs by exogenous Noggin alters the sclerotic ring

To determine whether bone morphogenetic proteins (BMPs) are involved in the development of scleral ossicles, we blocked their activity with Noggin, a specific inhibitor of bmp2 and bmp4, which is commonly used with beads for in vivo implantation (Botchkarev et al., 2001; Chang et al., 1999; Chung et al., 2007; Hosoya et al., 2008). We found that Noggin soaked beads implanted at HH 35, the onset of ossicle induction, has no effect on the maintenance of the papilla adjacent to the bead (Fig. 3A). However, 2–4 days later, when condensations are normally visible; the condensation underlying the bead implantation site is missing (n=6) (Fig. 3E–J). Control experiments with PBS-soaked beads show no effect on papillae or ossicle development (Fig. 3C and D). This experiment suggests that one of the BMP family members, likely bmp2 and/or bmp4, is required for the proper development of the sclerotic ring.

Unexpectedly, the majority of the embryos that survived to develop ossicles consistently have larger ossicles in the surgery eye compared to the control eye (5/6 embryos). These larger ossicles occurred in two places – directly adjacent to the missing ossicle, or in the next group of ossicles to be induced (Table 1). In the oldest of these embryos (HH 41) the ossicle directly adjacent to the bead implantation site, grew large enough to close the gap that appeared as a result of the inhibited ossicle (Fig. 3I and J). Despite this compensation in the ring of scleral ossicles, the diameter of the complete ring remains unchanged (average diameter control eyes: 3.72 mm ± 0.23 mm; average diameter bead implant eyes: 3.68 mm ± 0.46 mm; n = 6 embryos). This data is tabulated in Table 1 and suggests that the sclerotic ring has an intrinsic ability to compensate for missing individual ossicles.

### Bmp2 is mapped to the conjunctival papillae and interacts with ihh

To determine which BMP family members are involved in the scleral ossicle system we performed in situ hybridizations of the likely candidates; namely bmp2, bmp4 and bmp7. No bmp4 or bmp7 expression was found in stages HH 30 through 37. Bmp2 expression was found in the conjunctival papillae, but not in the underlying mesenchyme, at HH stage 34.5 through to early HH stage 36. Taken together with the aforementioned Noggin data, these results suggest that bmp2 is a key player in scleral ossicle development.

To determine whether the Hedgehog and BMP families interact, we performed a Noggin bead implant experiment followed by in situ hybridization for ihh. One day after localized BMP inhibition, we observe dramatically reduced ihh expression in the bead implantation area (n = 6, Fig. 3O). In the HH stage 35 contralateral control eye, ihh expression is normal (Fig. 3P). This result indicates that the BMP and Hedgehog pathways do interact during scleral ossicle development and that BMP is required for the expression of ihh.

### Discussion

#### Location of ptc1 and ihh expression suggests ihh is a key player in ossicle induction

Previous research from our lab has shown that shh is present during the induction of scleral ossicles and that inhibition of the Hedgehog family through the localized application of exogenous cyclopamine prevents ossicle as well as papillae formation (Franz-
It was concluded that shh is likely a maintenance factor for the papillae, and possibly involved in subsequent ossicle induction. We have now confirmed the presence of the ptc1 receptor in the same location as shh providing support for this hypothesis. Since cyclopamine is a ubiquitous Hedgehog inhibitor, we could not at the time eliminate the possibility of other members of the Hedgehog family playing a role in ossicle development. In the present study, we show that ihh expression is present during a very small developmental window of about one day. Although the temporal expression of ihh is brief compared to ihh expression in other areas of development such as the limb, the expression is continuous during the induction phase (HH stages 35–36) of ossicle development (previously determined by Franz-Odendaal, 2008). This expression is in the conjunctival papillae and in the underlying mesenchyme, and coincides with the spatial location of ptc1, however it is present within a narrower temporal range (Fig. 4). This confirms that ihh is likely acting both in the conjunctival epithelium and in the underlying mesenchyme adjacent to the papillae, probably as a short range signal. The ihh expression pattern is also different to that of shh, which is expressed in a narrower spatial domain (i.e. only the conjunctival papillae) and over a wider temporal range (HH stage 35 until late stage 36, 8.5 to 10 days) (Fig. 4). These results support the hypothesis that shh could be acting on the papilla itself as a proliferation factor, as suggested by Franz-Odendaal (2008). We cannot however rule out whether shh might also be acting as a long range signal to the underlying mesenchyme.

Abzhanov et al. (2007) have recently shown that Indian hedgehog plays a role during intramembranous ossification of the neural crest.

Odendaal, 2008).
BMPs are critically important to the development of the sclerotic ring

Previous investigations performed by our lab, using real-time PCR, have shown that there is an increase in expression of bmp2 and 4 (3.5 and 2.7 fold respectively) at HH stage 36, relative to stages HH 33 (Franz-Odendaal, 2008). Since the eye tissues that were used for the real-time PCR data included the conjunctival epithelium, underlying scleral mesenchyme, RPE, and potentially parts of the neural retina, it was not understood if these genes were specifically playing a role in the development of scleral ossicles. We therefore attempted to locally inhibit BMP using its inhibitor Noggin, during the development of a scleral ossicle and then investigated the expression patterns of candidate BMP family members.

Our results show that exogenous Noggin directly affects the development of the ossicle at the implantation site and has an indirect effect on the rest of the sclerotic ring. It is difficult to determine where the exogenous Noggin diffuses to or which tissues it acts on, since the Noggin bead was placed directly under the conjunctival epithelium. BMP signaling could have been inhibited in the papillae, the underlying scleral mesenchyme, and or retinal pigmented epithelium (RPE). Therefore it was not surprising to find Ihh involved in the development of the intramembranous bones of the avian eye.

Previously, Ihh-null mouse embryos had no endochondral bone in the trunk and the dermal bones of the skull although present are markedly reduced in size (Karp et al., 2000; St-Jacques et al., 1999). Additionally, several researchers have shown the involvement of the Hedgehog family in osteoblast differentiation and bone patterning (e.g. Ehlen et al., 2006; Hu et al., 2005; Laforest et al., 1998; Quint et al., 2002; Rodda and McMahon, 2006). Therefore it was not surprising that Ihh was again detected within the conjunctival epithelium and adjacent ossicles during the development of scleral ossicles (Palmoski and Goetinck, 1970). Further investigation is required to determine the underlying mechanisms for compensation to perturbations in the system.

BMPs are not crucial for the maintenance of this epithelial structure (Fig. 3A and B) unlike Shh. The inhibition of Shh by Noggin therefore suggests two possible roles for BMPs. Firstly, BMPs could be active in the mesenchyme and play a role in the induction of mesenchymal cells to condense into scleral ossicle condensations (Hall, 2005). Alternatively, BMPs could be present in the papillae and involved in an epithelial–mesenchymal signaling event, by diffusing to the underlying mesenchyme. bmp2 and 4 are likely candidates, as they have been implicated in skeletogenesis in other areas of vertebrate embryos (e.g. Abe et al., 2000; Abzhanov et al., 2007; Quint et al., 2002) and we have previously detected elevated expression levels within the chicken eye at HH stage 35 and 36 (Franz-Odendaal, 2008). Another likely candidate is bmp7, which also has an affinity for Noggin and is known to regulate many of the morphogenetic factors (Dkk, Msx 2) that are crucial for skeletal cell differentiation (Shea et al., 2003). We could not detect bmp4 or bmp7 expression within the scleral mesenchyme or conjunctival epithelium; however bmp2 transcripts were localized to the conjunctival papillae at HH stage 35 to 36 (Fig. 4). This finding indicates that bmp2 is possibly involved in the epithelial–mesenchymal induction of the scleral ossicles. bmp2 was also localized to the RPE, indicating that there is the potential for signaling from the RPE. However this signaling is most likely received by the scleral mesenchyme, since it has been previously shown that the RPE is cruical in the induction of scleral cartilage (Thompson et al., 2010). Several researchers have shown that BMPs play an important role in determining the size and shape of skeletal elements (e.g. in the chick limb, Duprez et al., 1996; in the dermal fin rays, Laforest et al., 1998) and that BMPs are one of the major targets of Hh signaling (Bitgood and McMahon, 1995; Methot and Basler, 1999; Quint et al., 2002 and others). More recently, Abzhanov et al. (2007) have specifically shown that BMPs also play an important role during dermal bone development by regulating the earliest cell differentiation decisions. It is therefore not surprising that we find that the local inhibition of BMPs affects Ihh expression. Our study also demonstrates that inhibiting one ossicle can affect the size of other ossicles within the sclerotic ring (also shown by Franz-Odendaal and Vickaryous, 2006) and suggests that there is an intrinsic mechanism preventing the formation of an incomplete ring.

The ability of skeletal tissues within the eye to compensate to perturbations is intriguing. The unique sequential induction of groups of papillae/ossicles during an extended period of time (two days) gives the scleral mesenchyme many opportunities to compensate for the potential loss of an ossicle. This ability to compensate is also demonstrated in the chicken mutant scaleless, which only has a few large scleral ossicles (Palmoski and Goetinck, 1970). Further investigation is required to determine the underlying mechanisms for compensation and how inhibiting one ossicle can affect other ossicles in the ring. When a papilla is inhibited (or removed) the absence of this inhibitory signal could enable adjacent scleral ossicles to enlarge. Alternatively, the physical presence of a condensation might inhibit enlargement of adjacent ossicles. These two possibilities are not mutually exclusive. A developmental window likely exists during which adjacent ossicles can respond by enlarging, if they do not respond
within this window, then the next group of ossicles are affected. We have recently shown that the development of the vasculature in the eye does not correlate with ossicle development [Jourdeuil and Franz-Odendaal, in press]. Although very intriguing, this ability to compensate is beyond the scope of the current study.

Conclusions

This study has unraveled more of the signaling mechanism involved in the development and induction of scleral ossicles, which has been a 30-year mystery until the research performed by our lab. Specifically, this research provides insight into the roles of the Hedgehog and BMP family of genes. We show that shh is directly involved in the development of this dermal bone and that together with bmp, may be acting as short range and long range signals. We hypothesize that shh within the conjunctival papillae signals to the epithelium in a positive feedback mechanism regulating shh production in the papillae in order to produce more shh and to maintain the papillae. During this phase of papillae maintenance and induction, Ihh is produced in the conjunctival papillae and in the underlying mesenchyme, and is likely received in the mesenchyme where it interacts with bmp2 to induce ectomesenchymal cells to aggregate into skeletogenic condensations. Scleral ossicle development therefore seems to follow typical dermal bone development in which Hh and BMP families interact.

Several studies have shown that BMP, Hh and FGF pathways form complex networks regulating tissue interactions, for example, during skeletal development [Bitgood and McMahon, 1995; Kim et al., 1998]. Our results have contributed to filling in several gaps in the understanding of the molecular pathways and networks involved in scleral ossicle development, specifically the involvement of the Hh and BMP families. The role of the FGF family remains to be determined.

The scleral ossicle system provides a unique opportunity to investigate the epithelial–mesenchymal induction of intramembranous bones as well as the interactions between the BMP and Hh families in regulating tissue differentiation in general. This system is also highly accessible, offering a way to investigate compensation of the vertebrate craniofacial skeleton and its adaptability to exogenous and environmental influences.

References


