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Review

Growth factor regulation of lens development

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Abstract

Lens arises from ectoderm situated next to the optic vesicles. By thickening and invaginating, the ectoderm forms the lens vesicle. Growth factors are key regulators of cell fate and behavior. Current evidence indicates that FGFs and BMPs are required to induce lens differentiation from ectoderm. In the lens vesicle, posterior cells elongate to form the primary fibers whereas anterior cells differentiate into epithelial cells. The divergent fates of these embryonic cells give the lens its distinctive polarity. There is now compelling evidence that, at least in mammals, FGF is required to initiate fiber differentiation and that progression of this complex process depends on the synchronized and integrated action of a number of distinct growth factor-induced signaling pathways. It is also proposed that an antero-posterior gradient of FGF stimulation in the mammalian eye ensures that the lens attains and maintains its polarity and growth patterns. Less is known about differentiation of the lens epithelium; however, recent studies point to a role for Wnt signaling. Multiple Wnts and their receptors are expressed in the lens epithelium, and mice with impaired Wnt signaling have a deficient epithelium. Recent studies also indicate that other families of molecules, that can modulate growth factor signaling, have a role in regulating the ordered growth and differentiation of the lens.

Keywords: Lens development; Growth factors; Lens fiber differentiation; Lens epithelial differentiation; FGF; BMP; TGFβ; Wnt

Introduction

Over the years, the eye lens has been a popular system for studying mechanisms of development. For example, at the beginning of the 20th century, there was much interest in embryonic induction, and studies on the lens can be credited for gaining early insights into the nature and importance of this phenomenon. More recently, the application of new molecular technologies has provided a major impetus to lens developmental research. Consequently, there have been major advances in the identification of key regulatory molecules that mediate the main processes of lens development, including induction, morphogenesis, differentiation, and growth. The aim of this brief review is to give an overview, within a historical context, of what is known about growth factor regulation of these processes and identify some of the major questions that remain outstanding.

Lens induction

Reviews ranging from early (Coulombre, 1965; Grobstein, 1956; Jacobson, 1966) to more recent works (Fisher and Grainger, 2004; Goudreau et al., 2004; Lang and McAvoy, 2004; Weaver and Hogan, 2001) discuss various aspects of lens induction in depth. The aim of this section is to outline the main steps in the story of lens induction and establish the extent of our understanding of growth factor regulation of the processes involved.

Lens arises from head ectoderm that is associated with outgrowths of the developing forebrain, the optic vesicles (Fig. 1A). Soon after ectoderm and neuroectoderm become closely associated, lens morphogenesis begins and the ectoderm thickens to form the lens placode (Fig. 1B).

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Because of their close spatial association, embryologists hypothesized that the optic vesicle was the lens inducer. While early experiments with amphibians showed that removal of the optic vesicle primordium resulted in no lens formation (Spemann, 1901), later experiments such as those of King (1905), showed that in some cases, lens-like structures formed from ectoderm even when the optic vesicle was removed at the early neurula stage. These contradictory results generated further experimental activity in the first half of the 20th century with different species of amphibians. From these, convincing evidence emerged that structures bearing at least some resemblance to lenses can develop in the absence of optic vesicle (reviewed in Fisher and Grainger, 2004; McAvoy, 1981).

The concept that tissue interactions that take place earlier in the embryo are important for lens development began to gain momentum in the 1950s. For example, in transplantation experiments with newts, Liedke (1951) showed that ectoderm from gastrula formed lens if it was transplanted to early neurula but not to late neurula. In the latter case, the ectoderm missed out on association with the anterior mesodermal mantle which underlies presumptive lens ectoderm during neurulation. Such results stimulated research into the roles of other tissues that are associated with presumptive lens cells, besides the optic vesicle. Jacobson and colleagues conducted a series of explantation experiments with amphibians during the 1950s and 1960s and provided evidence that the endodermal wall of the future pharynx and presumptive heart mesoderm had some lens-inducing capacities (Jacobson, 1955, 1958, 1963a,b,c). More recently, from a comprehensive series of transplantation experiments with amphibians, Grainger and colleagues identified a key role for the anterior neural plate as an early inducer of lens ectoderm (Henry and Grainger, 1990). Thus, over the years, the view that lens induction is a simple one-step model involving an interaction between presumptive lens ectoderm and optic vesicle has evolved into the recognition that lens induction is a multi-step process that involves a series of inductive interactions. In their recent detailed review of the results of their extensive experiments, as well as critical evaluation of the literature on amphibians, chicks, and mice, Fisher and Grainger (2004) propose a current model for lens determination that includes five stages: competence, bias, specification, inhibition, and differentiation.

While many of the tissue interactions involved in lens induction have been defined, not so much is known about the regulatory factors that mediate these inductive events. Most progress has been made in the area of identification of key transcription factors that are expressed in the presumptive lens ectoderm and that are required for lens formation. Various genetic manipulations in *Drosophila* and mice as well as studies on human mutations have resulted in the identification of many different classes of transcription factors that have roles in lens development (reviewed in Goudreau et al., 2004; Lang and McAvoy, 2004). Foremost among these is the highly conserved Pax6 which appears to be at the top of a regulatory hierarchy (see Punzo et al., 2004). However, how these key genes are themselves regulated is little understood. Mutant mouse studies indicate that fibroblast growth factor (FGF; Faber et al., 2001) and bone morphogenetic protein 7 (BMP7; Wawersik et al., 1999) receptor signaling are required for lens induction and that they cooperate to promote Pax6 expression (Faber et al., 2001; Lang and McAvoy, 2004). Other studies also show that BMP4 is required for the optic vesicle to manifest a lens inducing capability (Furuta and Hogan, 1998). However, the observation that BMP4 does not effect Pax6 expression in the presumptive lens indicates that it may be involved in another induction pathway with, as yet, undefined factors from the optic vesicle (Furuta and Hogan, 1998; Lang and McAvoy, 2004).

Lens morphogenesis and differentiation

As described above, the early stages of lens morphogenesis are characterized by a close physical association between the presumptive lens and optic vesicle (Fig. 1). Outgrowth of the optic vesicle results in its coming to lie directly under the presumptive lens ectoderm (Figs. 1A, B). Although they are closely associated, the optic vesicle and presumptive lens ectoderm do not make complete contact; a narrow gap, across which the basal surfaces of the cells face each other, is maintained (Fig. 1B). Mesodermal cells which generally underlie other regions of ectoderm are largely excluded from this gap. Once in close proximity to each other, the presumptive lens ectoderm and optic vesicle send out thick cytoplasmic processes from their basal surfaces. These processes extend only partly across the gap, although occasionally a bridging process is detected (McAvoy, 1981). A fibrillar extracellular matrix (ECM) builds up between the two tissues and appears to be the basis for the strong adhesion between them (McAvoy, 1981; Wakely, 1977). During this time, the presumptive lens thickens to form the placode and then invaginates together with the optic vesicle to form lens pit and optic cup, respectively (Figs. 1B, C). ECM-mediated adhesion between these two tissues is probably important for coordinating their morphogenetic movements; however, it is likely that components of this ECM, including laminin and fibronectin, also play a role in mediating the fate and behavior of lens cells, as studies have shown that ECM components influence their migration, differentiation, and phenotype (Blakely et al., 2000; Parmigiani and McAvoy, 1991; Zuk and Hay, 1994). Integrin signaling has also been implicated, mainly through involvement of Src family tyrosine kinase activity, in regulating the transition of lens cells from proliferative to differentiative states (Menko, 2002; Walker et al., 2002a), and switches in integrin expression have been shown to be important for the progression of lens cell differentiation (Walker et al.,

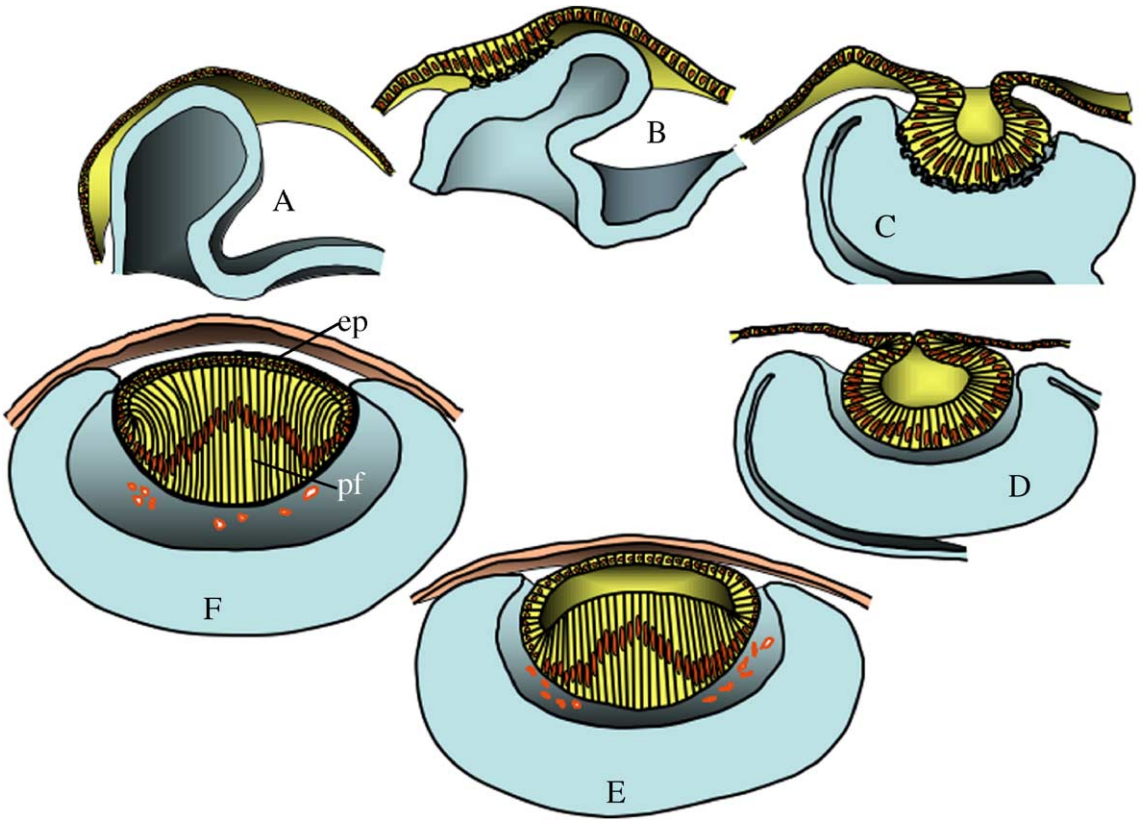

Fig. 1. Schematic diagrams A–F represent sections through the developing eye of a rodent embryo from embryonic days (E) 8.5 to 13.5. By E8.5 (A), the optic vesicle (blue) has grown out from the developing forebrain to lie close to a region of head ectoderm (yellow). By E9.5 (B), the optic vesicle is closely associated with head ectoderm and basal extensions from the opposing tissues can be detected at this stage. Ectodermal thickening in this region forms the lens placode. Invagination of the placode and optic vesicle occurs at E10.5 (C) leading to the formation of the lens pit and optic cup, respectively. By E11.5 (D), the lens pit has deepened to form the lens vesicle. By E12.5 (E), the lens vesicle has completely closed and detached from the optic cup. Lens cells in the posterior half of the vesicle elongate to form primary lens fiber cells. By E13.5 (F), the lens vesicle lumen has disappeared and the primary lens fibers (pf) are in contact with the anterior lens vesicle cells that form the epithelium (ep). Vitreous humor and hyaloid vasculature (orange, E, F) form between the developing lens and retina (arises from the optic cup). Ectoderm that forms over the lens gives rise to cornea (pink).

2002b). How growth factors and integrins cooperate to influence the developmental fate and behavior of lens cells is a key area for future lens research.

The lens pit deepens and it finally breaks away from the ectoderm to form the lens vesicle (Fig. 1D). The next major event in lens development involves the differentiation of two forms of lens cells from this vesicle. Cells in the posterior half of the vesicle elongate and differentiate to form the primary fibers, whereas cells in the anterior part of the vesicle differentiate into the epithelium (Figs. 1E, F). In this way, the lens acquires its distinctive polarity. The lens grows rapidly during late embryonic and early postnatal stages by cell division and differentiation. Cell divisions occur in the epithelial region just above the lens equator known as the germinative zone (Fig. 2; Harding et al., 1971; McAvoy, 1978a,b). The progeny of cell divisions migrate, or are displaced, below the equator into the transitional zone, where they elongate and differentiate into fiber cells. In this way, new fiber cells are continuously added to the fiber mass throughout life. Thus, the lens continually grows and maintains its distinct polarity with the monolayer of epithelial cells restricted to the anterior compartment (Fig. 2).

Lens polarity is maintained throughout life and there is clear evidence that it is tightly regulated by the ocular environment. This is best highlighted through the elegant experiments of Jane and Alfred Coulombre in the 1960s, using the embryonic chick. These experiments clearly demonstrated not only the impact that the ocular environment has on lens polarity, size, shape and growth, but also the influence that the lens in turn has on eye development and growth. One of their most cited experiments, which was later reproduced in mice (Yamamoto, 1976), involved the removal of the embryonic chick lens which was inverted and replaced in the eye so that the lens epithelium which normally faced the cornea, now faced the vitreous. After 5 days, all the epithelial cells facing the vitreous elongated into lens fibers, while those situated at the lens equator continued to divide and reconstituted a new anterior epithelium. The lens fibers that were initially repositioned away from vitreous and now faced the cornea, had ceased to elongate (Coulombre and Coulombre, 1963). This experiment clearly showed that the lens can readily reorganize, reversing its polarity under the influence of the surrounding ocular environment. This reorganization of cells was also apparent

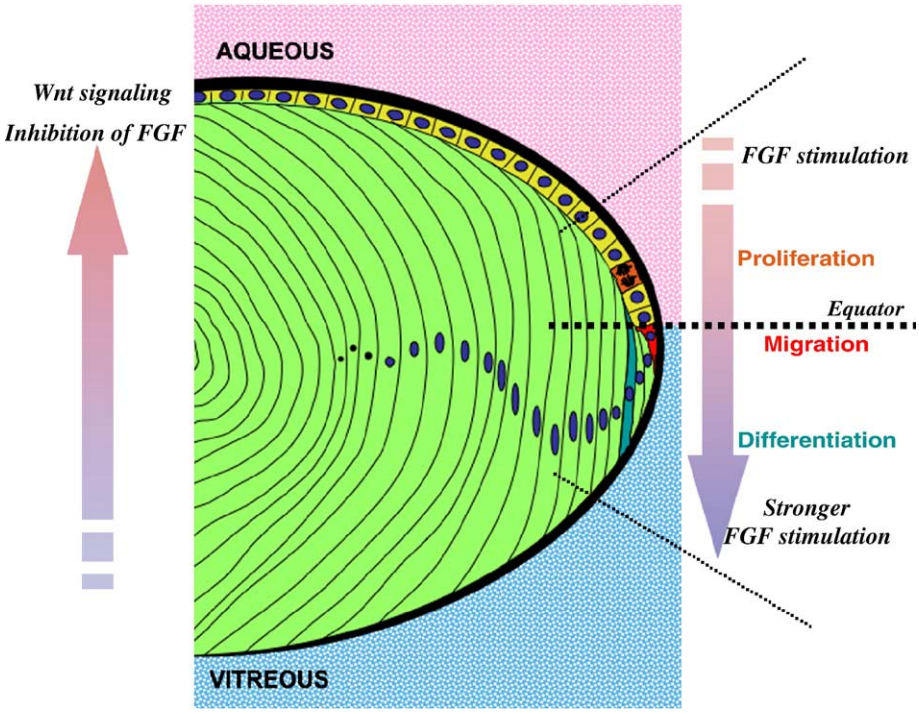

Fig. 2. Diagram indicating how the ocular media and a gradient of FGF stimulation may determine antero-posterior patterns of lens cell behavior. In the postnatal lens, cell proliferation is restricted to the epithelium and predominantly occurs in a band of cells above the equator known as the germinative zone. Progeny of proliferative activity migrate (or become displaced) below the equator where they initiate fiber elongation. These zones coincide with compartments defined by the anatomy of the eye: the epithelial cells are exposed to aqueous (pink background) and the fiber cells are exposed to vitreous (blue background). The cellular behaviors indicated, proliferation (orange), migration (red), and fiber differentiation (blue), are observed both in vivo (in an antero-posterior direction) and in lens epithelial explants (sequentially as the concentration of FGF is increased; McAvoy and Chamberlain, 1989). The right-hand arrow indicates the gradient of FGF stimulation that is proposed to govern this antero-posterior pattern of cell behavior in vivo. The left-hand arrow indicates that other factors, including inhibitory influences, may also contribute to the proposed FGF gradient.

if the lens was replaced with two identical lenses placed in unusual orientations. In this case, the epithelial cells facing the cornea of each lens continued to divide, while those facing the vitreous elongated to form lens fibers, in the process establishing a new equatorial zone. Although two lenses were implanted in place of one, their combined volume, shape, and position of their epithelium was normal, relative to the eye of the host (Coulombre and Coulombre, 1969). Other experiments showed that even if the lens was surgically replaced by an isolated lens epithelium, attached to its lens capsule (whether cells faced the cornea or vitreous), this implant reconstituted a lens vesicle, and subsequently formed a lens with the appropriate polarity; epithelial cells facing the vitreous exited the cell cycle and elongated, while those facing the cornea formed an epithelial sheet (Coulombre and Coulombre, 1971). This latter model was shown to have relatively low species specificity as when fetal mouse lens tissue (lens epithelium attached to its capsule) was substituted for the embryonic chick lens, the chick eye cup supported the development and growth of a properly oriented mouse lens (see Coulombre, 1969).

From the studies above, it became clear that the lens is a target of influences from the surrounding ocular environment. In turn, however, the lens has also been shown to influence some of these neighboring ocular tissues. For example, the lens induces the anterior corneal epithelium to differentiate from the overlying head ectoderm (Lewis, 1907), as without this influence, a scleral-like tissue develops in place of the cornea (Zinn, 1970). The lens is also indirectly involved in regulating eye growth through its ability to promote the accumulation of vitreous. As expansion of the vitreous body accounts for the normal increase in gross size of the eye (influencing the superficial eye coats such as the sclera and choroid, Coulombre and Herrmann, 1965), failure of the vitreous to accumulate (as is the case in the absence of a lens) results in smaller eyes, that is, microphthalmia (Coulombre and Coulombre, 1964). The lens appears to play an instructive role in this case because if the lens is removed, boiled, and then returned, the vitreous still fails to accumulate. However, if the lens is removed and returned without boiling, this ‘living’ lens will continue to induce vitreous accumulation and subsequently eye growth (Coulombre and Coulombre, 1964). How the lens influences vitreous accumulation is little understood, as are the soluble factors from the lens that influence the formation and growth of other ocular tissues. In contrast, as a result of intense research activity over the last 30 years, there is a growing understanding of the growth factors and signaling pathways that regulate the growth and differentiation of lens cells.

Lens fiber differentiation

As a result of the lens manipulation experiments described above, attention has been focused on identifying the factors that are responsible for directing the fate and behavior of lens cells. Most of the work, until recently (see Lens epithelial differentiation section), has been concentrated on identifying the factor(s) that regulate fiber differentiation; that is, the extensive cell elongation and the acquisition of fiber-specific patterns of gene expression (particularly the accumulation of crystallin proteins) that characterize this process. Early experiments showed that lens growth is independent of the growth of other ocular tissues with the exception of the neural retina. The dependence of lens on the neural retina was first reported nearly a century ago in amphibia (le Cron, 1907), and later experiments with chicks also showed that removal of the embryonic chick retina resulted in the arrest of lens growth. Re-introduction of the retina (even a small piece) in the eye cavity influenced the continual growth of the lens (see Coulombre, 1965). An important finding that stemmed from mouse studies in vitro showed that the retinal factor(s) that influence this lens growth could act across a Millipore© filter, indicating that these factors are most likely diffusible molecules (Muthukkaruppan, 1965). Taken together, these studies set in motion the quest, which continues to this day, to identify the nature of these diffusible lens fiber differentiation factor(s).

Although some early attempts (as early as 1926) were made to grow the chick and mouse lens in tissue culture (see Mann, 1948), it was not until the mid-1960s that Philpott and Coulombre (1965) developed an in vitro system whereby cells of the embryonic chick lens epithelium (still attached to their lens capsule) could be isolated from the fiber cells and induced to elongate in tissue culture. It was the adoption of this epithelial explant system that has provided many past and present lens cell biologists with important leads in identifying the molecules regulating lens fiber differentiation. Some of the first studies to use this system were primarily interested in the mechanism of early fiber cell elongation (Piatigorsky and Rothschild, 1971, 1972; Piatigorsky et al., 1970, 1972a,b); a process earlier shown to be readily induced in vitro by serum (Philpott and Coulombre, 1965). Insulin was soon after shown to substitute for serum in inducing lens fiber cell elongation in chick lens epithelial explants (Piatigorsky, 1973; Piatigorsky et al., 1973). Studies to follow identified 'lentropin' from the vitreous (Beebe et al., 1980), a protein later shown to be functionally and immunologically related to insulin-like growth factor-1 (IGF-1; Beebe et al., 1987), to also induce epithelial cell elongation and specialization for lens crystallin synthesis in chick lens explants.

At the time the chick lens explant system was being utilized, a mammalian lens epithelial explant system was established (McAvoy, 1980). In line with earlier studies identifying neural retina as a key regulator of lens growth, co-culture experiments showed that cells in rat lens epithelial explants underwent proliferation and differentiation in response to neural retina (McAvoy, 1980; McAvoy and Fernon, 1984). The fact that retina-conditioned media induced these same effects in rat lens explants (Campbell and McAvoy, 1984; Walton and McAvoy, 1984) led to the identification of a retina-derived diffusible lens 'fiber differentiation factor' (Campbell and McAvoy, 1986) which was soon after identified as a member(s) of the FGF family (Chamberlain and McAvoy, 1987, 1989). Since then, over the last 15 years, numerous studies from a range of laboratories have provided compelling evidence that members of the FGF family play key roles in mammalian lens biology, particularly in relation to their ability to induce lens fiber differentiation. In vitro studies showed that, of the range of growth factors investigated, FGF was the only growth factor with the ability to induce mammalian lens epithelial cells to undergo many of the fiber-specific morphologic (Lovicu and McAvoy, 1989, 1992) and molecular (Chamberlain and McAvoy, 1989; Kok et al., 2002; McAvoy and Chamberlain, 1989) changes including elongation, structural specialization, and onset of specialized crystallin gene expression. Both FGF prototypes, FGF-1 and FGF-2 (de Iongh and McAvoy, 1992, 1993; Lovicu and McAvoy, 1993; Lovicu et al., 1997; Schulz et al., 1993), and high-affinity FGF receptors (de Iongh et al., 1996, 1997) were shown to be expressed throughout the eye, in particular in the lens. While evidence has grown for a role for FGF in fiber differentiation in mammals, the situation with the chick lens has not been so clear. Insulin and IGF-1 were previously reported to induce lens cell elongation and specialized crystallin gene expression in the chick (Beebe et al., 1987; Piatigorsky et al., 1973) but FGF could not be shown to have similar effects (see Huang et al., 2003). For many years there were suggestions that the effect of FGF on the lens was specific to mammals (see Lang, 1999). Other studies with chick explants, however, have shown that FGF, like IGF, can indeed induce lens fiber differentiation markers including the intermediate filament CP49 and delta-crystallin, providing that the cells are exposed to it for a sufficient length of time (Le and Musil, 2001). In vivo studies have also shown that FGF coated beads implanted into mesenchyme surrounding the optic vesicle/cup of young chick embryos can induce formation of ectopic lens tissue including fiber cells (Vogel-Hopker et al., 2000).

One of the most significant findings to come from the rat lens explant system arose from dose response studies with FGF. Interestingly, it was shown that FGF could induce different responses in lens epithelial cells with increased dosage; a low concentration of FGF induced lens cell proliferation, whereas sequentially higher doses were required to induce epithelial cell migration and fiber cell differentiation (McAvoy and Chamberlain, 1989). This finding, together with the fact that FGF bioavailability appears to differ throughout the eye (e.g., more FGF can be recovered from the vitreous than aqueous; Schulz et al.,

1993), led to the proposal that the distinct polarity of the lens in the eye may be determined by a FGF gradient (Fig. 2; see Chamberlain and McAvoy, 1997). This also fits well with the fact that the antero-posterior patterns of lens cell behavior correlate with the distribution of the ocular media, and that vitreous (which bathes lens fiber cells in vivo) but not aqueous (which bathes the lens epithelium) can induce fiber differentiation in rat lens explants (Lovicu et al., 1995). Fractionation of the vitreous showed that most of its fiber-differentiating activity was associated with FGF-1 or FGF-2 (Schulz et al., 1993); however, the observation that the fiber differentiating activity in a small percentage of vitreous fractions was not blocked by neutralising FGF-1 or FGF-2 antibodies, indicated the involvement of factors other than FGF-1 and FGF-2 (Schulz et al., 1993). This does not exclude other members of the FGF family, such as FGF-23 that has recently been reported to be present in vitreous of human patients (Nakanishi et al., 2002).

Strong support for the presence of a FGF gradient in the eye has come from studies on transgenic mouse models that utilized a modified alpha A-crystallin promoter (Overbeek et al., 1985) to overexpress different FGFs specifically in the lens (Lovicu and Overbeek, 1998; Robinson et al., 1995a, 1998). These studies have clearly shown that altered levels of FGF in the eye can inappropriately induce lens epithelial cells to exit from the cell cycle and differentiate into fiber cells. Further support for FGF having a role in lens fiber differentiation has come from other transgenic studies involving expression of different forms of FGF receptor; overexpression of a truncated FGF receptor that acted in a dominant-negative manner (Chow et al., 1995; Robinson et al., 1995b; Stolen and Griep, 2000), or alternatively, overexpression of a specific secreted FGF receptor (sFGFR3 but not sFGFR1; Govindarajan and Overbeek, 2001) led to the inhibition of fiber differentiation in vivo. These findings not only indicated that FGF receptor signaling is essential for lens fiber differentiation but that a FGF ligand, with the ability to bind a specific FGF receptor isoform (FGFR3), is the likely endogenous FGF for regulating lens growth. Whether this same FGF can also bind FGFR2 is yet to be determined. As multiple members of the FGF gene family (comprised of 22 members; Ornitz and Itoh, 2001; Yamashita et al., 2000) are expressed in the eye, it is apparent that redundancy may be active in this tissue. Support for this comes from the fact that lens cells express the gene products of at least three of the four FGF receptors, that many of the FGFs have overlapping effects in the lens (see Lovicu and Overbeek, 1998), and that mice null for a number of different FGFs have no abnormal lens phenotype (see Lang and McAvoy, 2004). Based on this, we are still far from identifying the endogenous FGF(s) involved in regulating lens development.

Early studies, taking advantage of conventional homologous recombination events to delete genes of interest, provided little advance to further understanding the role of FGF receptors in lens biology. For example, attempts to 'knockout' FGFR1 (Deng et al., 1994; Yamaguchi et al., 1994) and FGFR2 (Xu et al., 1998) resulted in early embryonic lethality. Indications that FGFR1 and FGFR3 were not required for lens fiber cell differentiation first emerged from transgenic studies (Lovicu and Overbeek, 1998); however, more definitive studies using an aphakia complementation system showed that FGFR1-deficient embryonic stem cells were capable of contributing to the development of normal murine lenses (see Zhao et al., 2002a). Further support for this came from studies using conditional deletion of 'floxed' FGFR1 in murine lenses, specifically expressing Cre recombinase (Zhao et al., 2002a; see also Zhao et al., 2004). More recent studies exploiting these conditional gene-targeting strategies have generated triple 'knockouts' of FGFR1, FGFR2, and FGFR3 in the lens. The observation that these mice undergo no fiber differentiation confirms the requirement for FGF receptor signaling in fiber differentiation and shows that no single FGF receptor is essential for regulating this process (Zhao et al., 2003). Although the promoter used for expressing Cre recombinase in this study only permitted analyzing the role of FGFR signaling from lens vesicle formation onwards, similar studies utilizing the Cre/loxP system, using a different promoter (Pax6 PO; Ashery-Padan et al., 2000), reported that targeted deletion of FGFR2 in earlier stages of lens morphogenesis influenced lens epithelial cell growth/and or survival (Garcia et al., 2002). Taken together, these studies emphasize the importance of FGF signaling in regulating fiber differentiation and other events in lens morphogenesis.

While there is compelling evidence that FGF signaling is necessary for fiber differentiation, it also appears that it is not sufficient; in recent years, there has been growing evidence that other growth factor-induced signaling pathways are required for the regulation of this complex process. In particular, members of the TGFβ superfamily appear to have a prominent role in regulating aspects of fiber differentiation. Bmps, TGFβs, and their respective receptors are expressed in the lens (see de Iongh et al., 2001; Faber et al., 2002). Bmps, for example, aside from their role(s) in lens induction (see earlier), appear to be important for the promotion of fiber cell elongation (Belecky-Adams et al., 1997; Faber et al., 2002). In vitro studies have shown that noggin (a Bmp ligand inhibitor) can block vitreous-induced cell elongation in chick lens epithelial explants and this can be restored if Bmps are added to the vitreous depleted of noggin-binding proteins (Belecky-Adams et al., 1997). In mice, in vitro experiments have also shown that primary fiber cell elongation can be suppressed in the presence of noggin (Faber et al., 2002). Consistent with this, primary fiber cell elongation is inhibited when a dominant-negative form of Alk6 (*Bmpr1b*; a type I Bmp receptor) is overexpressed in the lens (Faber et al., 2002). The asymmetric suppression of primary fiber cell elongation in this model would suggest that other primary fiber cell differentiation stimuli (inde-

pendent of Alk6 signaling) are also present at this stage of development. Interestingly, overexpression of noggin in lenses of transgenic mice, although functionally impairing formation of the ciliary body, does not appear to impact directly on lens development (Zhao et al., 2002b). Other transgenic mouse studies indicate that TGFβ receptor signaling may also be required for secondary fiber differentiation, as overexpression of a truncated type 2 TGFβ receptor, acting in a dominant-negative fashion, results in impaired secondary lens fiber cell maturation and/or maintenance (de Iongh et al., 2001). In addition to the TGFβ superfamily, in vitro studies on chicks have indicated a role for EGF/TGFα signaling in regulating early fiber differentiation events, particularly expression of the fiber-specific cytoskeletal protein, filensin (Ireland and Mrock, 2000, 2004). Further ongoing studies characterizing the role of intracellular signaling pathways initiated by different growth factors in the lens, will no doubt shed more light on this important area of research.

Although a number of different growth factor family members have been reported to influence lens fiber differentiation, transgenic studies overexpressing many of these different ocular growth factors imply that, at least in mammals, only FGFs (see Lovicu and Overbeek, 1998) can initiate this differentiation. When members of the EGF (TGFα, Reneker et al., 1995), PDGF (PDGF-A, Reneker and Overbeek, 1996), TGFβ (TGFβ1, Srinivasan et al., 1998), IGF (IGF-1, Shirke et al., 2001), or BMP (BMP7, Hung et al., 2002; Zhao et al., 2002b) growth factor families are expressed in the lens, none of these can directly induce mammalian lens epithelial cells to differentiate into fibers. Although some of these studies, for example, expressing PDGF-A (Reneker and Overbeek, 1996) or TGFβ-1 (Lovicu et al., 2004a), have shown fiber-specific beta-crystallin expression in the disrupted epithelium of lenses from the resultant mice, in vitro studies have confirmed that neither of these growth factors can directly induce lens fiber differentiation (Kok et al., 2002; Lovicu et al., 2004a). Thus, while many of these factors, particularly the TGFβ superfamily, have been shown to influence processes important in fiber differentiation, their inability to directly induce the differentiation process indicates that they are downstream of initiating events. The picture that is emerging so far is that FGF appears to be a key initiator but that the complex processes of fiber differentiation are likely to depend on the synchronized and integrated action of a number of distinct growth factor-induced signaling pathways. With the establishment of effective lens-specific promoters (Ashery-Padan et al., 2000; Zhao et al., 2004) described above, and the more recent development of lens-specific inducible promoters (Overbeek et al., 2004), future studies targeting the expression and/or deletion of a number of genes, in a more spatial- and temporal-specific fashion, will no doubt greatly expand our understanding of the role of different molecules, including FGFs, in the processes involved in lens induction, differentiation, and growth.

Lens cell proliferation

In addition to FGF, a wide range of growth factors has been shown to be mitogenic for lens epithelial cells. Ocular growth factors, such as PDGF-A, insulin/IGF-1, EGF/TGFα, and HGF, have also been shown to be effective lens mitogens in a range of species (Choi et al., 2004; Hyatt and Beebe, 1993; Kok et al., 2002; Liu et al., 1996; Reddan and Wilson-Dziedzic, 1983; Wormstone et al., 2000; Wunderlich and Knorr, 1994). A number of studies have also shown that these growth factors and/or their receptors are expressed in the lens (de Iongh and McAvoy, 1993; Ireland and Mrock, 2004; Lovicu et al., 1997; Potts et al., 1994; Reneker and Overbeek, 1996; Shirke et al., 2001; Weng et al., 1997; see also Chamberlain and McAvoy, 1997). In some cases, the expression pattern of the ligand and its receptor (e.g., for PDGF) coincide with the region at the lens equator where epithelial cells proliferate (Reneker and Overbeek, 1996). Transgenic mouse studies have shown that overexpression of either IGF-1 (Shirke et al., 2001) or PDGF-A (Reneker and Overbeek, 1996) in the eye leads to increased DNA synthesis in the lens epithelium, resulting in the expansion of the epithelium (germinative zone) towards the posterior pole of the lens, in the case of IGF-1 (Shirke et al., 2001), or epithelial multilayering in the case of PDGF-A (Reneker and Overbeek, 1996). Interestingly though, lenses of mice lacking the PDGF-A receptor (PDGFR-α) are relatively normal (Soriano, 1997) and show normal levels of lens cell proliferation (Potts et al., 1998) indicating that PDGF receptor signaling is not essential for this process. Since a number of mitogens and their receptors are expressed in the lens, a likely scenario is that no one mitogen is essential, but that they all cooperate to regulate cell proliferation in the lens epithelium.

Growth factor signaling

The fact that low doses of FGF can switch on the cell cycle machinery, while higher doses promote exit from the cell cycle (by upregulating specific cell cycle inhibitors, e.g., $p57^{Kip2}$; see Lovicu and McAvoy, 1999; Lovicu et al., 2004b) leading to fiber cell differentiation, highlights the need for a better understanding of the intricate signaling pathways involved in determining different cell behaviors and fates. The identification and characterization of the growth factor-induced intracellular signaling pathways involved in regulation of lens cell proliferation and differentiation are still very much in their infancy. To date, one of the most studied groups of signaling molecules are the mitogen-activated protein kinases (MAPKs), more specifically, the extracellular-regulated kinases (ERKs), as they are the most abundant MAPKs in lenses of a number of species (Li et al., 2003). The levels of activation of these terminal-signaling enzymes have been shown to be linked closely to the different FGF stimuli in rat lens explants, with a

'differentiating' dose of FGF able to induce a greater level of ERK phosphorylation than a lower 'proliferation' dose of FGF (Lovicu and McAvoy, 2001). Furthermore, in the rat, FGF-induced lens cell proliferation and early morphological stages of fiber differentiation have been shown to be dependent on ERK activation (Golestaneh et al., 2004; Lovicu and McAvoy, 2001). Interestingly, in the chick, FGF-induced lens cell proliferation and differentiation was shown to be independent of ERK signaling, whereas ERK signaling was required for insulin or IGF-induced fiber differentiation (Le and Musil, 2001). Also in chicks, EGF/TGF alpha has been shown to activate ERKs and, in an interesting contrast to mammals, low and high levels of ERK activation appear to be related to differentiation and proliferation, respectively (Chen et al., 2001).

Although many studies have shown that elongation and a range of molecular markers expressed during fiber differentiation, such as filensin (Lovicu and McAvoy, 2001), CP49 (Le and Musil, 2001), and MIP (Golestaneh et al., 2004), are dependent on ERK-activation, it has been shown that other growth factor-induced fiber-specific markers, such as FGF-induced beta-crystallin accumulation in rats (Lovicu and McAvoy, 2001), and FGF-induced delta-crystallin accumulation in chicks (Le and Musil, 2001) are independent. These studies clearly demonstrate the uncoupling of the fiber differentiation process in the lens and highlight the fact that multiple signaling pathways are involved in the regulation of the lens fiber differentiation process. Although ERK-signaling, as in many other tissues, is important for regulating events in lens biology, there are a large number of other intracellular signaling pathways that may play just as important roles, including the PI 3-kinases (Chandrasekher and Sailaja, 2003; Souttou et al., 1997; Zatechka and Lou, 2002), JAK/STATs (Ebong et al., 2004; Potts et al., 1998), and Rho GTPases (Maddala et al., 2004), to name a few. Elucidating the exact nature of the endogenous signaling pathways in the lens required for the regulation of lens cell proliferation and differentiation may not only allow us to develop better strategies to regulate these processes, for example, in preventing lens pathology, but may also lead to identifying the endogenous factors primarily activating these events.

Modulators of growth factor signaling

To date, research has focused on the identification of the key molecules and specific signaling pathways that influence lens cell behavior, as it is the impaired function of these molecules, and the dysregulation of their respective signaling pathways that can be causally linked to the disease state. As the transgenic studies described earlier have shown, to ensure a physiologically appropriate biological outcome, growth factor signaling events must be precisely regulated, not only spatially but temporally as well. It is with this in mind that a lot of interest is now focused on identifying and characterizing the role of modulatory molecules that have the potential to regulate (either as agonists or antagonists) the bioavailability of specific growth factors. With FGFs playing such a key role in lens biology (see earlier), it is important to understand if and how these growth factors are regulated in the eye. As mentioned above, a 'gradient' of FGF stimulation in the eye may be critical for the regulation of the distinct spatial lens cell processes. However, in addition to antero-posterior differences in levels of FGF bioactivity, molecules that inhibit FGF signaling may also be involved. For example, in other developmental systems, repressors of receptor tyrosine kinases (RTK) belonging to the *sprouty* (Spry) and *sef* gene families have been reported to be negative-feedback regulators of FGF activity (for review, see Kim and Bar-Sagi, 2004; Tsang and Dawid, 2004). These antagonistic molecules can influence RTK signaling (including intracellular pathways activated by FGF) at the receptor level or at different intracellular targets, in particular those involved in the Ras-MAPK pathway (Tsang and Dawid, 2004). Recent studies have shown that both Sprouty and Sef are expressed in the lens, with strongest expression in the lens epithelium (Lovicu, Boros and McAvoy, unpublished data). Based on this, it is tempting to speculate that in vivo, high levels of such FGF antagonists may be important for the maintenance of the lens epithelium, and their down-regulation (making the cells more receptive to FGF) required for the epithelial cells to differentiate into fiber cells (see Fig. 2).

Another interesting regulatory molecule to be described recently in the literature is *Crim1*. This gene encodes a cysteine-rich protein that has been shown to bind and modulate the activity of members of the TGFβ-superfamily, in particular the Bmps (Wilkinson et al., 2003). Taken together with the fact that Crim1 is found in the ocular media (Lovicu et al., 2003), that it is highly expressed in the lens during morphogenesis (Lovicu et al., 2000), and that Bmps have been reported to play an important role in this early differentiation of the lens, Crim1 may be a strong candidate as a Bmp regulatory molecule in the eye. As preliminary reports have shown that a deficiency of Crim1 in vivo results in abnormal lens and/or ocular development (see Lovicu et al., 2003), a more thorough analysis of its role in the eye is clearly warranted. Further studies to determine the exact role of these regulatory molecules in the lens and how they influence growth factor activity (in particular intracellular signaling), will no doubt open up new avenues of investigation leading to an increased understanding of growth factor regulation of lens cell behavior.

Lens epithelial differentiation

To date, most of the studies on growth factor regulation of lens cell differentiation have concentrated on the lens fibers, and the lens epithelium has received little attention. A

number of growth factors, including FGFs, have been shown to be mitogenic for lens epithelial cells (see above). Survival roles have been attributed to FGFs (Renaud et al., 1994) and a more recently identified factor, lens epithelial-derived growth factor (Singh et al., 2000). However, until recently, there has been no information on factors that were involved in lens epithelial differentiation. One of the more recent growth factor signaling pathways to be investigated in this regard is the Wnt/β-catenin pathway. The localization of Wnts and their Frizzled (Fz) receptors in the mammalian lens epithelium during development is consistent with a role for Wnt-Fz signaling in its formation and maintenance (Ang et al., 2004; Chen et al., 2004; Liu et al., 2003; Stump et al., 2003). Also, recent studies have shown that mouse embryos homozygous for a mutation in the *lrp6* gene (coding for a co-receptor for Wnt signaling) did not form a normal lens epithelium (Stump et al., 2003). A common feature of these mice was the absence of the anterior epithelium and extrusion of the lens fibers into the corneal stroma. As LRPs are required for Wnt-Fz signaling through the β-catenin pathway, it was concluded that the normal formation of the epithelium requires β-catenin signaling. Independent evidence for a role for β-catenin signaling during early stages of lens epithelial differentiation comes from a recent study of reporter gene expression in the eyes of *TCF/Lef-LacZ* transgenic mice (Liu et al., 2003). As many studies have shown, β-catenin interacts with TCF/Lef transcription factors to form a transcriptionally active nuclear complex (Brantjes et al., 2002), expression of the *TCF/Lef* reporter construct at an early stage of differentiation of the lens epithelium (E13.5) indicates a role for Wnt/β-catenin signaling in the differentiation of the lens epithelium from the lens vesicle.

Fzs and Wnts continue to be expressed in the mouse lens during subsequent embryonic and postnatal development (Ang et al., 2004; Chen et al., 2004; Liu et al., 2003; Stump et al., 2003). Since there is no evidence of TCF/Lef activity, this raises the possibility that non-canonical Wnt-Fz signaling pathways may also have roles in regulating lens cell behavior. For example, Wnt-Fz signaling can also activate the planar cell polarity (PCP) pathway. This pathway does not require LRP co-receptors and appears to function independently of β-catenin (McEwen and Peifer, 2001). Changes in cell shape and polarity in other cellular systems are characteristically regulated through the PCP pathway (Huelsken and Birchmeier, 2001). Recent studies have also shown that many signaling molecules associated with Wnt/PCP signaling such as CDC42 (McAvoy et al., 2004) and Rac and Rho (Maddala et al., 2003) are expressed in the lens epithelium. Therefore, it is possible that this pathway may have a role in regulating cell polarity across the epithelium as well as the major cellular rearrangements that occur at the lens equator.

As with the other growth factors, modulators of Wnt-Fz signaling are also expressed in the lens. Members of the family of secreted frizzled-related proteins (Sfrps) expressed in the lens include *Sfrp1*, *Sfrp3*, *Sfrp4*, and *Sfrp5*. Essentially, they have similar patterns of expression to each other and to the *Fzs* during lens development (Chen et al., 2004; see also Leimeister et al., 1998; Liu et al., 2003). The exception is *Sfrp2*, which has a very restricted pattern of expression, being detected weakly at first in the central cells of the lens placode, and then is strongly expressed in all cells of the lens pit. *Sfrp2* then becomes restricted to the presumptive epithelial cells of the lens vesicle. By E14.5, *Sfrp2* is only present in a few cells above the lens equator. *Sfrp2* is not detected in the lens at E18.5 or at later stages. The Dikkopfs (Dkks) are another family of Wnt-Fz signaling regulators. *Dkks 1*, *2*, and *3* have similar patterns of expression to each other and to the majority of the *Wnts* and *Fzs* during lens development (Ang et al., 2004).

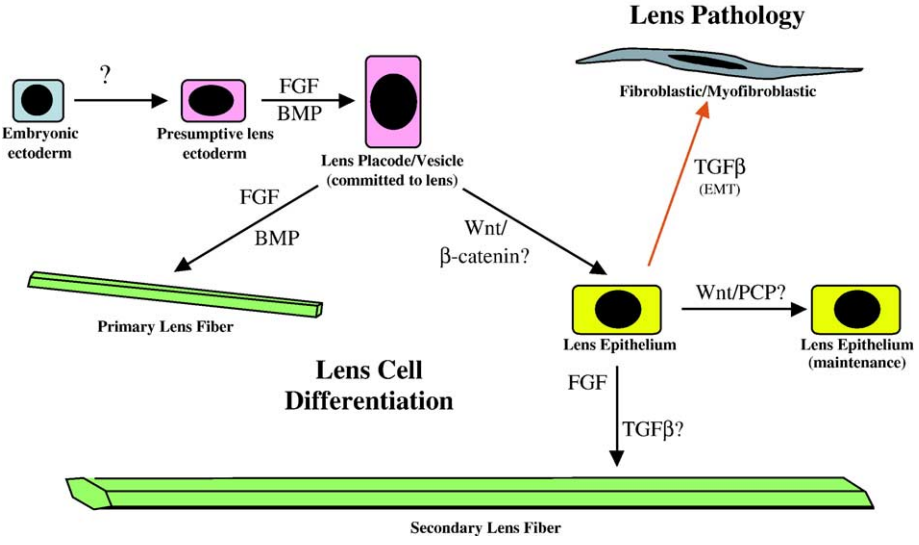

Fig. 3. Diagram illustrating major stages of lens determination and differentiation. Also included are the main growth factors that are thought to influence cell fate and behavior during embryonic and postnatal development.

How these widely expressed regulatory molecules influence Wnt-Fz signaling is being intensively explored in many developmental systems. The Sfrps through their ability to bind both Wnts and Fzs mostly appear to act as antagonists of Wnt-Fz signaling, although there are also reports of some Sfrps acting as agonists of Wnt-Fz signaling, perhaps through their ability to sequester and transport Wnts (Jones and Jomary, 2002). For the Dkks, it appears that their modulation of Wnt/β-catenin signaling relates to their ability to bind the LRP co-receptor. As mentioned above, LRP5/6 is required for β-catenin signaling. Recent studies indicate that LRP5/6, when part of the Wnt ligand–receptor complex, removes axin from the β-catenin destruction complex, resulting in stabilization of β-catenin, its translocation to the nucleus, and subsequent association with the TCF/Lef family of transcription factors (Mao et al., 2001; Zorn, 2001). In the absence of LRP5/6, the β-catenin pathway is inactive but Wnt signaling can proceed through the PCP pathway (see Semenov et al., 2001). Dkk1 has been shown to bind to LRP6 and specifically block the β-catenin signaling pathway (Semenov et al., 2001). This association does not inhibit PCP signaling, and in fact recent evidence indicates that when the canonical β-catenin pathway is antagonized, an alternate JNK pathway is activated (Park and Moon, 2002). Dkks in the lens therefore may have a role in regulating Wnt/β-catenin signaling so that cells in different functional domains can alternate between different Wnt signaling pathways.

Lens pathology

As in other developmental systems, aberrant growth and differentiation of epithelial cells can cause debilitating pathological conditions. This is the basis of some subcapsular cataracts including the posterior capsule opacification that is a major complication of modern cataract surgery. In this condition, fibrotic plaques grow across the visual axis and progressively restrict the ability of the lens to transmit light so that further surgery is required. Using rat, mouse, and human models, it has been shown that TGFβ is the key initiator of this condition. TGFβ destabilizes the epithelial phenotype and induces an epithelial mesenchymal transition (EMT; Hales et al., 1994; Liu et al., 1994; Lovicu et al., 2002; Srinivasan et al., 1998; Wormstone, 2002). Thus, one important approach to preventing or slowing this very common cataract will be not only to block TGFβ-induced signaling, but also to promote the signaling pathways, such as those activated by Wnt ligands, that are important for maintaining the normal epithelial phenotype.

Conclusions

Clearly, progress has been made, but we have still a long way to go to fill in all the gaps in our understanding of the complex growth factor signaling pathways that determine cell fate and behavior during lens development (see Fig. 3). In the latter part of the 20th century, concomitant with the growth of knowledge of growth factors in general, progress was made in identifying some of the key players in lens morphogenesis and differentiation, such as the FGFs, Bmps, TGFβs, and more recently, the Wnts. However, our knowledge is still rudimentary, particularly in relation to the mediators of the early inductive interactions that confer lens competence to regions of the head ectoderm (Fig. 3). Similarly, we know little about the signaling pathways that are downstream of growth factor ligand/receptor interactions. Some encouraging progress has been made in recent years and, as is common in biological systems, the deeper we probe, the more complexities we uncover. The existence of several signaling pathways for most of these ligands and the relatively untouched area of their cross talk leaves much fertile ground for future investigations. Moreover, the growing appreciation of the existence of regulatory molecules for many of these factors and signaling pathways opens up further mechanisms for creating gradients of factors and exquisite regulation of dynamic developmental processes. It is also important to appreciate the remarkable technological advances and the emergence of new tools that have helped us to winkle out some of the secrets of the embryo and the cell. We shall need all of these tools, and more, before we fully understand the complex interweavings of growth factor signaling in lens development.

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