Which Factors Stimulate Lens Fiber Cell Differentiation In Vivo?

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The lens of the eye is polarized with the anterior hemisphere covered by a cuboidal epithelium and lens fiber cells occupying the posterior surface and interior. Lens epithelial and fiber cells represent a single-cell lineage; epithelial cells are the progenitor population and fiber cells the terminally differentiated form (Fig. 1). In 1965 it was demonstrated that when a lens of a chicken embryo was rotated through 180° to orient the epithelium toward the retina, it would completely repolarize over a period of days. This simple experiment implied that all the stimuli required to establish polarization would be found in the surrounding ocular media. As a consequence of this observation, the question of how polarization is controlled has been of central interest to developmental biologists interested in the eye. The purpose of the current review is to examine how recent experimental analysis has advanced our understanding of how lens polarization is controlled.

The Components of Lens Polarization

One of the features of the lens that makes it attractive as a model system is its regional compartmentalization. Proliferation within the lens lineage is restricted to the epithelial cells. Even within the epithelium, there are zones where the proliferative index is distinct. Epithelial cells adjacent to the ciliary process show the highest proliferative index, whereas the adjacent region extending to the tip of the iris shows an intermediate rate of proliferation. The lowest level is found in the central region of the epithelium (Fig. 1). These observations have led to the suggestion that factors regulating proliferation in the lens epithelium may be produced in the ciliary process and that their availability may be restricted by the surrounding tissues of the eye.

A number of experiments have suggested that the retina is a source of fiber cell differentiation factors. When mouse lenses were rotated in situ in the presence or absence of retina, lens repolarization occurred only when retinal tissue remained. Consistent with this observation was analysis showing that retina-conditioned medium could stimulate fiber cell differentiation.

The observations and manipulations described suggest that soluble signaling molecules present in ocular media control lens polarization by regulating the rate and location of cell division in the lens epithelium as well as the differentiation of lens fiber cells. It has been tacitly accepted in turn that the concentration or activity of these signaling molecules must be closely regulated if lens polarization is to be maintained. The signaling molecules that may control lens polarization are discussed later.

Evidence for Fibroblast Growth Factor Involvement in Lens Polarization

One of the most interesting series of experiments examining control of lens polarization has come from Chamberlain and McAvoy. Initial experiments showed that at least some of the fiber cell differentiation activity in retina-conditioned media was FGF2 (basic FGF). These experiments showed that FGF2 could function to stimulate fiber cell differentiation in vitro, but given the nature of the analysis, fell short of showing that an FGF pathway was necessary for fiber cell differentiation.

Circumstantial evidence has suggested that a gradient of FGF may be in part responsible for controlling lens polarization. This idea is derived from the observation that different cellular responses within the lens lineage are optimally stimulated by different concentrations of FGF. Specifically, the half-maximal concentration of FGF required for epithelial cell proliferation is low (0.15 ng/ml), whereas the half-maximal concentration required for differentiation is high (40 ng/ml). This arrangement implies that if an FGF gradient existed in the eye (anterior, low concentration; posterior, high concentration), a polarized lens might be the result. The best evidence that such a gradient exists comes from measurements of FGF activity in ocular media. Using a variety of techniques, it has been shown that FGF activity levels are higher in vitreous than in aqueous. The distribution of immunoreactive FGF1 and FGF2 is also consistent with a role for FGFs in the control of lens polarization.

Experiments using transgenic mice have shown that various members of the FGF family can act as fiber cell differentiation stimuli in vivo. The first of these was generated by Robinson et al. and Lovicu and Overbeck who showed that FGF1 (acidic FGF) expressed from the αA-crystallin promoter and secreted from lens fiber cells would stimulate adjacent epithelial cells to form fibers. A number of experiments since then have shown that various FGFs including FGFs 1, 3, 4, 7, 8, and 9 are also able to stimulate fiber cell differentiation. Overexpression studies are limited to showing what a factor can stimulate. For this reason, a number of groups have used dominant-negative FGF receptors as a means of inhibiting FGF action and asking whether this signaling pathway is necessary. Although the degree of the phenotypic response varies in different transgenic lines, it is clear that an inhibition of FGF signaling results in diminished fiber cell differentiation. This provides evidence that the FGF signaling pathways normally function in vivo for fiber cell differentiation.

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FGFs are required for fiber cell differentiation and probably for other aspects of lens development.

However, a number of recent results have initiated a degree of rethinking. Perhaps most surprising is that adult mice made homozygous null for both FGF1 and FGF2 show no defect in lens or eye development on histologic examination (Claudio Basilico, personal communication). These FGF ligands have been the prime candidates for fiber cell differentiation factors, and the absence of phenotype in the null mice makes a clear statement that, although they may participate, they are not necessary for fiber cell differentiation. It is, of course, possible that one of the other FGFs (currently, a family of at least 18 molecules) is critical for fiber cell differentiation or that a number of FGFs are responsible for fiber cell differentiation when their activities are combined. Presumably, as more FGFs are tested for their activity in lens development through overexpression and activity inhibition, we will learn whether this explanation is valid.

With the information available to date, we should also consider the possibility that stimulation of fiber cell differentiation may not normally function as an FGF function in vivo. Fiber cell differentiation in response to FGF overexpression in the lens could be explained as an aberrant response to a level of FGF ligand that is not physiological (although the presence of FGF receptors suggests a role for FGFs in some aspect of lens fiber cell physiology). If FGFs do not normally function in fiber cell differentiation, the most difficult experimental results to explain are those in which differentiation was inhibited with a dominant-negative FGFRI.14,15 The biochemical specificity of such mutant receptors appears unchallenged. If these experiments have been misinterpreted, it is a consequence of our lack of understanding of the complexity of the vivo response.

Experiments performed using the chick as a model system have also contributed to uncertainty about the function of FGFs in fiber cell differentiation. It has been presumed that the mechanism of lens development in different vertebrates would be conserved, at least in the major elements. For this reason, it is surprising that neither recombinant FGFs nor eye-derived growth factor (containing a mixture of FGFs) can stimulate the differentiation of fiber cells from chick lens epithelial explants. In addition, unlike the mouse, expression of a dominant-negative acting FGF receptor in the chick lens (using a retroviral expression vector) does not result in changes to fiber cells that are consistent with an inhibition of fiber cell differentiation (David Beebe, personal communication). As in the mouse,16 the chick lens is known to express FGFRI,17 and it is therefore likely that the signal transduction machinery is present. Thus, we are left with apparently conflicting results in chick and mouse that may argue for evolutionary divergence in the mechanism of lens development.

Consistent with the idea that FGF pathways may not be required for normal lens development and fiber cell differentiation is the recent observation that in chimeric mice generated with ES cells without the FGFRI gene, the mutant cells make a substantial contribution to the lens, suggesting that FGFRI is not required for normal lens development.14,15 These data are not necessarily at odds with previous experiments inhibiting FGF receptor function in the lens,15 because messenger RNAs for FGFRI and FGFRII are expressed in elongating lens fiber cells.16,18,19 To summarize, although none of the evidence available so far is conclusive, the requirement for FGF signaling during normal lens development has become less certain. In the remaining sections of the review, we consider alternative stimuli.

Evidence for Insulin-like Growth Factor-I Involvement in Fiber Cell Differentiation

Insulin-like growth factor-I (IGF-I) was first implicated in lens development in experiments from Beebe et al.20 that showed that a factor in chick vitreous could stimulate fiber cell differentiation in cultured explants. First called lentropin, this factor was later shown to be identical with or very similar to IGF-I. In explant assays, IGF-I is a potent differentiation agent in chick lens epithelial cells but is minimally active in stimulating proliferation.21

In contrast, rat lens epithelial explants differentiate poorly in response to IGF-I alone with a limited increase (compared with the response to FGF) in their content of α-, β- and γ-crystallin.22 However, it has been shown that when combined, IGF-I and FGF synergize in stimulating differentiation22 and that a brief (2-hour) exposure of rat lens epithelial cells to FGF permits fiber differentiation when IGF-I is subsequently applied.23 From these data, it can be suggested that in rodents FGF and IGF-I may act in concert to guide the differentiation of lens fiber cells. Somewhat at odds with these in vitro data is the recent observation that IGF-I overexpression in the mouse lens results in increased lens epithelial proliferation but not in
differentiation (S. Shirke, M. Robinson, P. Overbeek and RAL, unpublished data). This tends to argue that in vivo, the primary function of IGF-I in the rodent lens may be a stimulus for cell division. A detailed characterization of lens development in mice in which IGF-I signaling is deficient may provide additional evidence for the role of this signaling pathway. As in the case of FGF, data examining IGF-I function in lens development may argue that birds and rodents have diverged in the mechanism for control of fiber cell differentiation. It will be particularly interesting to determine in vivo whether a dominant negative IGF-I receptor can block fiber cell differentiation in the chick.

Other Candidates for Fiber Cell Differentiation Factors

With few firm conclusions about the identity of fiber cell differentiation factors, it is worth asking whether alternative molecules may serve this role. Of interest is the recent demonstration, in which mice with targeted deletion of the BMP4 gene were used, that bone morphogenetic protein (BMP)-4 is required for normal development of the lens beyond the placode stage.24 As part of this analysis, Furuta and Hogan24 showed that lens development, as assayed through the appearance of lens crystallins, could be rescued if explanted eye primordia were provided with exogenous BMP4. Although the experiments performed do not indicate whether BMP4 acts directly, it is possible that this factor can stimulate fiber cell differentiation. This is consistent with expression of BMP receptors in the developing lens.

The signal transducers and activators of transcription (STAT) proteins are substrates of the receptor-associated Janus kinases (Jaks). When Jaks are activated by ligand binding, STATs become phosphorylated and translocate to the nucleus. In turn, they are involved in transcriptional activation.25 The presence of a phosphorylated form of STAT 3 in freshly isolated chick lens cells implies that a ligand that can activate the Jak–STAT signaling pathway may be involved in regulating fiber cell differentiation.26 Because none of the candidate fiber cell differentiation factors identified to date is known to signal through the Jak–STAT pathway, this observation identifies a promising possibility.

CONCLUSIONS

The lens represents a complex developmental system in which the characteristic morphogenesis is likely to be imposed by multiple signaling pathways. Future directions in the field are clear. First, we must seek confirmation that FGF signaling is required for fiber cell differentiation and, if this is obtained, determine which ligands provide stimulation. Manipulations producing lens-specific deletion of FGF receptor genes may be the most definitive. Second, in the event that FGFs are not essential for fiber cell differentiation, the search for alternative signaling pathways is of great importance. Candidates have already arisen with the recognition that BMPs and ligands for the Jak–STAT signaling systems may be involved. Perhaps the most critical aspect of future work is to try to understand how the different signaling pathways involved in morphogenesis are coordinated to produce a structure as well defined in size, shape, and cellular characteristics as the lens.

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References


ERRATUM

In the article entitled “Transforming Growth Factor-β1, -β2, and -β3 In Vivo: Effects on Normal and Mitomycin C-Modulated Conjunctival Scarring” by Cordeiro et al. (*IOVS* 1999;40:1975–1982), an author’s last name was inadvertently misspelled on page 1975. The name should have read: Fabiana D’Esposito.