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An Essential Role for FGF Receptor Signaling in Lens Development

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Abstract

Since the days of Hans Spemann, the ocular lens has served as one of the most important developmental systems for elucidating fundamental processes of induction and differentiation. More recently, studies in the lens have contributed significantly to our understanding of cell cycle regulation and apoptosis. Over twenty years of accumulated evidence using several different vertebrate species has suggested that fibroblast growth factors (FGFs) and/or fibroblast growth factor receptors (FGFRs) play a key role in lens development. FGFR signaling has been implicated in lens induction, lens cell proliferation and survival, lens fiber differentiation and lens regeneration. Here we will review and discuss historical and recent evidence suggesting that (FGFR) signaling plays a vital and universal role in multiple aspects of lens development.

Keywords

FGFR; FGF; Lens; Development; Eye

1. Introduction

The focus of this review is to examine what is known about the role of FGFs and FGFRs in the development of the vertebrate ocular lens. As this topic has been addressed using many different model systems, lens development will largely be considered in aggregate, and species differences will be highlighted when necessary. This is not meant to suggest that there are no differences between lenses of different species. In fact, we know that there are significant species differences in the size and shape of lenses as well as in the arrangement of suture patterns [reviewed in 1]. Lenses of different species also differ in major crystallin proteins [reviewed in 2]. Nonetheless, a strong argument can be made that the major genetic pathways and signaling molecules involved in vertebrate embryonic lens development are largely, if not entirely, conserved. With that being said, we will first launch into a brief overview of the major events in vertebrate lens development that transform a layer of surface ectoderm in the early embryo into the transparent organ responsible (in collaboration with the cornea) for gathering and focusing light onto the retina. This will be followed by a brief review of the FGF and FGFR family, focused on those members of the family that are present in the developing or mature eye. The remainder of our discussion will focus on what we have learned about the role of FGFR signaling in different aspects of lens development and what questions remain to be answered.

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1.1 Overview of Embryonic Lens Development

Although detailed reviews of numerous aspects of lens development can be found elsewhere [3] here we will focus on the major events that are common to vertebrate lens development and result in the major structural features of the lens. In vertebrates, the lens begins development as a sheet of surface ectoderm that is exposed to multiple inductive influences during embryogenesis starting around late gastrulation and culminating when the presumptive lens ectoderm (PLE) overlies the embryonic optic vesicle (OV) (Figure 1A). Shortly after physical contact between the PLE and OV is established, the lens ectoderm begins to thicken forming the lens placode (Figure 1B). The lens placode subsequently invaginates forming the lens pit as the OV invaginates to form the optic cup (Figure 1C). As the lens pit deepens, the connection to the surface ectoderm narrows forming the lens stalk. The lens stalk is a transient structure that eventually degenerates, by mechanisms that are currently unclear, separating the initially hollow lens vesicle from the overlying surface ectoderm that will differentiate into the corneal epithelium (Figure 1D). The cells that were at center of the lens placode form the posterior half of the lens vesicle and continue to elongate toward the anterior, eventually filling the lumen of the vesicle as they form the primary lens fiber cells (Figures 1E, F). The peripheral invaginating cells of the lens placode develop into the anterior half of the lens vesicle forming the lens epithelium. Initially all of the cells of the lens vesicle are capable of proliferation, but the primary fiber cells quickly lose their ability to proliferate as fiber differentiation progresses. While all lens epithelial cells retain the ability to undergo proliferation, lens cell proliferation normally becomes largely restricted, as development progresses, to a band of epithelial cells slightly anterior to the lens equator known as the germinative zone (Figure 1G). As these cells proliferate, adjacent epithelial cells move closer to the lens equator where they withdraw from the cell cycle and elongate forming secondary lens fiber cells. The post-mitotic region of lens epithelial cells posterior to the germinative zone is known as the transitional zone, as these are the epithelial cells in transition to becoming secondary fiber cells. It is within the transitional zone that the expression of many genes characteristic of fiber cell differentiation first becomes evident. Immediately posterior to the transitional zone (at the lens equator) cells line up into columns called meridional rows and begin elongating into secondary fiber cells. In this way the lens continues to grow throughout the life of the vertebrate organism by progressively adding on layer upon layer of secondary fiber cells onto the lens nucleus composed of the primary fiber cells formed during embryonic development.

Both primary and secondary fiber cells undergo a series of characteristic changes, among the first of which is permanent withdrawal from the cell cycle. Cell cycle withdrawal is followed by morphological elongation and eventual degeneration of all intracellular organelles. All of these changes are orchestrated by changes in gene expression triggered by an unidentified fiber cell differentiation factor or factors. Although the cyclin dependent kinase inhibitor p57^{KIP2} and transcription factors Prox1 and c-maf/L-maf (c-maf in mammals and L-maf in the chick) are expressed in the lens epithelium, transcripts from all of these genes are markedly increased early in the lens fiber differentiation process. Likewise, lens fiber cell differentiation is characterized by the appearance or massive increase in abundance of several specific crystallin proteins. In birds, δ -crystallin levels increase and β -crystallins appear as fiber cell differentiation is induced. In mammals, the expression of β - and γ -crystallins are generally associated with lens fiber differentiation, although some specific β - and γ -crystallins are expressed at lower level in the lens epithelium [4]. Other proteins such as CP49 (phakinin), filensin (CP115), aquaporin0 (MIP) and connexin 46 (or the chick ortholog Cx45.6 [5]) are also commonly used as markers of lens fiber cell differentiation, although CP49 and filensin are also expressed at low levels in the post-mitotic lens epithelial cells of the annular pad in avian lenses [6]. The annular pad cells of avian lenses are analogous to the transitional zone epithelial cells of mammalian lenses.

In all vertebrates, the lens maintains a distinct polarity. Epithelial cells line the anterior face of the lens which is exposed to the aqueous humor and faces the cornea. The remainder of the lens is composed of fiber cells which are exposed to the vitreous humor between the lens and the neural retina. The ciliary body is also found adjacent to the lens equator where lens epithelial cells continuously begin their differentiation into lens fiber cells. From the elegant chick lens reversal experiments from Coulombre and Coulombre in the mid 1960s, it was apparent that the polarity of the lens was dependent on its position within the eye [7]. In these experiments when the chick lens was removed and replaced with the epithelial side of the lens facing the neural retina, the cells that were in the center of the anterior epithelium began to elongate and undergo fiber cell differentiation while the epithelial cells at the equator proliferated and migrated over the former posterior (epithelial-free) side of the lens forming a new epithelial layer in facing the cornea. This observation led to the conclusion that there was some fundamental difference between the environments in contact with the anterior and posterior surfaces of the lens, with the posterior environment (vitreous) promoting lens fiber cell differentiation and the anterior environment (aqueous) promoting the maintenance of the lens epithelium.

1.2 The FGF and FGFR Family

Affinity for heparin or heparin sulfate proteoglycans is a hallmark of all members of the FGF family and these co-factors are essential for effective activation of FGFRs [reviewed in 8]. FGF1 and FGF2, the first members of the FGF family to be isolated, were independently purified by several laboratories and known by a variety of different names including acidic FGF (aFGF) [9,10], endothelial cell growth factor (ECGF) [11], retina-derived growth factor (RDGF) [12], eye-derived growth factor-II (EDGF-II) [13,14] and brain-derived growth factor-II (BDGF-II) [15] for FGF1, and basic FGF (bFGF) [16], eye-derived growth factor-I (EDGF-I) [17] and brain-derived growth factor-I (BDGF-I) [18] for FGF2. As the names RDGF and EDGF-I and EDGF-II suggest, these prototypical FGFs are abundantly present in the mature eye. In mammals, the FGF family currently consists of 22 distinct genes named *FGF1-23* in mice and humans. *Fgf15* is the mouse ortholog of human *FGF19* and will hereafter be referred to as *FGF15/19*. The orthologous nature of these genes was not discovered until after they were named, therefore, there is no human FGF15 and there is no mouse FGF19 [19,20]. In addition to FGF1 and FGF2, transcripts and/or protein for FGF3 [21], FGF5 [22], FGF7 [23], FGF8 [24], FGF9 [25], FGF10 [26], FGF11-13 [27] and FGF15/19 [28] are present in the normal eye either during development and/or at maturity. In addition to published reports, evidence for FGF14 expression in the developing mouse lens can be found using the Open Access database of *in situ* hybridization results (www.genepaint.org) [29]. Therefore, at least 13 of the known 22 FGF genes are expressed in the eye and could potentially influence lens development or function.

Chicks and mammals have four FGFR tyrosine kinase genes (FGFR1-4). The prototypical FGFR extracellular domain contains a signal peptide and three immunoglobulin-like (Ig) domains as well as a cluster of acidic and serine/threonine amino acids known as the “acid box” between Ig domains I and II. FGFRs also contain a single transmembrane domain and a split intracellular tyrosine kinase domain (Figure 2). Transcripts from all four FGFR genes are known to undergo alternative splicing generating numerous receptor isoforms [reviewed in 30,31,32]. The complexity of this alternative splicing is too great to review here, but a few of the major isoforms generating diversity in ligand affinity and specificity are important to mention. Alternative splicing of FGFR1 and FGFR2 can lead to receptors with either two (β -form) or three (α -form) extracellular Ig domains, with the β -form (at least of FGFR1) exhibiting higher ligand affinity [33]. It is the first Ig domain that is alternatively missing in this case as Ig domains II and III are critical for ligand binding. FGFR1-3 also undergo alternative splicing resulting in the use of one of two alternative exons encoding the carboxy terminal half of the

third Ig-domain (closest to the membrane) generating receptors (IIIb or IIIc) having different ligand specificity (Figure 2A, B) [34,35]. The classical example of this splicing variation is the IIIb and IIIc isoforms of FGFR2. FGFR2-IIIb is also known as the keratinocyte growth factor receptor (KGFR) and has high affinity for FGF7 and low affinity for FGF2. FGFR2-IIIc is also known as bek and has high affinity for FGF2 and low affinity for FGF7 [36]. FGFR1 and FGFR2 additionally exist in a IIIa isoform which introduces a stop codon prior to the transmembrane domain resulting in a secreted extracellular domain [37,38]. Secreted isoforms of FGFR3 [39] and FGFR4 [32] have also been described. It is thought that these secreted isoforms of FGFRs may negatively regulate FGFR signaling. A more in depth discussion of alternative splicing and structure of FGFRs can be found in an excellent recent review by Eswarakumar, Lax and Schlessinger [40]. Dimerization of FGFRs is induced by ligand binding in complex with heparin or heparin sulfate proteoglycan. Dimerization of the FGFR monomers results in transphosphorylation and activation of the cytoplasmic domains of the receptor that initiates an intracellular signal transduction cascade (Figure 2C, D) [reviewed in 41]. In addition to FGFR1-4, the vertebrate genome expresses another gene capable of FGF binding alternatively named FGFR5 or FGFR-like-1 for FGFR-like-1 [42]. FGFR-like-1 differs from the other FGFR genes in that it does not encode an intracellular tyrosine kinase domain, and thus is not likely to be independently capable of initiating an intracellular signal transduction cascade.

1.3 Expression Pattern of FGFR Genes in the Vertebrate Lens

The developing vertebrate lens expresses all four FGFR genes [43–49]. The expression pattern of these genes in the lens is not identical. Although the presence of FGFR1, 2 and 3 transcripts in the mouse lens has been published [43–45], a detailed developmental expression pattern for these receptors during lens morphogenesis was carried out in John McAvoy's laboratory, and these rat expression patterns will be referred to here [46,47]. Rat FGFR1 expression was detected in the PLE and lens pit and in the lens vesicle with levels increasing in later lens development within the transitional zone and decreasing in the lens epithelium, particularly at postnatal stages [47]. The expression pattern of FGFR1 in the chick is similar during embryonic development, with transcripts being detected in the lens vesicle and in both the epithelial and fiber cells of the embryonic lens [50]. FGFR1 is also expressed in the intact and regenerating newt lens [51]. The rat FGFR2-IIIb isoform (KGFR) was first detected in the early elongating primary fiber cells within the lens vesicle. Later in embryonic development, KGFR transcripts were detected in the lens epithelium but were much more numerous in the transitional zone. This pattern of KGFR expression in the lens persisted into postnatal stages with transcript abundance generally increasing in an anterior to posterior direction within the lens epithelium, with the exception of the germinative zone where transcript levels are reported to be slightly lower than the surrounding epithelium [46]. This same study simultaneously examined the expression of the FGFR2-IIIc isoform (bek). FGFR2-IIIc is expressed earlier than KGFR in the lens, being first detected within the lens pit. Later in lens development, FGFR2-IIIc expression is notable in that it is the FGFR most uniformly expressed in the lens epithelium without showing an increase in abundance at the transitional zone. Neither FGFR2 isoform continued to be expressed in mature fiber cells [46]. Transcripts for both isoforms of FGFR2 were also readily detected in the developing newt lens [52]. In contrast, FGFR2 expression was not detected in the lens vesicle of the developing chick eye nor at later stages of lens development [50,53]. FGFR3 expression is abundant in the developing lens of rodents [45, 46], chick [53] and amphibians [52,54]. In rats, FGFR3 expression initiates later than FGFR1 or FGFR2 with transcripts first being detected in the elongated primary fiber cells at embryonic day 14 (E14). Later, FGFR3 expression was also detected in the lens epithelium, but transcripts were markedly more abundant in the cells at the transitional zone similar to the pattern seen for KGFR. In the chick, FGFR4 is expressed during lens placode formation and later becomes restricted to cells of the transitional zone [48,49]. An *in situ* hybridization survey in the

amphibian *Pleurodeles waltl* failed to detect FGFR4 expression in the lens [55]. A detailed expression pattern of FGFR4 expression in the lens has not been published in mammals, but transcripts for FGFR4 are evident in E14.5 mouse lenses and can be seen using the Open Access database (www.genepaint.org) generated by a robotic *in situ* hybridization platform [29]. Given this evidence, it is highly likely that all vertebrate lenses express multiple FGFR genes in the lens during development.

While the presence of multiple FGF ligands in the eye and multiple FGFRs in the lens are suggestive of an important function for these molecules in lens development and homeostasis, the precise roles played by each ligand and receptor in lens biology remains an area of active investigation. Do these ligands and receptors perform unique roles in lens biology or are they simply redundant? While these questions can't yet be answered definitively the remainder of this review will focus on insights from recent experiments that provide useful clues.

2. Lessons learned from FGF studies on lens cells *in vitro*

The stimulation of proliferation of cultured mammalian lens epithelial cells by exogenous FGF1 or FGF2 has been known for many years [13]. FGFs, however, are neither unique nor the most potent stimulators of lens epithelial cell proliferation. A number of other growth factors including PDGF, insulin, IGF-1, IGF-2, EGF, TGF α and HGF have all shown to be effective mitogens for lens cells from several species [for review see 56]. FGF-stimulated lens cell proliferation *in vitro* requires both ERK activation and PI3-K/AKT signaling [57,58]. Cultured lens epithelial cells synthesize both FGF1 and FGF2 [59]. FGF1 and FGF2 lack typical signal peptide sequences and are secreted via a nonclassical, ER/Golgi-independent mechanism, with the secretion of FGF1 being induced by cell stress [reviewed in 60]. In cultured lens cells FGF1 protein levels increase upon contact inhibition or serum starvation [61]. Antisense oligonucleotides specific for FGF1 were shown to decrease lens cell survival in serum free medium approximately 50% relative to similar cultures treated with sense FGF1 or pBluescript vector oligonucleotides [61]. These experiments suggested that the main role for endogenously expressed FGF1 might be as a lens cell survival factor.

2.1 Studies in Lens Epithelial Explants

The seminal observation concerning FGFs and lens biology were made by the McAvoy lab in the 1980s using a rat lens explant system. In the lens explant system, the lens capsule and adherent lens epithelial cells are removed from the fiber cell mass and cultured *in vitro*. In contrast to many previous studies in which lens epithelial cells were cultured on plastic or various other matrices, epithelial cells are maintained on their natural basement membrane (the capsule) in the lens explant system. Co-culture of these lens epithelial explants with neural retina induces epithelial cell elongation as well as the expression of mammalian lens fiber cell differentiation markers such as β - and γ -crystallins [62]. In an attempt to identify the lens fiber differentiating factor from retina, Chamberlain and McAvoy purified FGF1 and FGF2 from the retina and found that these factors were both capable of inducing β - and γ -crystallin accumulation as well as morphological changes consistent with fiber cell differentiation [63, 64]. Furthermore, McAvoy and Chamberlain discovered that FGF2 was capable of stimulating explanted lens epithelial cells to proliferate, migrate, or differentiate at concentrations of 0.15, 3 and 40 ng/ml respectively [65]. Taken together with the asymmetric distribution of FGF1 within the eye [66], these observations formed the basis of the hypothesis that a gradient of FGF concentration might be responsible for lens polarity within the eye, with lower levels of FGF stimulating proliferation in the germinative zone and higher concentrations stimulating fiber differentiation in the transitional zone. This hypothesis was supported by findings indicating that 70% of the fiber cell differentiating activity on lens explants present in bovine vitreous humor could be blocked by a mixture of antibodies to FGF1 and FGF2 [67].

Similar experiments in David Beebe's laboratory using chick lens explants found that chicken vitreous humor [68,69], as well as purified insulin, IGF-I and IGF-II were all capable of promoting lens epithelial cell elongation in serum-free medium within 4–5 hrs at a concentration of 1 µg/ml. Several other growth factors, including FGF, did not show this activity in the chick explants under identical conditions [69]. In other chick explant experiments, FGF2 (either 100 ng/ml or 1 ng/ml) failed to induce significant entry of lens epithelial cells into S-phase of the cell cycle following a 3 hour incubation [70].

These apparently different effects of FGFs on rat and chick lens explants led to the speculation, by some in the field, that the regulation of lens cell proliferation and differentiation might be fundamentally different in mammals and birds. Subsequent experiments demonstrating that vitreous humor, from either bovine or chicken embryo sources, induced similar effects in chick lens explants makes this explanation extremely unlikely [71]. In these experiments, bovine and chick vitreous humor were able to induce rapid (within 6 hours) elongation of explanted chick lens epithelial cells, and this effect could not be entirely reproduced by recombinant FGF1, FGF2 or FGF8, although FGF2 did induce a statistically significant increase in cell length [71]. The rapid cell elongation promoting effects of vitreous humor could not be prevented by co-incubation with the FGFR inhibitor SU5402, or by preincubation of the vitreous with heparin sepharose beads [71].

Another explanation for the apparent differences in response between chick and rat lens explants to FGFs was in the way the experiments were done. In the rat experiments, proliferation, migration and differentiation were measured at 18 hours, 24 hours and 5 days after the addition of FGF respectively to explants prepared from 3 day old (neonatal) animals, whereas chick explants were from 6 day old embryos and responses to FGF were measured within 3–6 hours. Chick explant experiments performed by Le and Musil found that FGF2 at 25ng/ml stimulated lens epithelial cells to enter S-phase (approximately 2 fold over control explants) following 16 hour incubation, similar to that seen with insulin at 1 µg/ml [72]. Furthermore, the same concentration of FGF2 induced dramatic cell elongation and the expression of the fiber cell-specific protein CP49 following 6 days of culture [72]. In a final set of experiments, Le and Musil showed that the ability of chick vitreous humor conditioned medium to promote increased δ -crystallin protein synthesis was abolished by the pre-incubation of the conditioned medium with immobilized heparin beads in low salt conditions. Significantly, this δ -crystallin promoting activity of the vitreous conditioned medium was capable of being eluted from the heparin beads under high salt conditions, suggesting that this activity behaved like a molecule from the FGF family [72]. Subsequent experiments performed in the Beebe laboratory demonstrated that although the absolute accumulation of proteins characteristic of lens fiber differentiation (δ -crystallin, β -crystallins and CP49) was reduced in chick explants cultured with heparin depleted vitreous or with vitreous in the presence of SU5402 relative to control vitreous, these proteins were produced in all vitreous treated explants and not induced in explants cultured in defined media following three days of incubation.

Therefore, while vitreous, insulin and members of the IGF family initiate very rapid elongation in chick lens explants, FGF2 alone also promoted a differentiation response following a longer incubation period. These results reinforce the notion that FGF signaling most likely plays a similar role in mammalian and avian lens development. In the course of embryonic development, no growth factor acts in isolation. Experiments in chick explants suggest that factors in the vitreous that do not act through FGFRs are capable of inducing a differentiation response that is significantly enhanced by FGFR activity [71], and several sets of experiments in postnatal rat lens explants demonstrated that insulin or IGF-I potentiated the activities of FGF with respect to lens fiber differentiation [73–76].

Other experiments in chick lens explants supported a role for FGF/FGFR signaling in cell survival. Either treatment of chick lens explants with 20% vitreous humor in the presence of SU5402 or pretreatment of the vitreous humor prior to addition to explant media with heparin sepharose beads caused significant increases in lens cell apoptosis relative to control media supplemented with 20% vitreous. Apoptosis in these experiments was measured by TUNEL assay after 18 hours or 2 days in SU5402 or heparin depleted vitreous treated cultures respectively [71].

3. Ectopic or Over-expression of FGFs in the Lens of Transgenic Mice

The result of studies with FGF1 and FGF2 on lens epithelial explants inspired Paul Overbeek's laboratory to over-express members of the FGF family during lens development in transgenic mice. The first of these experiments demonstrated that FGF1 was capable of inducing central lens epithelial cells to elongate and accumulate β -crystallins (Figure 3A) provided the FGF1 transgene contained a signal peptide sequence derived from FGF4 [77]. Subsequent experiments demonstrated that transgenic expression of FGF3 [78], FGF4, FGF7, FGF8 or FGF9 in the lens exhibited similar differentiating effects on lens epithelial cells including cell cycle withdrawal [79]. The cumulative result of these experiments was that changing the concentration and/or the relative distribution of several different FGFs during development could alter the normal polarity of the lens. In addition to FGFs, a number of other growth factors and cytokines have been expressed in the lens during lens development. These include TGF α [80–82], EGF [82], TGF β [83–85], BMP7 [86], VEGF [87], IGF-I [88], PDGF [89], NT3 (ML Robinson, unpublished data), insulin [90], optineurin [91], IL-1 β [92] and LIF [93]. Only members of the FGF family, however, have demonstrated a clear differentiation phenotype including cell cycle withdrawal, elongation and the accumulation of fiber cell characteristic crystallins in transgenic mice.

Curiously, despite the strong differentiating activity of FGF2 on rat lens epithelial explants [64], transgenic mice over expressing FGF2 in the lens exhibited an inhibition of fiber cell differentiation, despite the inclusion of a signal peptide on the FGF2 transgene [94]. This inhibition of differentiation was characterized by a failure of primary and secondary lens fiber cells to properly elongate and fill the lumen of the lens vesicle. In these same studies, FGF2 was shown to protect neonatal lens cells from the apoptotic effects of pRb sequestration mediated by the papillomavirus oncoprotein E7 [94]. Thus, in contrast to the actions of FGF2 in rat lens explants and to several other FGFs in transgenic mice, transgenic expression of FGF2 acted to inhibit fiber cell differentiation and to promote lens cell survival. Transgenic expression of FGF15 in the lens failed to influence lens fiber differentiation in several independent transgenic lines (Paul Overbeek, personal communication). It is still unclear why FGF2 and FGF15 over-expression in the lens leads to a dramatically different lens phenotype in transgenic mice than the other FGFs tested.

The general theme that emerged from transgenic over expression of FGF ligands in the eye was that ectopic FGF presented to lens epithelial cells is capable of inducing lens fiber differentiation and protecting lens epithelial cells from at least some forms of apoptotic stress. These experiments alone, however, could not address the requirement of FGFR signaling for normal lens fiber differentiation or cell survival during normal development.

4. Interference with Endogenous FGFR Signaling in Transgenic Lenses

Further insight into the effects of FGFs during lens development came from transgenic experiments designed to express alternate forms of FGFRs in the lens. Membrane bound FGFR molecules lacking an intracellular domain heterodimerize with endogenous full-length FGFRs and act as dominant negative inhibitors of FGFR signaling (Figure 2E) [95,96]. Three laboratories undertook similar experiments to express an FGFR1 molecule with an intact ligand

binding and transmembrane domain but lacking an intracellular tyrosine kinase domain. In all cases, lens expression of the dominant negative FGFR1 inhibited fiber cell elongation and ultimately caused fiber cell apoptosis [97–99]. Fiber cell differentiation, while inhibited, was not prevented in any of these dominant negative transgenic FGFR studies. This was not surprising because even if FGFR signaling were an essential component in initiating fiber cell differentiation, the transgene promoters utilized in these experiments did not express the dominant negative transgene until fiber differentiation had commenced.

In another transgenic approach to inhibit FGFR signaling during mouse lens development Govindarajan and Overbeek expressed self-dimerizing, secreted versions of the extracellular domain of FGFR1 and FGFR3 in the lens [100]. The idea being that secreted FGFRs would bind and sequester FGF ligands present in the ocular media and thus inhibit FGFR signaling in the lens cells (Figure 2F). In each case the IIIc splice forms of the receptor were used. In these experiments several transgenic mouse lines secreting the FGFR3-IIIc extracellular domain exhibited a postnatal inhibition of lens fiber differentiation, but those lines secreting FGFR1-IIIc did not. This inhibition induced by secreted FGFR3-IIIc was characterized by a posterior displacement of the transitional zone and consequent expansion of the lens epithelium (Figure 3B). The morphological displacement of the transitional zone was accompanied by regional displacement of several molecular markers of fiber cell differentiation including p57^{KIP2}, c-maf and Prox1 [100]. These observations suggested that an endogenous postnatal fiber promoting activity was sequestered by secreted FGFR3-IIIc but was not by FGFR1-IIIc. The most comprehensive analysis of FGFR ligand binding specificity to date is based on the ability of particular FGF ligands to induce mitogenesis in transfected BaF3 cells expressing defined FGFR isoforms [34,35]. FGF1 is the only member of the FGF family that binds all known splice isoforms of FGFR genes [34,101]. In light of this data, if we consider effective ligand-receptor binding as that inducing at least 50% of the mitogenic activity of elicited by FGF1 stimulation, FGFR1-IIIc would be expected to bind FGF1, 2, 4, 5, 6, 8 and 15/19 and FGFR3-IIIc would be expected to bind FGF1, 2, 4, 9, 17, 18, 15/19 and 20. While other interpretations are possible, taken together, the results of Govindarajan and Overbeek [100] suggest that FGF4, 9, 17, 18 or 20 might be involved in the regulation of lens fiber differentiation in mice, and of these only FGF9 is known to be expressed in the eye. This is particularly compelling in light of the observation that at least a portion of FGF9 null embryos demonstrate delayed primary fiber cell elongation [25]. It is also worth noting that several activating mutations have been described for FGFRs that result in ligand-independent enhancement of intracellular FGFR signaling [reviewed in 40]. These mutations are often dominant and responsible for several human genetic syndromes, many of which have mouse models. None of these activating FGFR mutations have been associated with primary lens abnormalities in humans or mice.

Elegant experiments initiated in the laboratory of Richard Lang demonstrated that FGFR signaling plays an important role in mouse lens induction. Several pieces of evidence supported this conclusion. First, primordial eye explants cultured in the presence of the specific pharmacological FGFR inhibitor SU9597 demonstrated a reduction both in the expression of Pax6 in the PLE and the OV, and in the size of the lens pit. Second, expression of a dominant negative FGFR1-IIIc in the PLE using the Pax6 P0 promoter and ectoderm enhancer resulted in a decreased lens placode thickness, delayed lens placode invagination, smaller lens vesicles with delayed primary fiber cell elongation, reduced proliferation in the lens pit and lens epithelium and overall smaller lenses with occasional failure of lens stalk degeneration [102]. The defects present in the dominant negative FGFR1 transgenic mice were exacerbated by the deletion of one allele of Bmp7, revealing a genetic interaction between FGF and BMP signaling in early lens formation. Subsequent work by Belecky-Adams, Adler and Beebe demonstrated that purified BMP2 was able to enhance chick lens epithelial cell elongation induced by either

FGF1 or FGF2 alone, further suggesting cooperation between BMP and FGF signaling in avian lens fiber differentiation [103].

5. A Conserved Role for FGF/FGFR Signaling in Non-Mammalian Lens Development

Several studies in amphibians also suggest that FGF/FGFR signaling plays an important role in lens morphogenesis. One of the strengths of some amphibian species is their capacity for regeneration. *Xenopus laevis* larvae are capable of regenerating lenses following lentectomy via transdifferentiation of the outer cornea under the influence of neural retina [reviewed in 104]. Using explants from outer cornea, Bosco et al., were able to show that transdifferentiation of the outer cornea into lens fibers could take place in the absence of neural retina when FGF1 was supplied at 500 ng/ml to the culture media. The transdifferentiated lenses induced by FGF1 contained only fiber cells, in contrast to lenses regenerated *in vivo*, and formed without the requirement of new cell division [105]. It has also been recently suggested that FGF/FGFR signaling is an essential component for maintaining lens forming competence in the epidermis of *Xenopus laevis* larvae [106].

The champion of amphibian lens regeneration is undoubtedly the newt, and several studies in this organism demonstrate the requirement of FGF/FGFR signaling in this process. In contrast to *Xenopus*, lens regeneration in newts occurs via transdifferentiation of pigmented cells from the dorsal iris [reviewed in 107]. FGF1, FGFR1, FGFR2 (both KGFR and bek) and FGFR3 are expressed in the newt lens during the regeneration process [51,52]. Exogenous FGF1, supplied as a heparin bead during lens regeneration, resulted in slightly elongated anterior lens epithelial cells. In contrast, beads supplying exogenous FGF4 induced multiple lenses to form from the dorsal iris and these lenses displayed abnormal polarity [52]. Interestingly, during newt lens regeneration, FGFR1 expression is specifically induced in the depigmenting cells of the dorsal iris and pharmacological inhibition of FGFR signaling by SU5402 arrested lens regeneration at the dorsal iris depigmentation stage [51].

In a set of more recent experiments carried out in the laboratory of Hisato Kondoh the actions of FGF/FGFR signaling during lens regeneration were analyzed in more detail. In these a single 50 ng injection of FGF2 in the intact newt eye reproducibly induced the formation of a second lens from the dorsal iris, without the need to remove the original lens [108]. In contrast, similar single injections of FGF1, FGF4, FGF7, FGF8, FGF9, FGF10, EGF, IGF or VEGF did not elicit lens regeneration from the dorsal iris. The investigators then analyzed and compared FGF2 initiated lens regeneration from the dorsal iris with lens regeneration initiated by the removal of the lens and they found that in both cases the ventral as well as dorsal iris up regulate the endogenous expression of FGF2, Pax6, and Sox2 and initiate the expression of MafB within 6 days (in FGF2 injected eyes) or within 8 days (following lens removal). Only the dorsal iris, however, went on to express Prox1, Sox1 and β B1-crystallin 14 or 16 days after FGF2 injection or lens removal respectively, coincident with the appearance of a new lens [108]. Furthermore, the authors demonstrated that daily 100 ng intraocular injections of dimerized, soluble FGFR2-IIIc (bek) were able to completely block lens formation from the dorsal iris following lens removal. Identical injections of soluble FGFR2-IIIb (KGFR) were completely ineffective at preventing lens regeneration [108]. These studies demonstrated that a single FGF2 injection was sufficient to induce lens development from the dorsal iris and suggested that FGF2 was the endogenous molecule responsible for the initiation of lens regeneration in the dorsal iris following lens removal. The failure of FGF1, 4, and 9 to exhibit a similar effect is puzzling in light of predictions that these growth factors should also bind and activate FGFR2-IIIc [34]. How different FGF ligands acting through common FGFRs elicit different effects *in vivo* remains a great mystery to be elucidated.

Experiments in chick also support a possible role for FGF/FGFR signaling in lens induction. In early chick development as the OV comes in close contact with the PLE, the OV expresses both FGF8 and FGF19. The patterns of FGF8 and FGF19 are complimentary within the OV with FGF8 present in the ventral portion of the vesicle with the FGF19 expression domain being slightly more dorsal and extending to the end of the OV/PLE contact. FGF19 is also expressed by the chick PLE at this stage [28]. Within the eye at this stage, FGFR4, the preferred receptor for FGF15/19 [109,110], is simultaneously expressed only in the PLE and adjacent surface ectoderm [48]. A number of interesting observations were made during a series of *in ovo* electroporation experiments designed to mis-express L-Maf, FGF8, FGF19 and a dimerized secreted FGFR4 during chick eye development [48]. In these experiments expression patterns of lens marker genes such as L-Maf, Prox1 and δ -crystallin were examined by whole mount in situ hybridization as readout of lens patterning modifications. No alterations in the expression patterns of these genes were observed following mis-expression of FGF19. In several cases, however, FGF19 expression was induced by mis-expression of L-Maf as was the expression of Prox1 and δ -crystallin as had been shown previously [111]. Mis-expression of FGF8 induced the expression of both L-Maf and FGF19, and mis-expression of the secreted FGFR4 also induced the expression of L-Maf. The authors suggest that L-Maf is a positive regulator of FGF19 expression which in turn negatively regulates L-Maf expression via activation of FGFR4 in a negative feedback loop [48]. The significance of these findings awaits further investigation. While it is clear that FGF8 is capable of inducing L-Maf expression, and the developmental localization of FGF8, FGF19 and FGFR4 in the chick eye is compelling, concrete evidence that any of these particular molecules are *required* for chick lens induction is presently lacking. There are also legitimate reasons to suspect that there may be some species differences in lens induction with respect to FGF8, FGF19 and FGFR4. First, while FGF19 is clearly expressed in the chick PLE and lens, there is no evidence that the mouse homologue (FGF15) is ever expressed in these tissues [28]. Likewise, FGF8 is expressed in both zebrafish [112] and chick retina but evidence for FGF8 expression in the mammalian retina or OV is lacking, although the FGF8 is expressed in the optic stalk [79,113]. Also, despite strong expression of FGF8 in the zebrafish retina, *ace* mutants lacking functional FGF8 undergo normal lens induction [114]. Therefore the roles of, or requirements for, FGF8, FGF15/19 and FGFR4 in lens induction remain unclear.

The potential role of endogenous FGF/FGFR signaling in chick lens fiber differentiation was examined by several experiments in the laboratory of David Beebe. In one such experiment, recombinant avian retrovirus vectors were used to express FGFR1 or FGF1 constructs in the developing chick lens. In each case, the FGFR1 or FGF1 expression cassette was separated from a β -galactosidase (LacZ) reporter gene by an internal ribosome entry site to mark those infected cells that were expressing the recombinant FGFR1 or FGF1 [71]. The virions contained either full length FGFR1, a dominant negative FGFR1 (without an intra cellular tyrosine kinase domain), an FGF1 fused to a signal peptide or simply the LacZ reporter. Virus was injected into the lens vesicle of 3 day old chick embryos and lenses were removed several days later. While the proportion of infected cells per lens was very small, several examples of elongated, X-gal stained fiber cells were evident in lenses previously infected with the dominant negative FGFR1 (Figure 3C), and infection of isolated lens epithelial cells with the secreted version of FGF1 did not result in significant elongation of these cells. Curiously, expression of either the full length FGFR1 or secreted FGF1 led to fragmented lens fiber cells, which the authors attributed as evidence that the constructs were indeed expressed [71]. As the proportion of infected cells in these experiments was small and no quantitative measures were made to compare the relative level of dominant negative transgene expression versus endogenous FGFR expression within infected cells, it is difficult to interpret the results of this experiment. One interpretation is that FGFR signaling is not required for lens fiber differentiation in the chick. It is also possible that the expression levels of the truncated FGFR1 were not high enough to effectively block endogenous FGFR signaling. Other studies have

demonstrated that dominant negative receptor expression must exceed endogenous receptor expression by several fold to block endogenous FGFR signaling [95]. It is also possible that when isolated or small patches of lens epithelial cells secrete recombinant FGF1 in a background of non-transduced cells, that the amount of FGF1 signal produced is not strong enough to initiate global differentiation in the epithelium.

6. Targeted Mutations in Mice: Do They Clarify or Confuse?

If FGF/FGFR signaling plays an essential role in vertebrate lens development, what are the specific receptors and ligands required and what is their essential role? The definitive answers to these questions are still elusive, but a few distinct themes are emerging. Perhaps no other technique has proven more powerful to elucidate the role(s) specific genes play during vertebrate development than that of targeted mutagenesis in mice. Other model systems have their advantages, but at present the mouse is the only vertebrate model system where specific genetic manipulation is simple and efficient. For these reasons, the remainder of this review will discuss what targeted mutations have taught us about FGF/FGFR signaling in lens development and where opportunities for further clarification exist.

6.1 No Single FGF Emerges as an Essential Lens Development Factor

As discussed above, more than half of the known FGF ligands are expressed in the eye. Mice with null mutations in FGF1 [115], FGF2 [116,117], FGF3 [118], FGF4 [119], FGF5 [120], FGF6 [121], FGF7 [122], FGF8 [123], FGF9 [124], FGF10 [125], FGF11 [126], FGF14 [127], FGF15 [128], FGF17 [129], FGF18 [130,131] and FGF23 [132,133] display a diverse range of phenotypes. With the exception of some FGF9 deficient mice displaying delayed primary lens fiber cell elongation, [25], none of the FGF ligand mutations published to date display any reported primary defects in lens development. The same is true for mice deficient for both FGF1 and FGF2 [115]. Deficiency for FGF4 leads to peri-implantation lethality [119], but as FGF4 expression has not been reported in the eye, it is unlikely that this FGF plays a unique role in lens development. FGF8 null mutants also die early in embryogenesis making evaluation of a possible role for this ligand in lens development difficult. FGF8 expression, however, has not been reported in the murine OV or retina. The *ace* mutation in zebrafish suggests that FGF8 is dispensable for early lens development [114], however it must be noted that during the evolution of zebrafish, there was a genome duplication making it likely that another FGF8 ortholog may exist in this species. Also, no lens abnormalities are associated with hypomorphic alleles of FGF8 that result in severe craniofacial and cardiac malformations in mice [134]. Considering the proposed importance of FGF8 in chick lens development (discussed above), further experiments may be required before this ligand can be discounted in mouse lens development. Of the other FGF ligands where null mutations are available, all are either viable or capable of survival to the perinatal period. FGF11-14 are also known as fibroblast growth factor homologous factors (FHF1-4) and while they show significant structural similarity to other members of the FGF family, they are not likely to be secreted and they do not activate any of the known FGFR isoforms [reviewed in 126]. Therefore, despite the expression of FGF11-13 in the retina [27] and the possible expression of FGF14 in the lens (see above), these FGFs are unlikely to play essential, independent roles as signaling molecules for lens development.

6.2 Null and Conditional Mutations in FGFRs

Null mutations in all four FGFR genes are also available in mice. Mice lacking FGFR4 or FGFR3 or both FGFR3 and FGFR4 and do not display obvious abnormalities in lens development or function [135,136]. Homozygous null mutations in FGFR1 [137,138] or FGFR2 [139,140] are lethal during early embryonic development, but alternate strategies have been devised to explore the role of these receptors during lens development.

The function of FGFR1 in lens development was explored by two complementary approaches. One of these involved the production of chimeric embryos by the aggregation of LacZ marked FGFR1 null ES cells with embryos homozygous for the aphakia mutation. This lens complementation system had previously been shown to result in completely ES cell derived lenses in chimeric mice [141]. In a few cases, chimeric embryos developed morphologically normal FGFR1 null lenses displaying typical expression patterns for α -, β -, and γ -crystallins [142]. Likewise, a conditional (lox P- activated) mutation in FGFR1 [143] was used to selectively delete FGFR1 in cells of the lens lineage with two different transgenic Cre alleles. The Cre alleles utilized in these experiments were MLR10, where ocular Cre expression is restricted to the lens lineage subsequent to the lens pit stage (E10.5) [144], and Le-Cre where ocular Cre expression is present at the lens placode stage (E9.0) resulting in conditional gene deletion of all surface ectoderm-derived ocular structures [145]. No lens abnormalities were evident when the conditional FGFR1 mutation was activated by either MLR10 [144] or Le-Cre (unpublished results from the D. C. Beebe and M. L. Robinson laboratories). Similar results were seen when a conditional mutation in FGFR2 [146] was deleted with MLR10 (M. L. Robinson laboratory, unpublished observations). Embryonic lethality in FGFR2 deficient embryos is a consequence of placental failure [140] and can be rescued by complementation provided by tetraploid embryos that contribute extensively to the placenta, but are unable to contribute significantly to the embryo proper [reviewed in 147]. Chimeric embryos produced by the aggregation of homozygous FGFR2 mutant morulae with tetraploid morulae produced fetuses that survived to term, but died at birth due to lung agenesis [148]. One of the striking features of the FGFR2 deficient pups was the absence of eyelids. Several eye sections from mid- to late gestation embryos were examined. Although in most sections presented, mutant lenses looked slightly smaller, no major lens abnormalities were noted [148]. Similar lung and eyelid abnormalities were evident in mice specifically deficient for the FGFR2-IIIb isoform, again with no lens abnormalities noted [149,150]. A specific deletion of the FGFR2-IIIc isoform has also been described, and the resultant mice are viable with no reported lens abnormalities. [151]. In contrast, Cre-mediated deletion of FGFR2 catalyzed by Le-Cre resulted in mice with several distinct lens phenotypes (Figure 3D) [152]. These lenses were typically smaller than control lenses, evident as early as E12.5. Formation of primary fiber cells was also delayed, although immunologically detectable α -, β - and γ -crystallins were present in these FGFR2 deficient lenses in the appropriate pattern. Lens abnormalities typically progressed with developmental age such that lenses were typically absent or severely disorganized in adult mice. Although elongation of fiber cells and the appearance of crystallins associated with fiber cells demonstrated that fiber differentiation was not blocked in the absence of FGFR2, there was a significant reduction in the proportion of fiber cells expressing the cyclin dependent kinase inhibitor p27^{KIP1} suggesting that cell cycle withdrawal was not complete in these cells. Consistent with this interpretation, 3.5 % of E12.5 FGFR2 deficient fiber cells were positive for BrdU incorporation compared to just 0.13% of Le-Cre negative fiber cells [152]. Perhaps the most striking phenotype of Le-Cre/FGFR2 deficient lenses was a several fold increase in the apoptotic index of both lens epithelial cells and lens fiber cells. This increase in apoptosis was noted as early as E12.5 and was still evident at postnatal day 1 [152]. In light of findings with the Le-Cre/FGFR2 mutant mice, it would be interesting to determine if the lenses of either the FGFR2-IIIb or FGFR2-IIIc mutant mice display similar decreased lens cell survival, or if both isoforms must be missing to create this phenotype.

7. Conclusions

Some obvious conclusions descend from applying mouse genetics to understanding the role of FGF/FGFR signaling in lens development. First, it would appear that no individual FGF ligand is likely to be essential for lens development. Although null mutations in FGF 12, 13, 16, 20, 21 and 22 have yet to be reported, of these only FGF12 and FGF13 are known to be expressed in the eye (retina) and these are unlikely to be secreted or to activate FGFRs present on

the lens cell surface. Likewise, no single FGFR gene appears to be essential for lens induction or for the initiation of lens fiber cell differentiation, although FGFR2 signaling plays an essential, non-redundant role in lens cell survival. Could this mean that previous experiments demonstrating a role for FGF/FGFR signaling in lens induction and fiber differentiation in embryos, explants and in transgenic mice have led the scientific community astray? Further genetic evidence would suggest otherwise. FGFRs mediate much of their intracellular signaling through a membrane anchored docking molecule known as FRS2 [reviewed in 40,153]. FRS2 mediation distinguishes FGFR signaling from many other growth factor receptors such as PDGF, insulin, IGF-I, IGF-II, TGF β , etc. Upon FGFR activation, six separate tyrosines on FRS2 α become phosphorylated. Two of these tyrosines recruit the protein tyrosine phosphatase, Shp2 and the other four recruit Grb2. Mice homozygous for targeted mutations (Y-F) in the two Shp2 binding sites of FRS2 α (FRS2 α^{2F}) displayed variable, but severe ocular defects. In mutant embryos, the lens placode failed to induce strong expression of Pax6, and Pax6 levels in the lens pit and developing optic cup were similarly reduced. Likewise, Six3 levels are reduced in both the OV and PLE of FRS2 α^{2F} mutants, while Bmp4 and Chx10 expression was reduced specifically in the OV and optic cup. These findings provide genetic support for the importance of FGF/FGFR signaling during lens and retina induction [154]. Whether the lens and retinal roles of FRS2 α are independently required for eye development awaits further clarification.

So how do we reconcile the many pieces of experimental evidence suggesting that FGF/FGFR signaling plays a major role in the regulation of lens induction, lens cell proliferation, lens cell survival and lens fiber differentiation with the fact that, in mice, none of the known FGFs appear essential for these processes? Also, why would the lens express so many different FGFR genes if only one, FGFR2, was essential for lens development? Certainly the preponderance of evidence (dominant negative strategies, pharmacological inhibition, deletion of FGFR2 and mutations in FRS2 α) suggests that the FGFR is the endogenous mediator of FGF activities in the lens. Is it possible that multiple FGF ligands play redundant roles during different stages of lens development? Perhaps FGF/FGFR signaling required for lens development depends only on a quantitative level of FGFR signaling that can be achieved, in part, by many FGFs having overlapping receptor specificities. There is some precedent for this in the development of the mouse ear, where deletion of either FGF3 or FGF10 does not interfere with early otic vesicle morphogenesis, but the deletion of both interferes with the induction of the otic placode [155]. Another intriguing possibility is that FGFR activation in the lens may be mediated by a signal other than an FGF. FGFR activity can be activated by NCAM [reviewed in 156], and NCAM activation of FGFRs is thought to be essential for neuritogenesis [157]. Similar activation of FGFRs can be achieved by interactions with N-cadherin, and L1 [158,159]. Although it is difficult to imagine how these molecules could act as a diffusible activator of FGFRs on the lens cells, it is possible that these molecules would participate in signaling between the OV and the SLE during lens induction. At present, the endogenous ligands responsible for FGFR activation relevant for lens development remain a mystery.

While FGFR2 signaling is genetically required for lens cell survival, lens induction and at least early stages of lens fiber differentiation are intact in the absence of FGFR2. Also, loss of FGFR2 at a stage subsequent to the formation of the lens pit has no clear negative consequence on lens development (M. L. Robinson, unpublished result). Here too, it is likely that multiple FGFRs signal redundantly to mediate essential intracellular signals. In support of this notion, lens fiber differentiation is aborted in lens cells deficient for FGFR1, FGFR2 and FGFR3 subsequent to the lens pit stage (M. L. Robinson, unpublished observations). Loss of multiple FGFRs during earlier stages of lens development is likely to have profound effects on the lens induction phase as well. Another question worth asking is if different FGFRs mediate different intracellular responses, or is the importance of one FGFR isoform over another simply a matter of ligand specificity and spatio-temporal expression patterns. In other words, is the unique importance

of FGFR2 for lens cell survival unique to this receptor, or is the essential role of this receptor simply related to its relative abundance at a critical stage of lens development?

So what is the role of FGFR signaling in lens development? Is it important in lens induction? Does it participate in lens fiber differentiation? Does it participate in the regulation of lens cell proliferation? Is it essential for lens cell survival? It is required for lens development in all vertebrates? I would argue that the preponderance of evidence suggests that the answer to all of these questions is “yes”. How could one growth factor/receptor system regulate such pleiotropic responses (survival, proliferation and differentiation) on lens cells? Recent studies of FGF signaling suggest that differential ligand concentration can translate into just such a range of responses to FGF2 in NIH3T3 cells [160]. Although some mysteries remain as to the relative importance of each of the various FGF ligands and receptors to lens development, the future challenges lie in the elucidation of the relevant molecular events subsequent to receptor activation that mediate FGFR responses in lens cells. The diverse responses of lens and lens precursor cells have to FGF stimulation is likely to be regulated at many levels. These are likely to include regulation at the extracellular level via ligand and receptor concentration as well as regulation of FGFR signaling inside the cell via feedback inhibition of FRS2 α [161], or by other intracellular regulators of FGFR signaling including sprouty (Spry) [162,163] and Sef [164]. Extracellular regulation of FGFR activity during lens development via heparan sulfate proteoglycans was recently demonstrated with mutations in the heparan sulfate proteoglycan gene *Ndst1*. Mice homozygous for null mutations in *Ndst1* display severe lens hypoplasia and reduced FGFR signaling. *Ndst1* null lenses also exhibit reduced levels of AP2 α , α A-crystallin, Pitx3 and Prox1 suggesting that FGFR signaling may be an important upstream regulator for these molecules [165]. Studies on the intracellular regulators of FGFR signaling in the lens are in their infancy, but Spry1, Spry2 and Sef are expressed in the normal lens [166,167] and transgenic over-expression of Spry2, a dominant negative version of Spry2, or Sef in the lens leads to abnormal lens development [166–169]. Perhaps the most significant future challenge for developmental studies in the lens is to understand the intracellular language spoken by growth factor activation and how that is translated into specific changes in gene expression. While we are knocking on the door of such understanding we have not yet been invited in.

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Figure 1.

(A) Morphological development of the lens begins as the optic vesicle (OV) approaches the presumptive lens ectoderm (PLE). (B) Upon physical contact of the OV with the PLE, cells within the PLE elongate forming the lens placode. (C) The lens placode invaginates forming the lens pit and the OV invaginates forming the optic cup. (D) The lens pit deepens and the connection of the lens pit and overlying surface ectoderm is lost forming the lens vesicle. (E) The overlying surface ectoderm differentiates into the corneal epithelium and the cells at the posterior of the lens vesicle elongate forming the primary fiber cells. (F) The primary fiber cells fill the lumen of the lens vesicle as they reach the anterior lens cells making up the lens epithelium. The inner layer of the optic cup differentiates into the neural retina. (G) The mature lens consists of an anterior epithelial layer composed of non-proliferating central lens epithelial cells (cuboidal cells with white cytoplasm) and a narrow band of proliferating cells known as the germinative zone (pink cells). Just posterior to the germinative zone is the transitional zone (blue cells) where many genes important for fiber cell differentiation are initially expressed. Just posterior to the lens equator (dotted line) transitional zone epithelial cells begin elongating forming secondary fiber cells (green cells). As secondary fiber cells progress through later stages of differentiation, they lose their intracellular organelles (represented by the shrinkage and loss of red nuclei). The lens nucleus (yellow) is composed of fiber cells that were present in the embryonic lens. The mature lens is bathed on the anterior surface by the aqueous humor

and on the posterior surface by the vitreous humor. Adapted from Lovicu and McAvoy, 2005 [170]

Figure 2.

(A) FGFR molecules typically consist of three extracellular immunoglobulin-like (Ig) domains (I, II, and III, of which domain I may or may not be present), a single transmembrane domain and a split, intracellular tyrosine kinase domain. The use of an alternative exon encoding the carboxyl terminal half of Ig domain 3 is the major determinant of FGF ligand specificity and FGFR1-3 are present in both IIIb (A) and IIIc (B) isoforms. (C, D) Ligand induced dimerization of either FGFR isoform results in trans-autophosphorylation of FGFR monomers as well as phosphorylation of the FGFR associated molecule FRS2, initiating an intracellular signal transduction cascade. (E) Ectopic expression of a membrane bound FGFR lacking an intracellular tyrosine kinase domain inhibits FGFR activity by heterodimerization with wildtype FGFRs. (F) FGFR activity can also be inhibited by the sequestration of extracellular FGF ligands by dimerized secreted FGFRs.

Figure 3.

(A) Transgenic expression of secreted FGF1 results in elongation of lens epithelial cells and the induction of immunologically detectable expression of β -crystallins (brown staining, arrowhead). An embryonic day 15 lens from Robinson et al., 1995 [74] is shown. (B) Transgenic expression of a dimerized secreted FGFR3-IIIc led to a postnatal (seven day old eye shown) posterior movement of the transitional zone (arrows), normally present at the lens equator (dotted line) to a region at the back of the lens (arrowhead). From Govindarajan and Overbeek, 2001 [97]. (C) Retroviral transduction of a dominant negative FGFR1 construct in the chick lens was not able to prevent lens fiber cell elongation in a cell autonomous fashion (shown in whole mount). In these experiments, the FGFR1 sequences were separated by the coding sequence for LacZ by an internal ribosome entry site allowing for the identification of transduced cells by X-gal staining. Note that transduced cells appeared both in the lens fibers (black arrowheads) and the lens epithelial cells (white arrowheads). Image modified from Huang et al., 2003 [68]. (D) Conditional deletion of FGFR2 at the lens placode stage led to several lens phenotypes including small size, increased apoptosis, incomplete cell cycle withdrawal of lens fiber cells and fiber cell degeneration [148]. A newborn FGFR2 deficient lens is shown.