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Pathways regulating lens induction in the mouse

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ABSTRACT For more than a century, the lens has provided a relatively simple structure in which to study developmental mechanisms. Lens induction, where adjacent tissues signal the cell fate changes that result in lens formation, have been of particular interest. Embryological manipulations advancing our understanding have included the Spemann optic rudiment ablation experiments, optic vesicle transplantations as well as more contemporary work employing lineage tracers. All this has revealed that lens induction signaling is a multi-stage process involving multiple tissue interactions. More recently, molecular genetic techniques have been applied to an analysis of lens induction. This has led to the identification of signaling pathways required for lens induction and early lens development. These include the bone morphogenetic protein (Bmp) signaling pathways where Bmp4 and Bmp7 have been implicated. Though no fibroblast growth factor (Fgf) ligand has been implicated at present, the Fgf signaling pathway clearly has an important role. A series of transcription factors involved in early lens development have also been identified. These include Pax6, the Meis transcription factors, Six3, Mab21l1, FoxE3, Prox1 and Sox2. Importantly, analysis has indicated how these elements of the lens induction pathway are related and has defined genetic models to describe the process. It is a future challenge to test existing genetic models and to extend them to incorporate the tissue interactions mediated by the molecules involved. Given the complexity of this and many other developmental processes, a second century of analysis will be welcome.

KEY WORDS: *development, eye, lens, induction, patterning*

Introduction

With Spemman's optic primordium ablation experiments of 1901, investigation of visual system development has auspicious beginnings. In 1901, the experimental advances of the time were the instruments used to perform embryological manipulations (Weaver and Hogan, 2001). These new instruments allowed tissue ablations and transplantations to define the interactions required for developmental processes. Spemann's work suggested that the optic vesicle was required for development of lens (Spemann, 1901). However, even then, scientific controversies existed; it was only 2 years later that Mencl characterized a mutant salmon in which lenses but not retinas existed (Mencl, 1903). Clearly, this suggested an alternative mechanism of lens development. Since then, many investigators have repeated Spemann's original experiment with varying results. In some cases lenses would form after optic primordium ablation, but in other cases, they would not. These different outcomes probably indicate that different species vary with respect to the timing of lens induction signaling (Servetnick *et al.*, 1996). Even today these experiments form an important backdrop as we try to understand the molecular basis of lens induction.

The embryological origin of the lens

The lens is derived from the head surface ectoderm of the vertebrate embryo (Fig. 1). The first morphological sign of lens development is the formation of the lens placode. This structure is a thickened region of the head surface ectoderm immediately adjacent to the optic vesicle. The thickening of the lens placode occurs only after the optic vesicle has evaginated from the forebrain and made close contact with the surface ectoderm. The interaction between the optic vesicle and presumptive lens ectoderm is extremely strong and mediated by cytoplasmic extensions between the two tissue layers (McAvoy, 1980). Subsequently there is a coordinated invagination of the lens placode and outer layer of the optic vesicle. This results in the formation of the lens pit and the

Abbreviations used in this paper: bmp, bone morphogenetic protein; EE, ectoderm enhancer region; fgf, fibroblast growth factor; MAPK, MAP kinase.

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optic cup. At this stage, the epithelium of the lens pit closest to the presumptive retina has begun to thicken in the first steps of lens fiber cell differentiation. In addition, the outer layer of the optic cup has folded back against the proximal layer of the optic vesicle to form the adjacent epithelia of the RPE and presumptive retina. Closure of the lens pit at the surface ectoderm results in formation of the lens vesicle. Thickening of the posterior epithelium of the lens vesicle continues as fiber cells differentiate and extend towards the lens epithelium. Clearly, the nature of eye development and, in particular, the close association between the optic vesicle and lens placode has suggested that the optic vesicle might have a critical role in lens induction.

Lens induction is a multi-step process

In recent times, embryological manipulations have become more sophisticated than the early efforts pioneered by Spemann, and have defined multiple stages in the lens induction process. In particular, the group of Robert Grainger has been able to define at least four stages in lens development (Grainger *et al.*, 1992; Grainger, 1996). These correspond to a period of lens-forming competence (Servetnick and Grainger, 1991) in the mid/late gastrula ectoderm, the acquisition of a lens-forming bias throughout the head ectoderm during neurulation (Grainger *et al.*, 1997), specification of lens cell fate towards the end of neurulation, and differentiation, an aspect of lens development which continues throughout life (Grainger, 1992). While the definition of these stages are described in more detail in another chapter of this volume (Sullivan *et al.,* 2004) it is worth noting that one of the current challenges is to mesh the embryological and molecular genetic definitions of lens induction.

Induction genes become differentiation genes

While this review emphasizes the early steps in lens development, a large body of experimental work has shown us that many of the developmental pathways critical for lens induction are also critical for later stages of lens development. For example, the Fgf signaling pathway is involved both in inductive signaling (see below) and in the regulation of lens fiber cell differentiation (McAvoy *et al.*, 1999). It is also the case that some transcription factors critical for induction have later roles in differentiation. For example, in the mouse, Pax6 is required for lens induction, but also activates *αB-crystallin* later in lens development (Piatigorsky, 1998). There are also emerging links between induction genes like *Pax6*, transcription factors that are genetically downstream (like the Mafs and *Prox1*) and the regulation of differentiation genes like the crystallins (Cui *et al.*, 2004). Increasingly therefore, we will have the opportunity to draw a continuous developmental pathway from lens induction to lens function.

Pax6 gene function and regulation

The Pax6 *gene*

The role of the *Pax6* gene in eye development has been thoroughly investigated over a number of years. Pax6 function is critical for eye development as indicated by the absence of eyes in humans, mice, and flies that carry loss of function mutations in *Pax6* (Hill *et al.*, 1991; Glaser *et al.*, 1992; Jordan *et al.*, 1992; Quiring *et al.*, 1994). In vertebrates, *Pax6* is expressed in a variety of tissues including those that participate in the early phases of eye development. *Pax6* is first expressed in the anterior neural plate region that will eventually give rise to the retina (Grindley *et al.*, 1995). Somewhat later, *Pax6* is also expressed in a broad region of the head surface ectoderm including the domain that gives rise to the lens. While *Pax6* expression is retained in the presumptive retina and the retinal pigmented epithelium as the optic cup develops, the domain of expression in the surface ectoderm becomes restricted to the region of the lens placode and the surface ectoderm immediately surrounding. The expression level of *Pax6* increases in the lens placode after close contact with the optic vesicle (Grindley *et al.*, 1995).

Pax6 loss-of-function mutations have indicated a critical role for Pax6 in eye development, but in addition, gain-of-function experiments have revealed a remarkable activity in precipitating all of the events required for development of this complex structure. In the first experiment of this type, the Gehring lab demonstrated that misexpression of *Drosophila Pax6* (the *eyeless* gene) could result in the formation of ectopic eyes in multiple locations (Halder *et al.*, 1995). It was shown that this activity was evolutionarily conserved in that both fly and mouse *Pax6* could induce ectopic eye formation. This observation was a very powerful argument to suggest that *Pax6* occupied the apex of a genetic hierarchy that regulates eye development. In the holometabolous insects, the situation turned out to be slightly more complex in that there are two orthologues of *Pax6* called *eyeless* and *twin of eyeless* (Czerny *et al.*, 1999). These two genes are very closely related and both have the ability to induce ectopic eyes. This has suggested an adaptation in which this group of organisms has duplicated a primordial *Pax6* gene and subsequently the two genes have shared duty. A similar situation is also observed in Zebrafish where the duplicated *Pax6.1* and *Pax6.2* genes are expressed in distinct but overlapping domains (Nornes *et al.*, 1998). In this case, gene duplication is probably a consequence of a much larger partial genome duplication.

Experiments performed in *Xenopus leavis* have shown that *Pax6* can induce ectopic eyes in a vertebrate (Chow *et al.*, 1999). Such ectopic eyes form only in the head region, perhaps because there is a restricted domain of eye competent ectoderm in the early *Xenopus* embryo (Servetnick and Grainger, 1991). Remarkably, ectopic eyes contain all the mature cell types that might be expected, including a selection of appropriately laminated mature retinal neurons, cells of the retinal pigment epithelium as well as those of the lens. The importance of *Pax6* for the induction of ectopic eye structures has been confirmed by experiments in which different combinations of presumptive eye region genes were misexpressed in *Xenopus* (Zuber *et al.*, 2003). The ability of Pax6 to induce ectopic eyes in both invertebrates and vertebrates has suggested an evolutionarily conserved function at the apex of a genetic hierarchy controlling eye development (Callaerts *et al.*, 1997).

Pax6 *is necessary and sufficient for lens development*

A number of experiments indicate that *Pax6* has a critical, autonomous function in development of the lens. In the first of these, tissue recombination experiments were performed using wild type and Pax6 mutant (*small eye*) optic vesicle and presumptive lens ectoderm (Fujiwara *et al.*, 1994). It was shown that a combination of wild type optic vesicle and wild type presumptive lens would, as expected, result in lens formation. Similarly, recombining small eye optic vesicle and wild type presumptive lens

ectoderm also resulted in lens formation. By contrast, recombining wild type optic vesicle with *small eye* presumptive lens ectoderm failed to give lens formation (Fujiwara *et al.*, 1994). This indicated that lens development required *Pax6* function in the surface ectoderm but not the optic vesicle.

A similar conclusion can be drawn from experiments in which wild type/*small eye* chimeric mice were generated (Quinn *et al.*, 1996; Collinson *et al.*, 2000). In this circumstance it was observed that *small eye* mutant cells did not contribute to the lens placode at E9.5 or subsequently to the differentiating lens. The selective aggregation of wild type cells in these chimeric mouse experiments also suggested that Pax6 may have an important role in regulating the adhesive interactions. In the most direct demonstration of the requirement for *Pax6* in lens development a floxed allele of the *Pax6* gene was conditionally deleted in the lens placode (Ashery-Padan *et al.*, 2000). This experimental strategy resulted in a failure of lens development beyond the placode stage. Interestingly, although the retina was misshapen, retinal neuron differentiation and lamination occurred normally.

Gain-of-function experiments have also indicated a central role for *Pax6* in lens development. If the experimental conditions used to generate ectopic eyes in *Xenopus laevis* are modified slightly, the result is induction of ectopic lenses at high-frequency (Altmann *et al.*, 1997; Chow *et al.*, 1999). These lenses express lens-specific markers such as β-crystallin and many have the polarized morphology of a normal lens. In some experiments, remarkably, ectopic lenses are perfect mimics of the endogenous lens in size, morphology and marker expression. Interestingly, lineage tracing studies indicate that these ectopic lenses are a results of the cell autonomous activity of Pax6 (Altmann *et al.*, 1997; Chow *et al.*, 1999). These lenses also form in the absence of any retinal tissue. Combined, gain- and loss-of-function experiments indicate that *Pax6* is necessary and, in the context of the *Xenopus* embryo, sufficient for development of the lens.

Pax6 *expression in the lens lineage is controlled by at least two enhancers*

The important role of *Pax6* in many developmental processes, as well as its complex expression pattern, has motivated analysis of transcriptional regulation. The identification of transcription control elements in *Pax6* has rested on the simple strategy of sequence alignment to identify conserved regions and functional assessment using transgenic reporter constructs (Williams *et al.*, 1998; Kammandel *et al.*, 1999; Xu *et al.*, 1999). If long-range sequence alignments are generated to compare the mouse and human *Pax6* genes, it immediately becomes apparent that transcriptional regulation of this gene is complex. Indeed, there are upwards of 40 highly conserved, non-coding sequence regions in Pax6 (see trafac.cchmc.org).

A number of these conserved regions have transcriptional enhancer activity. Approximately 3.5kb upstream of the P0 promoter in *Pax6*, there is a highly conserved region of 340 base pairs that has activity as a lens lineage enhancer (Williams *et al.*, 1998; Kammandel *et al.*, 1999; Xu *et al.*, 1999). This region has been designated *EE* for ectoderm enhancer. When incorporated into a reporter transgene, the *EE* gives expression in the developing lens placode and adjacent ectoderm starting at E8.75 in the mouse (Williams *et al.*, 1998; Kammandel *et al.*, 1999; Xu *et al.*, 1999). Subsequently, the *EE* has activity in the entire lens vesicle but

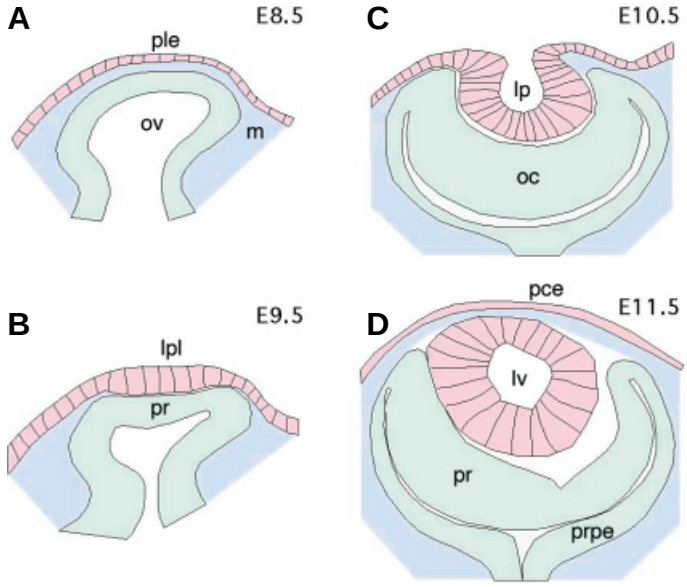

Fig. 1. Morphogenesis of the lens. (A-D) *show the stages of lens development in the mouse from E8.5 to E11.5 in daily intervals. The three tissue layers involved in eye development include the surface ectoderm (red) the mesenchyme (blue) and the neuroepithelium of the optic vesicle (green). ple – presumptive lens ectoderm; ov, optic vesicle; m, mesenchyme; lpl, lens placode; pr, presumptive retina; lp, lens pit; oc, optic cup; pce, presumptive corneal ectoderm; lv, lens vesicle; prpe, presumptive pigmented retinal epithelium.*

beyond E11.5 is restricted to the lens epithelium and the epithelia of the lacrimal gland and conjunctiva (Williams *et al.*, 1998; Kammandel *et al.*, 1999; Xu *et al.*, 1999).

The lens lineage enhancer designated *SIMO* was identified in a more roundabout and interesting way. The van Heyningen group was examining the nature of mutations in *Aniridia* patients and found a translocation break point situated 3' to the last exon of *Pax6* (Kleinjan *et al.*, 2001). Upon further investigation it was shown there were several highly conserved regions of sequence just distal to the translocation breakpoint. Testing of these regions in transgene reporter constructs indicated that one of them, the *SIMO* element, had activity in the lens lineage (Kleinjan *et al.*, 2001). While this has not been tested directly, there is a good possibility that *SIMO* and *EE* work cooperatively to give the full breadth and level of *Pax6* expression in the presumptive lens region (Treisman and Lang, 2002; Lang and McAvoy, 2003).

There are two phases of Pax6 *expression*

Straightforward expression analysis for *Pax6* in *Pax6*^{Sey1Neu} homozygotes has indicated that there are two distinct phases of *Pax6* expression in the presumptive lens ectoderm of the mouse embryo (Grindley *et al.*, 1995). The *Pax6*^{Sey1Neu} allele is a point mutation that still permits gene transcription but does not permit production of functional protein (Grindley *et al.*, 1997). An assessment of *Pax6* gene expression on this mutant background indicates that expression of *Pax6* in the head surface ectoderm is retained but that expression in the lens placode (designated *Pax6*^{placode}) is lost (Grindley *et al.*, 1995). This indicates that there are two distinct phases of *Pax6* expression and that the later

expression phase (designated *Pax6*$^{pre\text{-}placode}$) is dependent on the first phase of expression. These two phases of *Pax6* expression constitute the first steps in a genetic pathway describing lens induction in the mouse (Fig. 2). For the reasons indicated above, it is likely that *EE* and *SIMO* work in concert to give *Pax6*^{placode}.

The EE is required for normal development of the lens

To assess the role of the *EE* in development of the lens, gene targeting experiments were carried out in which the entire *EE* was deleted. Homozygous *EE* deletion mice showed abnormal lens development (Dimanlig *et al.*, 2001). The lens placode was thinner than normal in mutant mice though this occurred primarily on the nasal side of the structure. Proliferation of presumptive lens cells was also diminished, and, at all stages of development lens structures were smaller than in wild type mice. Consistent with the Peters' anomaly observed in some cases of *Pax6* heterozygosity, *EE* homozygous mice showed a persistent lens stalk and a failure of the lens vesicle to separate from the surface ectoderm. At E9.5 interestingly, Pax6 immunoreactivity could still be detected in the lens placode though it was very much reduced on the nasal side. When combined with reporter construct analysis this suggests that the function of the *EE* is to promote *Pax6* expression primarily on the nasal side of the lens placode. The observation that deletion of the *EE* does not block lens development completely is consistent with the idea that at least one additional enhancer, perhaps the *SIMO* element (Kleinjan *et al.*, 2001), is required for the full level of *Pax6* expression in the lens placode (Fig. 2). It will be very interesting to compare the phenotype of a *SIMO* enhancer deletion mouse with that of the EE deletion.

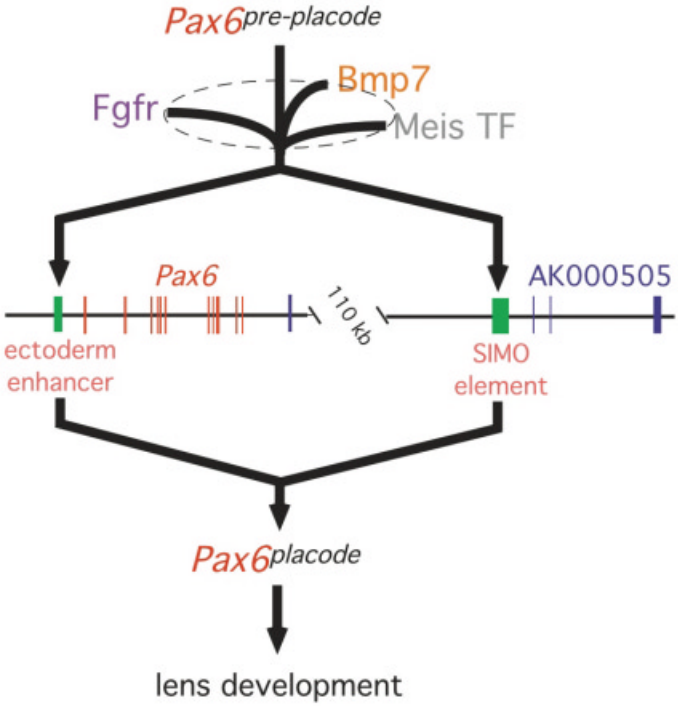

Fig. 2. Inductive signals regulate *Pax6* expression in presumptive lens: a genetic model. *The arrows indicate genetic interactions except in the case of the Meis transcription factor-enhancer interactions. At the apex of the hierarchy is the pre-placodal phase of* Pax6 *expression* (Pax6$^{pre\text{-}placode}$). *It is understood that the later phase of* Pax6 *expression in the lens placode (*Pax6^{placode}*) is dependent upon earlier activity of Pax6.* Pax6^{placode} *is apparently regulated by at least two enhancers that are represented by the green vertical bars on the schematic of the* Pax6 *gene (exons, red vertical bars). One is the so-called ectoderm enhancer and the other the SIMO element that is located in the final intron of the adjacent gene, AK000505, that is transcribed in the opposite direction to* Pax6. *The Meis transcription factors (Meis TF) are likely to regulate the expression of Pax6 directly by binding to the ectoderm enhancer, and possibly to the SIMO element. Since both placodal Pax6 is reduced in mouse embryos that express a dominant-negative Fgf receptor in the lens lineage it is likely that* Pax6^{placode} *is genetically downstream of Fgf receptor activity. Given that an even lower level of Pax6 can be recorded in embryos that express the dominant-negative Fgf receptor* and *have half the normal level of Bmp7, we can suggest that Fgf receptor and* Bmp7 *signaling cooperate to maintain the placodal phase of* Pax6 *expression. Previous analysis has shown that the early phase of* Pax6 *expression is unaffected in the* Bmp7 *null mice and thus, FGF and* Bmp7 *signaling must converge on the pathway downstream of* Pax6$^{pre\text{-}placode}$.

Pax6 lens enhancers may be regulated by Meis *transcription factors*

The mammalian Meis family transcription factors have recently been implicated in early development of the lens. The Meis family are TALE-class homeodomain transcription factors (Burglin, 1997) and homologues of the *homothorax* gene of *Drosophila* (Bessa *et al.*, 2002). Meis binding sites have been identified in the ectoderm enhancer of *Pax6* (Zhang *et al.*, 2002) and this has suggested that *Pax6* may be regulated *in vivo* by this class of transcription factor. The experimental evidence for this suggestion includes, (1) immuno-identification of Meis in a complex with *EE* probes in mobility shift assays, (2) transgenic mice showing that the activity of *EE* is dependent upon Meis binding sites, (3) a genetic interaction between a *Meis2* transgene and the *Pax6*^{Sey1Neu} allele, and (4) the demonstration that suppressor forms of Meis1 can down-regulate *Pax6* expression when transiently expressed in the lens placode (Zhang *et al.*, 2002). More recently, it has been shown that a *Meis1* null mouse has defects in the lens, albeit mild and at a late stage of development (Hisa *et al.*, 2004).

Given the likelihood that at least two enhancers regulate *Pax6* expression in the lens placode (Fig. 2), the observed absolute regulation of *Pax6* by Meis transcription factors (Zhang *et al.*, 2002) requires that they function at both the *EE* and *SIMO* elements (Lang and McAvoy, 2003). Identification of Meis binding sites in the *SIMO* element is of great interest, but further experimentation will be required to precisely define the molecular genetics of this interaction. In particular, it will be very interesting to further analyze the eye phenotypes that arise in mice that are null or conditionally targeted for the combinations of the *Meis* genes (Zhang *et al.*, 2002). Since expression of the Meis genes is independent of *Pax6*, they are best incorporated into the model for genetic regulation of lens induction as an input upstream of both the *EE* and *SIMO* elements (Fig. 2).

Signaling pathways involved in lens induction

Bone morphogenetic proteins have important roles in lens development

The bone-morphogenetic proteins Bmp4 and Bmp7 are both proposed to have important roles in early development of the lens.

Bmp4 and *Bmp7* are expressed in the early eye tissues and have overlapping expression patterns (Dudley and Robertson, 1997; Furuta and Hogan, 1998; Wawersik *et al.*, 1999). Bmp7 is expressed in the presumptive lens ectoderm and presumptive RPE as well as the dorsal optic vesicle. Expression of *Bmp7* in the presumptive lens tissue ceases at about E11.5. Deletion of the *Bmp7* gene results in variably penetrant eye development defects that range from mild microophthalmia to anophthalmia (Dudley *et al.*, 1995; Luo *et al.*, 1995). The lens placode fails to form in severely affected animals and interestingly, the expression of *Pax6* in the lens placode is lost (Wawersik *et al.*, 1999). This has indicated that *Bmp7* is genetically upstream of the enhancers that control *Pax6* expression in the lens placode (Fig. 2).

While homozygous *Bmp4* mutant embryos do not survive past E10.5, lens formation from the presumptive lens ectoderm of null mutants can be rescued by recombining them with wild type optic vesicles in explant culture (Furuta and Hogan, 1998). This indicates that *Bmp4* expression in the presumptive lens ectoderm is not required for lens development. Explantation of eye rudiments from *Bmp4* null mice in the presence of recombinant Bmp4 also resulted in rescue of lens formation (Furuta and Hogan, 1998). However, recombinant Bmp4 was not sufficient to rescue lens formation when presumptive lens ectoderm from null mice was explanted. Combined, these data suggested that Bmp4 had to act in concert with at least one additional signal if lens development was to proceed. Interestingly, the absence of Bmp4 does not affect expression of *Pax6*, but does prevent the normal up-regulation of *Sox2*, an Sry-related transcription factor normally expressed in both presumptive lens and retina (see below). Combined with other information, this has suggested that Bmp4 and Sox2 may function in a parallel branch of the lens induction pathway (Fig. 2). This also suggests, despite the overlapping expression patterns, that Bmp4 and Bmp7 may have non-redundant functions in early eye development. The demonstration that Pax6 and Sox2 form a complex in the regulation of crystallin genes (Kamachi *et al.*, 2001) has indicated that these pathways may combine to regulate later steps in lens fiber cell differentiation.

FGF receptor signaling is required for lens induction

The Fgf signaling pathway has important functions at multiple stages of eye development. For example, Fgf signaling is both necessary and sufficient for differentiation of lens fiber cells according to loss- and gain-of-function experiments (McAvoy *et al.*, 1991; McAvoy *et al.*, 1999; Lang and McAvoy, 2003). More recently, we have understood that Fgf receptor activity has an important role to play in the inductive phases of lens development.

Explantation of eye primordia in the presence of a small-molecule inhibitor of the Fgf receptor kinase activity results in reduced levels of Pax6 in the lens placode (Faber *et al.*, 2001). Given the critical role of Pax6 in lens development, this has suggested that Fgf receptor activity lies upstream and is a lens induction factor. When a dominant-negative FgfR1 is expressed in the presumptive lens, early defects in lens placode formation and lens pit invagination are apparent. Importantly, Pax6 expression levels were reduced (Faber *et al.*, 2001). Genetic evidence of a lens induction function for Fgf receptor activity was pursued by determining whether Bmp7, an established lens inducer (Wawersik *et al.*, 1999) might cooperate with Fgf receptor activity. Indeed, crosses between *Bmp7* null (Dudley *et al.*, 1995) and *Tfr7/Tfr7* mice (transgene homozygotes expressing a dominant-negative FgfR1 in the lens placode) produced compound genotype mice with more severe lens development defects (Faber *et al.*, 2001). In particular, *Tfr7/Tfr7*, *Bmp7*$^{+/-}$ mice showed a very small lens pit and failed to separate and close the lens vesicle. In addition, Pax6 expression levels in the lens placode were at the lowest levels in *Tfr7/Tfr7*, *Bmp7*$^{+/-}$ mice, and at intermediate levels (compared with wild-type) in *Tfr7/Tfr7* mice (Faber *et al.*, 2001). This indicated that Bmp7 and Fgf receptor signaling converge upstream to give a full level of Pax6 expression in the placodal phase (Fig. 2). Though there have been some good candidates (McWhirter *et al.*, 1997; Lovicu and Overbeek, 1998; Vogel-Hopker *et al.*, 2000), the identity of the Fgf receptor ligands required for lens induction remains unclear.

Transcription factors genetically downstream of *Pax6*

FoxE3

Foxe3 is a transcription factor of the *forkhead* class that in mice has a very limited expression range in the developing neural tube and lens (Blixt *et al.*, 2000; Brownell *et al.*, 2000). In humans, mutations in *FOXE3* are associated with anterior segment ocular dysgenesis (Semina *et al.*, 2001). In the mouse, expression of *FoxE3* begins at E8.75 in the presumptive lens ectoderm (Brownell *et al.*, 2000) and represents a sub-domain of *Pax6*-positive presumptive lens ectoderm. This spatial relationship between *FoxE3* and *Pax6* expression domains continues through E9.5 (when the lens placode has formed), and E10.5 when the lens pit is invaginating. The *dysgenetic lens (dyl)* mouse carries mutations in *FoxE3* (Blixt *et al.*, 2000; Brownell *et al.*, 2000). This results in a failure of lens vesicle closure and separation, as well as reduced proliferation in lens epithelial cells (Blixt *et al.*, 2000; Brownell *et al.*, 2000). The expanded expression domain of Prox1 in the lens epithelium of the *FoxE3* $^{dyl/dyl}$ mouse (Blixt *et al.*, 2000) has suggested that in this location, *FoxE3* is a suppressor of *Prox1* (Fig. 3).

In the *Pax6* $^{Sey/Sey}$ background it has been shown that *FoxE3* expression is absent, (Brownell *et al.*, 2000) thus suggesting that *FoxE3* is genetically downstream of *Pax6* (Fig. 3) (Brownell *et al.*, 2000). The phenotypic resemblance of the *dyl* mouse with those in which the *Pax6* upstream ectoderm enhancer had been deleted (Dimanlig *et al.*, 2001) prompted an examination of a possible genetic relationship. *In situ* hybridizations for *FoxE3* in *Pax6*$^{\Delta EE/\Delta EE}$ embryos (that carry a homozygous deletion of the ectoderm enhancer) revealed that *FoxE3* expression was undetectable (Dimanlig *et al.*, 2001). This indicated that *FoxE3* is located downstream of the placodal phase of *Pax6* expression in a genetic pathway regulating lens development (Fig. 2).

Sox2

It is likely that Bmp4 functions in lens induction in a pathway that involves the transcriptional regulator Sox2. Sox2 is an HMG box transcription factor related to the sex-determining factor SRY (Kamachi *et al.,* 1995). Sox2 and family members Sox1 and Sox3 have been implicated in lens development through their expression patterns and through their regulation of crystallin genes (Kamachi *et al.,* 1995; Kamachi *et al.,* 1998). In particular, Sox2 is known to regulate δ-crystallin expression in the chick in a complex with Pax6 (Kamachi *et al.,* 2001). Thus, the observation that *Sox2* expression

in the lens lineage is not up-regulated in the usual way in *Bmp4* null embryos suggests the appealing model that Bmp4 stimulates *Sox2* expression in preparation for crystallin gene regulation by a Sox2-Pax6 complex (Fig. 3). When combined with the observation that *Sox2* is also not up-regulated in the usual way in *Pax6*$^{Sey/Sey}$ embryos (Furuta and Hogan, 1998), we can suggest that *Sox2* is downstream of *Pax6*$^{pre\text{-}placode}$ and participates with Bmp4 in the proposed parallel pathway (Fig. 3). It is likely that currently unpublished work examining the requirement for different Bmp receptors in lens induction will help refine our understanding of Bmp4 involvement in lens induction.

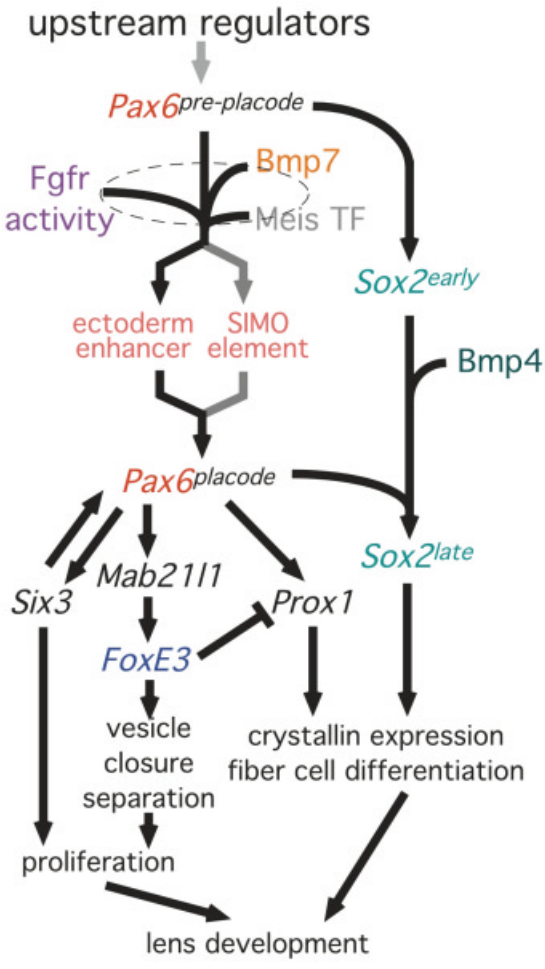

Fig. 3. An expanded model for the genetic pathways regulating lens induction. *See Fig. 2 for description of the central elements of this model. Based on the absence of* FoxE3 *expression in mice carrying a deletion of the Pax6 ectoderm enhancer (*Pax6$^{\Delta EE/\Delta EE}$) FoxE3 *is downstream of* Pax6^{placode}. *The similar phenotypes (reduced lens lineage proliferation and lens vesicle closure and separation failure) of the* dysgenetic lens (FoxE3) *and* Pax6$^{\Delta EE/\Delta EE}$ *mutant mice argue that* FoxE3 *is upstream of these cellular responses. Similarly, the loss of* FoxE3 *expression in* Mab21l1 *mutant mice and the loss of* Mab21l1 *expression in* Pax6$^{S\ ey/Sey}$ *embryos suggests the* Pax6-Mab21l1-FoxE3 *gene order indicated. The homeodomain transcription factor* Six3 *lies genetically downstream of* Pax6^{placode} *as mice that do not express placodal* Pax6 *also do not express placodal* Six3. *This is also true for* Prox1. *Since* FoxE3$^{dyl/dyl}$ *mice show an expansion of the* Prox1 *expression domain in the lens epithelium, this suggests that FoxE3 normally suppresses* Prox1 *at later stages of lens development. In* Pax6$^{Sey/Sey}$ *embryos,* Sox2 *expression is not up-regulated in the lens placode suggesting that the late phase of Sox2 expression is dependent on Pax6. Similarly, since* Sox2 *(but not* Pax6*) expression is not up-regulated in the lens placode of* Bmp4 *null mice, Bmp4 signaling likely contributes to the pathway between* Sox2^{early} *and* Sox2^{late}.

Mab21l1

The *Mab21l1* gene from mouse is the orthologue of *mab-21* from *C. elegans* (Mariani *et al.,* 1998; Mariani *et al.,* 1999; Yamada *et al.,* 2003). Interestingly, in the worm, *mab-21* is in the same genetic pathway as *mab-18*, the orthologue of vertebrate *Pax6* (Zhang and Emmons, 1995). Supporting the idea that Pax6 and *Mab21l1* might participate in the same developmental processes is the observation that like *Pax6*, *Mab21l1* is expressed in the eye primordium (both presumptive lens and retina) from an early stage (Mariani *et al.,* 1998; Yamada *et al.,* 2003). In particular, *Mab21l1* expression is up-regulated at approximately E9.5, thus suggesting it might be responsive to lens induction signals (Yamada *et al.,* 2003).

Recently, generation of a *Mab21l1* null mouse has suggested that *Mab21l1* has an important functional role in lens development. The *Mab21l1* null mouse displays lens development defects (in the form of lens placode invagination failure) from E9.5 and this results in a dramatic microophthalmia (Yamada *et al.,* 2003). Examination of *Mab21l1* expression in *Pax6*$^{Sey/Sey}$ mice indicated a failure of the normal up-regulation at E9.5. This suggested that *Mab21l1* was genetically downstream of *Pax6*. Detection of *FoxE3* and Pax6 expression in the *Mab21l1* null mutant indicated that Pax6 expression was normal, but that *FoxE3* expression was absent at E9.5 (Yamada *et al.,* 2003). Combined, these data suggest that *Mab21l1* lies between the placodal phase of *Pax6* expression and *FoxE3* in the model for genetic regulation of lens induction (Fig. 2). This analysis suggests that there is a direct analogy between the *mab-21,—mab-18* pathway in the worm and the *Pax6*, *Mab21l1* pathway in the mouse (Yamada *et al.,* 2003).

Six3

Six3 is a member of the six-homeodomain family of which *Drosophila sine oculis* is the founding member (Cheyette *et al.,* 1994; Serikaku and O'Tousa, 1994). *Six3* was first implicated in lens development by its expression pattern. In mice, *Six3* is expressed in the lens epithelium during differentiation stages but is first expressed in the lens placode (Oliver *et al.,* 1995). In *Medaka*, the expression pattern is somewhat distinct as it is present in the presumptive lens ectoderm but is down-regulated in the lens placode prior to lens differentiation (Loosli *et al.,* 1998). As in the mouse, chick *Six3* is expressed in the presumptive lens ectoderm overlying the optic vesicles but persists in the lens placode and is later localized to the lens epithelium (Bovolenta *et al.,* 1998).

Functional evidence for *Six3* involvement in lens induction came initially from misexpression studies that resulted in the formation of ectopic lenses in *Medaka* (Oliver *et al.,* 1996). In contrast to *Pax6*-induced ectopic lens formation in *Xenopus*, Six3-induced ectopic lenses appeared to arise as a result of the transformation of the otic vesicle (Oliver *et al.,* 1996). In addition, lineage tracing experiments revealed that *Six3* could direct ectopic lens formation in a cell non-autonomous manner. This led the authors to speculate that *Six3* misexpression may induce a soluble factor that changed the bias of the otic placode towards a lens fate (Oliver *et al.,* 1996).

There is now evidence to indicate that *Six3* and *Pax6* mutually activate at the transcriptional level (Ashery-Padan *et al.,* 2000; Goudreau *et al.,* 2002). As mentioned above, lens placode-specific conditional deletion of a floxed allele of *Pax6* results in lens development failure (Ashery-Padan *et al.,* 2000). An assessment of marker gene expression in these animals indicates that *Six3* expression is lost. Furthermore, it has been shown that an α-crystallin promoter-*Six3* transgene can up-regulate *Pax6* expression and rescue the lens defects that arise in *Pax6*$^{lacz/+}$ mice (Goudreau *et al.,* 2002). When combined with biochemical experiments showing that Six3 binding sites are located in the Pax6 ectoderm enhancer and that Pax6 binding sites are found in the Six3 gene (Goudreau *et al.,* 2002), this has suggested that Pax6 and Six3 can mutually transactivate. This information can be incorporated into the genetic model for mouse lens induction (Fig. 3). A direct assessment of the role of *Six3* in lens development, and its ability to feedback positively on *Pax6*, will come from conditional deletion of a floxed allele in the lens placode.

Prox1

Prox1 is a vertebrate homologue of *Drosophila prospero* that encodes a divergent homeodomain protein (Tomarev *et al.,* 1998). It is expressed in the presumptive lens and retina in the mouse from an early stage of development. Generation of *Prox1* null allele in the mouse revealed that this transcription factor is essential for the differentiation of lens fiber cells (Wigle *et al.,* 1999). Interestingly, there is now evidence from both *Drosophila* (Knoblich *et al.,* 1995; Spana and Doe, 1995) and mouse (Duncan *et al.,* 2002) that prospero and Prox1 change their sub-cellular localization at the developmental stage when their activity is critical.

Two analyses allow us to place Prox1 within the model for genetic regulation of lens induction. First, it has been shown that when Pax6 is conditionally deleted from the lens placode, Prox1 expression in this location is lost (Ashery-Padan *et al.,* 2000). Furthermore, In the *dysgenetic lens* mouse that carries a mutation in *FoxE3*, Prox1 expression in the lens epithelium is up-regulated. Combined, these data suggest that *Prox1* lies genetically downstream of *Pax6*^{placode} and that *FoxE3* is normally responsible for suppressing *Prox1* expression (Fig. 3). It remains to be determined whether these interactions represent many steps or whether there is the possibility of direct transcriptional regulation.

Speculation: is *Pax6* auto-regulation the result of MAPK-mediated activation?

Genetic analysis has indicated that *Pax6* autoregulates. Specifically, we understand that the placodal phase of *Pax6* expression is dependent upon functional Pax6 protein in the head ectoderm ((Grindley *et al.,* 1995); Figs. 2,3). Recent analysis has suggested that this genetic relationship may be the result of a direct regulatory interaction in which *Pax6* binds to the *EE* and activates *Pax6* (Aota *et al.,* 2003). The evidence for this comes from mobility-shift assays in which, through its *paired* domain, Pax6 can bind specifically to EE sequences and transfection experiments in which an EE-dependent expression construct is activated by Pax6 (Aota *et al.,* 2003). However, if *Pax6*^{placode} is directly regulated by *Pax6*$^{pre\text{-}placode}$, a problem arises in explaining how the two phases of expression are ever distinct. Two obvious possibilities are that the level of expression distinguishes these two phases or that another input is required. For example, there are no doubt many transcription factors that could function cooperatively with Pax6 at the EE (possibilities are Meis proteins (Zhang *et al.,* 2002), Sox2 or 3 (Aota *et al.,* 2003) or Six3 (Goudreau *et al.,* 2002)).

However, another mechanistic possibility has been raised by analysis indicating that Pax6 can be activated post-translationally (Mikkola *et al.,* 1999). The transactivation domain of Zebrafish Pax6 has a series of potential phosphorylation sites. Of these, serine 413 (Ser^{413}) is evolutionarily conserved from sea urchin to man. Interestingly, Ser^{413} can be phosphorylated *in vitro* by the mitogen activated protein kinases (MAPKs), extracellular-signal regulated kinase (ERK) and p38 kinase but not Jun N-terminal kinase (JNK). Mutation of Ser^{413} to appear similar to a phosphorylated residue enhances the transactivation potential of Pax6. Similarly, mutation of Ser^{413} to a residue that is the antithesis of a phophorylated side chain limits transactivation (Mikkola *et al.,* 1999). Finally, in NIH3T3 cells, it can be shown that Ser^{413} phosphorylation occurs in response to serum stimulation, a condition that activates the MAP kinases, and that this phosphorylation can by prevented by the MAP kinase inhibitor PD 98059 (Mikkola *et al.,* 1999).

It has clearly been shown that the Fgf signaling pathway is required for lens induction. The Fgf pathway includes, as one of its signaling arms, the Ras-Raf-MAP kinase pathway (Goldfarb, 2001). When combined with the observation that MAP kinases can enhance Pax6 transctivation, we can propose that the placodal phase of *Pax6* expression is the result of Fgf pathway-MAP kinase mediated activation of Pax6 that is available from *Pax6*$^{pre\text{-}placode}$ (Fig. 4). This proposal is entirely consistent with genetic analysis

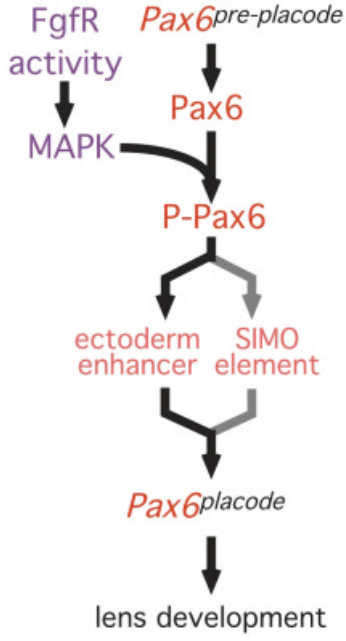

Fig. 4. Is Pax6 phosphorylation a step in lens induction? *Phosphorylation of Pax6 by MAP kinase (MAPK) enhances transactivation. Since MAP kinases are downstream of Fgf receptors (FgfR), this raises the possibility that Pax6 phosphorylation is an important step in Pax6 auto-regulation in the lens placode and therefore, lens development.*

suggesting that the Pax6 and Fgf pathways converge upstream of *Pax6 placode*. While this can explain all of the currently available data, there is clearly much experimental work required for a close examination of this potential developmental mechanism.

References

ALTMANN, C.R., CHOW, R.L., LANG, R.A. and HEMMATI-BRIVANLOU, A. (1997). Lens induction by Pax-6 in Xenopus laevis. *Dev Biol* 185: 119-23.

AOTA, S., NAKAJIMA, N., SAKAMOTO, R., WATANABE, S., IBARAKI, N. and OKAZAKI, K. (2003). Pax6 autoregulation mediated by direct interaction of Pax6 protein with the head surface ectoderm-specific enhancer of the mouse Pax6 gene. *Dev Biol* 257: 1-13.

ASHERY-PADAN, R., MARQUARDT, T., ZHOU, X. and GRUSS, P. (2000). Pax6 activity in the lens primordium is required for lens formation and for correct placement of a single retina in the eye. *Genes Dev* 14: 2701-11.

BESSA, J., GEBELEIN, B., PICHAUD, F., CASARES, F. and MANN, R.S. (2002). Combinatorial control of Drosophila eye development by Eyeless, Homothorax, and Teashirt. *Genes Dev* 16: 2415-27.

BLIXT, A., MAHLAPUU, M., AITOLA, M., PELTO-HUIKKO, M., ENERBACK, S. and CARLSSON, P. (2000). A forkhead gene, FoxE3, is essential for lens epithelial proliferation and closure of the lens vesicle. *Genes Dev* 14: 245-54.

BOVOLENTA, P., MALLAMACI, A., PUELLES, L. and BONCINELLI, E. (1998). Expression pattern of cSix3, a member of the Six/sine oculis family of transcription factors. *Mech Dev* 70: 201-3.

BROWNELL, I., DIRKSEN, M. and JAMRICH, M. (2000). Forkhead Foxe3 maps to the dysgenetic lens locus and is critical in lens development and differentiation. *Genesis* 27: 81-93.

BURGLIN, T.R. (1997). Analysis of TALE superclass homeobox genes (MEIS, PBC, KNOX, Iroquois, TGIF) reveals a novel domain conserved between plants and animals. *Nucleic Acids Res* 25: 4173-80.

CALLAERTS, P., HALDER, G. and GEHRING, W.J. (1997). PAX-6 in development and evolution. *Annu Rev Neurosci* 20: 483-532.

CHEYETTE, B.N., GREEN, P.J., MARTIN, K., GARREN, H., HARTENSTEIN, V. and ZIPURSKY, S.L. (1994). The Drosophila sine oculis locus encodes a homeodomain-containing protein required for the development of the entire visual system. *Neuron* 12: 977-96.

CHOW, R.L., ALTMANN, C.R., LANG, R.A. and HEMMATI-BRIVANLOU, A. (1999). Pax6 induces ectopic eyes in a vertebrate. *Development* 126: 4213-4222.

COLLINSON, J.M., HILL, R.E. and WEST, J.D. (2000). Different roles for Pax6 in the optic vesicle and facial epithelium mediate early morphogenesis of the murine eye. *Development* 127: 945-56.

CUI, W., TOMAREV, S.I., PIATIGORSKY, J., CHEPELINSKY, A.B. and DUNCAN, M.K. (2004). Mafs, Prox1, and Pax6 can regulate chicken betaB1-crystallin gene expression. *J Biol Chem* 279: 11088-95.

CZERNY, T., HALDER, G., KLOTER, U., SOUABNI, A., GEHRING, W.J. and BUSSLINGER, M. (1999). twin of eyeless, a second Pax-6 gene of Drosophila, acts upstream of eyeless in the control of eye development. *Mol Cell* 3: 297-307.

DIMANLIG, P.V., FABER, S.C., AUERBACH, W., MAKARENKOVA, H.P. and LANG, R.A. (2001). The upstream ectoderm enhancer in Pax6 has an important role in lens induction. *Development* 128: 4415-24.

DUDLEY, A.T., LYONS, K.M. and ROBERTSON, E.J. (1995). A requirement for bone morphogenetic protein-7 during development of the mammalian kidney and eye. *Genes Dev* 9: 2795-807.

DUDLEY, A.T. and ROBERTSON, E.J. (1997). Overlapping expression domains of bone morphogenetic protein family members potentially account for limited tissue defects in BMP7 deficient embryos. *Dev Dyn* 208: 349-62.

DUNCAN, M.K., CUI, W., OH, D.J. and TOMAREV, S.I. (2002). Prox1 is differentially localized during lens development. *Mech Dev* 112: 195-8.

FABER, S.C., DIMANLIG, P., MAKARENKOVA, H.P., SHIRKE, S., KO, K. and LANG, R.A. (2001). Fgf receptor signaling plays a role in lens induction. *Development* 128: 4425-38.

FUJIWARA, M., UCHIDA, T., OSUMI-YAMASHITA, N. and ETO, K. (1994). Uchida rat (rSey): a new mutant rat with craniofacial abnormalities resembling those of the mouse Sey mutant. *Differentiation* 57: 31-8.

FURUTA, Y. and HOGAN, B.L.M. (1998). BMP4 is essential for lens induction in the mouse embryo. *Genes Dev* 12: 3764-3775.

GLASER, T., WALTON, D.S. and MAAS, R.L. (1992). Genomic structure, evolutionary conservation and aniridia mutations in the human PAX6 gene. *Nat Genet* 2: 232-9.

GOLDFARB, M. (2001). Signaling by fibroblast growth factors: the inside story. *Sci STKE* 2001: PE37.

GOUDREAU, G., PETROU, P., RENEKER, L.W., GRAW, J., LOSTER, J. and GRUSS, P. (2002). Mutually regulated expression of Pax6 and Six3 and its implications for the Pax6 haploinsufficient lens phenotype. *Proc Natl Acad Sci USA* 99: 8719-24.

GRAINGER, R.M. (1992). Embryonic lens induction: shedding light on vertebrate tissue determination. *Trends Genet* 8: 349-55.

GRAINGER, R.M. (1996). New perspectives on embryonic lens induction. *Cell Dev. Biol.* 7: 149-155.

GRAINGER, R.M., HENRY, J.J., SAHA, M.S. and SERVETNICK, M. (1992). Recent progress on the mechanisms of embryonic lens formation. *Eye* 117-22.

GRAINGER, R.M., MANNION, J.E., COOK, T.L.J. and ZYGAR, C.A. (1997). Defining intermediate stages in cell determination: acquisition of a lens-forming bias in head ectoderm during lens determination. *Dev Genet* 20: 246-257.

GRINDLEY, J.C., DAVIDSON, D.R. and HILL, R.E. (1995). The role of Pax-6 in eye and nasal development. *Development* 121: 1433-42.

GRINDLEY, J.C., HARGETT, L.K., HILL, R.E., ROSS, A. and HOGAN, B.L. (1997). Disruption of PAX6 function in mice homozygous for the Pax6 Sey-1Neu mutation produces abnormalities in the early development and regionalization of the diencephalon. *Mech Dev* 64: 111-26.

HALDER, G., CALLAERTS, P. and GEHRING, W.J. (1995). Induction of ectopic eyes by targeted expression of the eyeless gene in Drosophila. *Science* 267: 1788-1792.

HILL, R.E., FAVOR, J., HOGAN, B.L., TON, C.C., SAUNDERS, G.F., HANSON, I.M., PROSSER, J., JORDAN, T., HASTIE, N.D. and VAN HEYNINGEN, V. (1991). Mouse small eye results from mutations in a paired-like homeobox- containing gene. *Nature* 354: 522-5.

HISA, T., SPENCE, S.E., RACHEL, R.A., FUJITA, M., NAKAMURA, T., WARD, J.M., DEVOR-HENNEMAN, D.E., SAIKI, Y., KUTSUNA, H., TESSAROLLO, L. *et al.,* (2004). Hematopoietic, angiogenic and eye defects in Meis1 mutant animals. *EMBO J* 23: 450-9.

JORDAN, T., HANSON, I., ZALETAYEV, D., HODGSON, S., PROSSER, J., SEAWRIGHT, A., HASTIE, N. and VAN HEYNINGEN, V. (1992). The human PAX6 gene is mutated in two patients with aniridia. *Nat Genet* 1: 328-32.

KAMACHI, Y., SOCKANATHAN, S., LIU, Q., BREITMAN, M., LOVELL-BADGE, R. and KONDOH, H. (1995). Involvement of SOX proteins in lens-specific activation of crystallin genes. *EMBO J* 14: 3510-9.

KAMACHI, Y., UCHIKAWA, M., COLLIGNON, J., LOVELL-BADGE, R. and KONDOH, H. (1998). Involvement of Sox1, 2 and 3 in the early and subsequent molecular events of lens induction. *Development* 125: 2521-32.

KAMACHI, Y., UCHIKAWA, M., TANOUCHI, A., SEKIDO, R. and KONDOH, H. (2001). Pax6 and SOX2 form a co-DNA-binding partner complex that regulates initiation of lens development. *Genes Dev* 15: 1272-86.

KAMMANDEL, B., CHOWDHURY, K., STOYKOVA, A., APARICIO, S., BRENNER, S. and GRUSS, P. (1999). Distinct cis-essential modules direct the time-space pattern of the Pax6 gene activity. *Dev Biol* 205: 79-97.

KLEINJAN, D.A., SEAWRIGHT, A., SCHEDL, A., QUINLAN, R.A., DANES, S. and VAN HEYNINGEN, V. (2001). Aniridia-associated translocations, DNase hypersensitivity, sequence comparison and transgenic analysis redefine the functional domain of PAX6. *Hum Mol Genet* 10: 2049-59.

KNOBLICH, J.A., JAN, L.Y. and JAN, Y.N. (1995). Asymmetric segregation of Numb and Prospero during cell division. *Nature* 377: 624-7.

LANG, R.A. and MCAVOY, J.W. (2003). Growth factors in lens development. In *Development of the ocular lens*, (ed. M. L. Robinson and F. J. Lovicu).

LOOSLI, F., KOSTER, R., CARL, M., KRONE, A. and WITTBRODT, J. (1998). Six3, a medaka homologue of the Drosophila homeobox gene sine oculis is expressed in the anterior embryonic shield and the developing eye. *Mech Dev* 74: 159-64.

LOVICU, F.J. and OVERBEEK, P.A. (1998). Overlapping effects of different members of the FGF family on lens fiber differentiation in transgenic mice. *Development* 125: 3365-77.

LUO, G., HOFMANN, C., BRONCKERS, A.L., SOHOCKI, M., BRADLEY, A. and KARSENTY, G. (1995). BMP-7 is an inducer of nephrogenesis, and is also required for eye development and skeletal patterning. *Genes Dev* 9: 2808-20.

MARIANI, M., BALDESSARI, D., FRANCISCONI, S., VIGGIANO, L., ROCCHI, M., ZAPPAVIGNA, V., MALGARETTI, N. and CONSALEZ, G.G. (1999). Two murine and human homologs of mab-21, a cell fate determination gene involved in Caenorhabditis elegans neural development. *Hum Mol Genet* 8: 2397-406.

MARIANI, M., CORRADI, A., BALDESSARI, D., MALGARETTI, N., POZZOLI, O., FESCE, R., MARTINEZ, S., BONCINELLI, E. and CONSALEZ, G.G. (1998). Mab21, the mouse homolog of a C. elegans cell-fate specification gene, participates in cerebellar, midbrain and eye development. *Mech Dev* 79: 131-5.

MCAVOY, J.W. (1980). Cytoplasmic processes interconnect lens placode and optic vesicle during eye morphogenesis. *Experimental Eye Research* 31: 527-34.

MCAVOY, J.W., CHAMBERLAIN, C.G., DE IONGH, R.U., HALES, A.M. and LOVICU, F.J. (1999). Lens development. *Eye* 13: 425-37.

MCAVOY, J.W., CHAMBERLAIN, C.G., DE IONGH, R.U., RICHARDSON, N.A. and LOVICU, F.J. (1991). The role of fibroblast growth factor in eye lens development. *Ann. N. Y. Acad. Sci.* 638: 256-74.

MCWHIRTER, J.R., GOULDING, M., WEINER, J.A., CHUN, J. and MURRE, C. (1997). A novel fibroblast growth factor gene expressed in the developing nervous system is a downstream target of the chimeric homeodomain oncoprotein E2A-Pbx1. *Development* 124: 3221-32.

MENCL, E. (1903). Ein fall von beiderseitiger augenlinsenausbildung wahrend der abwesenheit von augenblasen. *Arch. EntwMech. Org.* 16: 328-339.

MIKKOLA, I., BRUUN, J.A., BJORKOY, G., HOLM, T. and JOHANSEN, T. (1999). Phosphorylation of the transactivation domain of Pax6 by extracellular signal-regulated kinase and p38 mitogen-activated protein kinase. *J Biol Chem* 274: 15115-26.

NORNES, S., CLARKSON, M., MIKKOLA, I., PEDERSEN, M., BARDSLEY, A., MARTINEZ, J.P., KRAUSS, S. and JOHANSEN, T. (1998). Zebrafish contains two pax6 genes involved in eye development. *Mech Dev* 77: 185-96.

OLIVER, G., LOOSLI, F., KOESTER, R., WITTBRODT, J. and GRUSS, P. (1996). Ectopic lens induction in fish in response to the murine homeobox gene Six3. *Mech. Dev.* 60: 233-239.

OLIVER, G., MAILHOS, A., WEHR, R., COPELAND, N.G., JENKINS, N.A. and GRUSS, P. (1995). Six3, a murine homologue of the sine oculis gene, demarcates the most anterior border of the developing neural plate and is expressed during eye development. *Development* 121: 4045-55.

PIATIGORSKY, J. (1998). Multifunctional lens crystallins and corneal enzymes. More than meets the eye. *Ann N Y Acad Sci* 842: 7-15.

QUINN, J.C., WEST, J.D. and HILL, R.E. (1996). Multiple functions for Pax6 in mouse eye and nasal development. *Genes Dev* 10: 435-46.

QUIRING, R., WALLDORF, U., KLOTER, U. and GEHRING, W.J. (1994). Homology of the eyeless gene of Drosophila to the Small eye gene in mice and Aniridia in humans. *Science* 265: 785-9.

SEMINA, E.V., BROWNELL, I., MINTZ-HITTNER, H.A., MURRAY, J.C. and JAMRICH, M. (2001). Mutations in the human forkhead transcription factor FOXE3 associated with anterior segment ocular dysgenesis and cataracts. *Hum Mol Genet* 10: 231-6.

SERIKAKU, M.A. and O'TOUSA, J.E. (1994). sine oculis is a homeobox gene required for Drosophila visual system development. *Genetics* 138: 1137-50.

SERVETNICK, M. and GRAINGER, R.M. (1991). Changes in nerual and lens competence in Xenopus ectoderm: evidence for an autonomous developmental timer. *Development* 112: 177-188.

SERVETNICK, M.D., COOK, T., JR. and GRAINGER, R.M. (1996). Lens induction in axolotls: comparison with inductive signaling mechanisms in Xenopus laevis. *Int J Dev Biol* 40: 755-61.

SPANA, E.P. and DOE, C.Q. (1995). The prospero transcription factor is asymmetrically localized to the cell cortex during neuroblast mitosis in Drosophila. *Development* 121: 3187-95.

SPEMANN, H. (1901). Uber Correlationen in der Entwickelung des Auges. *Verh Anat Ges* 15: 61-79.

SULLIVAN, C.H., BRAUNSTEIN, L., HAZARD-LEONARDS, R.M., HOLEN, A.L. SAMAHA, F., STEPHENS, L. and GRAINGER, R.M. (2004). A re-examination of lens induction in chicken embryos: in vitro studies of early tissue interactions. *Int. J. Dev. Biol.* 48: 771-782.

TOMAREV, S.I., ZINOVIEVA, R.D., CHANG, B. and HAWES, N.L. (1998). Characterization of the mouse Prox1 gene. *Biochem Biophys Res Commun* 248: 684-9.

TREISMAN, J. and LANG, R. (2002). Development and evolution of the eye: Fondation des Treilles, September, 2001. *Mech Dev* 112: 3-8.

VOGEL-HOPKER, A., MOMOSE, T., ROHRER, H., YASUDA, K., ISHIHARA, L. and RAPAPORT, D.H. (2000). Multiple functions of fibroblast growth factor-8 (FGF-8) in chick eye development. *Mech Dev* 94: 25-36.

WAWERSIK, S., PURCELL, P., RAUCHMAN, M., DUDLEY, A.T., ROBERTSON, E.J. and MAAS, R. (1999). BMP7 Acts in Murine Lens Placode Development. *Dev Biol* 207: 176-188.

WEAVER, M. and HOGAN, B. (2001). Powerful ideas driven by simple tools: lessons from experimental embryology. *Nat Cell Biol* 3: E165-7.

WIGLE, J.T., CHOWDHURY, K., GRUSS, P. and OLIVER, G. (1999). Prox1 function is crucial for mouse lens-fibre elongation. *Nat Genet* 21: 318-22.

WILLIAMS, S.C., ALTMANN, C.R., CHOW, R.L., HEMMATI-BRIVANLOU, A. and LANG, R.A. (1998). A highly conserved lens transcriptional control element from the Pax-6 gene. *Mech Dev* 73: 225-9.

XU, P.X., ZHANG, X., HEANEY, S., YOON, A., MICHELSON, A.M. and MAAS, R.L. (1999). Regulation of Pax6 expression is conserved between mice and flies. *Development* 126: 383-95.

YAMADA, R., MIZUTANI-KOSEKI, Y., HASEGAWA, T., OSUMI, N., KOSEKI, H. and TAKAHASHI, N. (2003). Cell-autonomous involvement of Mab21l1 is essential for lens placode development. *Development* 130: 1759-70.

ZHANG, X., FRIEDMAN, A., HEANEY, S., PURCELL, P. and MAAS, R.L. (2002). Meis homeoproteins directly regulate Pax6 during vertebrate lens morphogenesis. *Genes Dev* 16: 2097-107.

ZHANG, Y. and EMMONS, S.W. (1995). Specification of sense-organ identity by a Caenorhabditis elegans Pax-6 homologue. *Nature* 377: 55-9.

ZUBER, M.E., GESTRI, G., VICZIAN, A.S., BARSACCHI, G. and HARRIS, W.A. (2003). Specification of the vertebrate eye by a network of eye field transcription factors. *Development* 130: 5155-67.