Lens induction in axolotls: comparison with inductive signaling mechanisms in *Xenopus laevis*

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ABSTRACT Amphibian lens induction is an embryonic process whose broad outlines are conserved between anurans and urodeles; however, it has been argued that some aspects of this process differ significantly between even closely related species. Classical embryologists concluded that in some species direct contact between the optic vesicle and ectoderm was both necessary and sufficient to induce the ectoderm to form a lens, while in other species tissues other than the optic vesicle induce lens formation. Recent studies of lens induction in *Xenopus* have argued that lens induction may be more conserved evolutionarily than was previously thought and that the different conclusions reached in the classical literature may be due more to experimental methodology than to actual differences in the process of lens induction. We have tested this hypothesis by examining the timing of lens induction in the axolotl and the ability of various tissues to induce lenses in explant cultures. We find that, despite the evolutionary divergence between *Xenopus* and *Ambystoma*, the mechanism of lens specification is substantially similar in the two species. These results support the hypothesis that the mechanism of lens induction is evolutionarily conserved among amphibians.

KEY WORDS: *lens, induction, determination, Xenopus, axolotl, embryo, evolution, eye*

Introduction

In the last decade, genes that regulate development have been shown to be conserved to a striking degree. These conserved regulatory genes include those involved in establishment of the body pattern (*Hox* genes: McGinnis and Krumlauf, 1992; Slack *et al.*, 1993) and in cell-to-cell signaling (for example, TGF-ß growth factors: Kingsley, 1994; the hedgehog family: Smith, 1994; the *Ras* pathway: Kayne and Sternberg, 1995; Wassarman *et al.*, 1995). The conservation of molecular genetic mechanisms can extend across a remarkable range of metazoan animals, from nematodes and fruit flies to frogs, mice, and humans.

Among the most striking examples of such conservation is the recent demonstration that cells that will give rise to the eyes in both fruit flies and mammals become committed to eye differentiation through the expression of homologous genes, termed *Pax-6* in vertebrates and *eyeless* in fruit flies (Quiring *et al.*, 1994; Strachan and Read, 1994; Halder *et al.*, 1995). The fly and vertebrate genes appear to be functionally interchangeable, since ectopic expression of the mammalian *Pax-6* gene in *Drosophila* embryos leads to the formation of ectopic eyes (Halder *et al.*, 1995). The discovery that eye determination is mediated by homologous genes in such widely divergent taxa was unexpected, given the assumption that animal eyes had evolved as many as 60 different times (Salvini-Plawen and Mayr, 1977), and

that arthropod and vertebrate eyes are organized in fundamentally different ways.

Against this backdrop of genetic evolutionary conservation, it remains clear that developmental mechanisms must differ to produce different body types. Given that diverse animal forms utilize genes that are in some cases functionally interchangeable, how are different body types generated? Elucidation of those mechanisms common to all developing animals, and of those mechanisms that differ so as to produce different animal forms, remains one of the fundamental goals of developmental biology.

While the question of conservation vs. divergence can be explored at many levels, we concentrate here on one model system that sheds light on such issues: the induction of the ocular lens in amphibian embryos. Lens induction holds a unique place in the history of embryology: it was experiments on the formation of the lens that led Spemann to postulate the concept of embryonic induction (Spemann, 1901; see Saha, 1991). Further examination of lens induction by others led to the idea that the mechanism of lens induction might differ in different species (reviewed by Spemann, 1938). This model suggested that the mechanisms of lens induction, and by inference other early developmental processes, appear to differ significantly in different amphibian species, implying that differences in cell-cell communication processes might underlie the development of different species. Whether there are significant variations in lens induction mechanisms among the
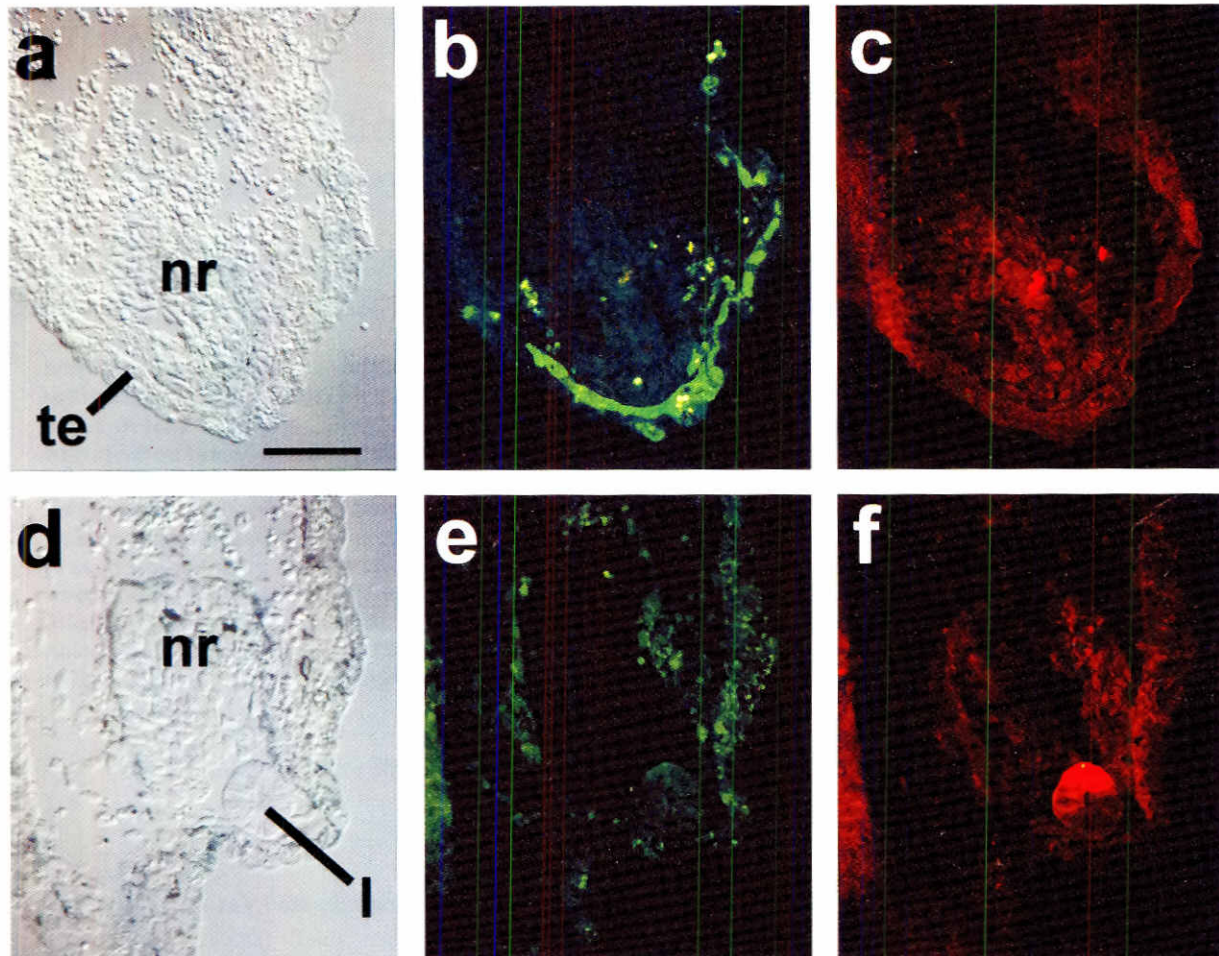
Fig. 1. Transplantation of gastrula ectoderm to the presumptive lens area of neural tube stage (a-c) and neural plate stage (d-f) embryos. (a and d) *Differential interference images of sections in the eye region. nr, neural retina; l, lens; te, transplanted gastrula ectoderm.* **(b and d)** *The same sections showing fluorescence from FDA, illustrating the sites of transplanted ectoderm.* **(c and f)** *Fluorescence resulting from reaction of sections with a lens-specific antibody. Bar, 0.1 mm.*

amphibians becomes a particularly intriguing question in light of the important role of the functionally conserved *Pax-6* gene, not only in eye formation in general but lens induction in particular (Hogan *et al.,* 1985). Since the function of *Pax-6* appears to be so conserved, other gene products would then be implicated in the mechanisms generating the variations in induction.

While lens induction has been extensively studied in the anuran *Xenopus* in recent years (Grainger, 1992), little work has been done in other species. As a test of the conservation of lens induction we have examined this issue in a urodele, the Mexican axolotl *Ambystoma mexicanum,* since the urodeles and anurans represent an ancient evolutionary split among the amphibians. It is estimated that *Xenopus* and the axolotl have not shared a common ancestor for at least 250 million years. In addition axolotl embryos are highly suited for performing the embryological manipulations required for these studies, since they develop slowly and are relatively large in comparison with *Xenopus* embryos.

Models of lens induction

In all amphibians studied, the lens forms from the ectodermal cells that overlie the optic vesicle, which forms during neurula stages of development. According to Spemann's original model, based on studies with the frog *Rana temporaria* (formerly *Rana fusca*) (Spemann, 1901), the lens is induced by direct contact from the underlying optic vesicle, which will later form the retina. Later studies, however, showed that, at least in some species, the lens cells can be determined without contact with the optic vesicle (reviews: Spemann, 1938; Jacobson and Sater, 1988; Saha *et al.,* 1989). These experiments suggested that other tissues, or diffusible factors, must be responsible for lens determination in at least some species.

These data were reconciled by presuming that "early inducing tissues," which were thought to include the endoderm and mesoderm which underlie the presumptive lens region during gastrulation, can also induce lens formation (Jacobson, 1966). "Late inducing tissues" (that is, the optic vesicle and optic cup), might also be able to induce the lens, but would not be necessary if the influence of the early tissues is sufficiently strong. Thus, the relative influence of early and late inducing tissues was thought to vary among different species, so that in some species the lens is determined prior to contact with the optic vesicle, while in others lens determination requires the optic vesicle.

The apparent variation in the relative importance of different inducing tissues came as a surprise even to Spemann, who was,

as he admits, "biased by the idea that similar animal forms must behave in a similar way" (Spemann, 1938). Nevertheless, he writes: *From all these results I considered myself obliged to draw the conclusion that the different vertebrates, indeed even different genera and species of a more restricted group, like that of the Anura or even of the frogs, behave in a different manner with regard to the mechanism of lens formation; that is to say the manner is not different in principle, but in degree, according to the importance of the optic vesicle in the process of lens formation* (Spemann, 1938).

Recent re-examination of lens induction in the anuran *Xenopus laevis* has addressed the issues of early and late inducing tissues (reviewed by Grainger, 1992). These studies have not only demonstrated that lens cells in *Xenopus* are determined prior to contact with the optic vesicle (Henry and Grainger, 1990), but have conclusively shown that the methods used in many previous studies of lens induction leave open the possibility that lenses are induced solely by early inducers (Grainger *et al.*, 1988; reviewed by Saha *et al.*, 1989).

In *Xenopus*, lens induction is initiated during gastrulation, a conclusion based on the finding that ectoderm is only responsive, or competent, to initiate a lens-forming response during mid to late gastrula stages (Servetnick and Grainger, 1991). Lens induction continues during neurulation (Henry and Grainger, 1987, 1990). Evidence indicates that planar (or horizontal) signals travel through the sheet of ectoderm from the presumptive neural plate (perhaps the optic primordia) to the ectoderm outside the neural plate, establishing a broad area that is predisposed, or biased, to form a lens. Subsequent signals, either from the neural plate, the underlying endoderm and mesoderm, or perhaps the optic vesicle, provide the final signals that induce the lens from a small area of the biased ectoderm (reviewed in Grainger, 1992). While the source of the final signals remains unclear, it is clear that the lens cells are specified prior to contact with the optic vesicle (Henry and Grainger, 1990), suggesting that lens specification is brought about by early inducing signals, and that the optic vesicle plays, at most, a relatively minor role in this process. Three major conclusions should be emphasized from the *Xenopus* studies: (1) ectoderm is competent for lens formation for only a short period during gastrulation; (2) the optic vesicle is neither necessary nor sufficient to elicit lens formation from ectoderm, and (3) the lens is determined prior to contact by the optic vesicle.

The variation in inductive mechanisms proposed for lens induction was, as noted above, derived from collation of studies from a number of amphibian species. This model is, however, subject to a number of caveats, principally concerning the experimental procedures used (Jacobson and Sater, 1988), and the criteria used to assess lens formation (Saha *et al.*, 1989). Re-examination of lens induction, using rigorous criteria (cell lineage labeling of transplanted tissues, and use of molecular markers to assess the response of transplanted tissue) has shown that in at least two anuran species, *Xenopus laevis* and *Rana palustris*, the optic vesicle is insufficient to induce a lens (Grainger *et al.*, 1988). In addition Jacobson and Sater (1988) report that the optic vesicle is not required for lens induction in a wide variety of amphibian species, also suggesting that this may be a general feature of amphibian lens induction. Thus the evidence has begun to accumulate in support of a more conserved mechanism for lens induction. As mentioned earlier, this study of lens induction in the axolotl was initiated to test in a rigorous way the conservation of

lens induction mechanisms between highly divergent amphibians.

Ambystoma has been used for previous studies of lens induction, but these experiments could not have used the rigorous criteria that have been developed for more recent experiments (Grainger *et al.*, 1988; Saha *et al.*, 1989). No uniform view of the lens induction process emerges from early studies on *Ambystoma* species. In *Ambystoma punctatum*, several investigators concluded that the optic vesicle is insufficient to induce lens formation (Harrison, 1920; Stone and Dinnean, 1943; Liedke, 1951, 1955; Reyer, 1958a,b; reviewed by Saha *et al.*, 1989), and that early inductive influences must be contributing to lens formation. In contrast, studies using *Ambystoma mexicanum* concluded that the optic vesicle is necessary (Ten Cate, 1953) and sufficient (Woerdeman, 1938) for lens induction. While these latter studies suggest that early inductive influences are insufficient to lead to lens determination, they are open to question, based on the methodology used (Saha *et al.*, 1989).

Despite the fact that *Xenopus* and *Ambystoma* are highly diverged amphibians, these two species use similar overall developmental strategies, have similar adult eye structures, and the morphological development of the eye is essentially the same in both species. Because of these fundamental similarities, similar experimental manipulations can be performed in the two species. Differences between the two species include an important factor: the rate of embryological development. Axolotls develop much more slowly than does *Xenopus*, requiring approximately 100 hours to reach the early neurula stage [at 18°, stage 20 of Schreckenberg and Jacobson (1975)], compared to approximately 21 h for *Xenopus* [at 23°, stage 19 of Nieuwkoop and Faber (1967)]. In fact it has been proposed that the rapid rate of early development in species such as *Xenopus* may lead to the commitment of tissues to specific fates at relatively earlier stages in development (Ten Cate, 1953), possibly implying that the role of early lens-inducing tissues might be more important in *Xenopus* than in axolotls.

Lens induction in axolotls

Experimental design

To study lens induction in *Ambystoma mexicanum*, we performed a series of tissue transplantations, explants and recombinants. In transplant experiments, a donor embryo was labeled by injection of the fluorescent lineage tracer fluoresceinated dextran amine (FDA) into embryos at the one- or two-cell stage. (Injections were performed at the Indiana University Axolotl Colony prior to shipment of injected embryos.) After embryos had developed to the appropriate stages, we transplanted tissue from the FDA-labeled donor to an unlabeled host embryo, allowed the host to develop to stage 39, and assessed the ability of the labeled transplant to form a lens by immunofluorescent staining of tissue sections with an antibody made against *Xenopus* lens proteins (Henry and Grainger, 1990); the *Xenopus* antibodies cross-react with axolotl lenses. Explants were removed from the embryos, cultured to the larval stage (stage 39 of Schreckenberg and Jacobson, 1975), and assayed to determine whether lens tissue had formed by immunofluorescence, as described above. Recombinants were made between an FDA-labeled and an unlabeled tissue and then cultured and assayed in a manner identical to explants. All

surgery was done in 3/4X Normal Amphibian Medium (NAM), and embryos or tissues subsequently cultured in 3/8X NAM or 1/10X NAM.

Is the optic vesicle sufficient to induce lenses?

In our first series of experiments, we tested whether the optic vesicle alone is capable of inducing a lens in *Ambystoma mexicanum*. To do this, we transplanted FDA-labeled ectoderm from gastrula embryos to the presumptive lens-forming region of a late neurula embryo (stage 20 of Schreckenberg and Jacobson, 1975). In such an experiment, the gastrula ectoderm would be exposed to the influence of the newly formed optic vesicle, which, at this stage, is just making contact with the ectoderm in the presumptive lens-forming region. However, the transplanted ectoderm would not be subject to any signals that would influence this region during neural plate stages of development. In 40 such transplants an unambiguous lens-like structure was induced in only a single case. A typical negative case is illustrated in Figure 1a-c. Results are summarized in Table 1. These experiments included transplants from early, middle, and late gastrula stages, which we show below are all competent to form lenses. Thus, the failure to form lenses in transplants to the late neurula embryos is not due to a lack of competence of the ectoderm to form lenses. These data suggest that, as in *Xenopus*, the optic vesicle is insufficient, on its own, to induce lens formation in the axolotl.

Does lens induction require early signals?

To determine whether gastrula ectoderm can form lenses if it is exposed to early inducing signals as well as to the optic vesicle, FDA-labeled gastrula ectoderm was transplanted to open-neural-plate stage embryos. The proportion of transplants that formed lenses after transplantation varied depending on the stage of the donor ectoderm (see Table 1). A typical positive case is seen in Figure 1d-1f. Early gastrula ectoderm did not form lenses (14 cases), mid-gastrula ectoderm formed lenses in 6 of 15 cases (40%), and late gastrula ectoderm in 4 of 12 cases (33%). Because late gastrula ectoderm can still form lenses in a substantial proportion of cases, we performed transplants at later stages as well to determine when the period of lens competence ends. Of 13 transplants made at early neural plate stage 7 (58%) formed lenses. At later neural plate stages lenses formed in 3 of 14 cases (21%).

In summary, results from transplantation experiments show that gastrula ectoderm cannot form lenses after transplantation to a late neurula embryo (that is, in response to the optic vesicle alone), but can form lenses after transplantation to an open neural plate stage embryo (that is, in response to earlier inducing signals as well as the optic vesicle). We conclude that early signals are necessary for lens induction in the axolotl, as they are in *Xenopus*.

The transplant experiments also show that the period of lens competence in *Ambystoma* appears longer than that of *Xenopus*. In *Xenopus*, ectoderm is competent to respond to lens induction only for a sharply restricted period, for a period of about 2-3 hours during mid-gastrula stages (Servetnick and Grainger, 1991). However a final conclusion regarding this point must await further experimentation. In *Xenopus* the temporal pattern of competence was established in two kinds of experiments. In one, ectoderm was removed from early gastrula stages and cultured to subsequent stages when its responsiveness was tested in transplants to neural

TABLE 1

TRANSPLANT EXPERIMENTS: TESTS OF THE TIMING OF LENS INDUCTION

Donor stage	Host stage	Number of transplants	Number of lenses formed
Gastrula	Neural tube		
st. 10	20	14	0 (0%)
st. 11	20	8	0 (0%)
st. 12	20	18	1 (6%)
Gastrula	Neural plate		
st. 10 to 11	14 to 14+	14	0 (0%)
st. 11 to 12	14 to 14+	15	6 (40%)
st. 12 to 13	14 to 14+	12	4 (33%)
st. 13 to 14	14 to 14+	13	7 (58%)
st. 14 to 15	14 to 14+	14	3 (21%)

Ectoderm was removed from labeled embryos [embryos injected with fluoresceinated dextran amine (see Henry and Grainger, 1990)], and transplanted to the presumptive lens region of an unlabeled host embryo. Donor and host embryos were at the stages indicated; all stages are according to Schreckenberg and Jacobson (1975). Embryos were cultured to stage 39, and were then fixed, sectioned, and stained with antibodies to *Xenopus* lens (Henry and Grainger, 1990). Lens formation was scored on the basis of immunofluorescence (to verify that the induced structure was a lens), and tissue labeling (to verify that the induced lens was derived from transplanted ectoderm).

plate stage hosts. This experiment has not yet been done in the axolotl. What was done in the axolotl was similar to the second series of *Xenopus* experiments: ectoderm was taken from the embryo at different stages and its competence directly assessed by transplantation into neural plate stage hosts. In the *Xenopus* experiments performed this way there was a slight increase in responsiveness in very late stage ectoderm (not seen in *in vitro* aging experiments), a result which was attributed to the possibility of inductive influences which may have acted on this ectoderm in the embryo. Likewise, the increase seen in responsiveness in late stage axolotl ectoderm might be due to inductive effects which biases ectoderm towards lens formation.

What tissues are responsible for the early lens-inducing signals?

To determine which tissues are responsible for transmitting early lens-inducing signals during axolotl development, we performed a number of explant and recombinant experiments. In the first series of experiments, the presumptive lens ectoderm (PLE) was removed from open-neural-plate stage embryos and cultured in isolation to the equivalent of the larval stage (stage 39), at which time it was assayed for lens formation. Nineteen explants were made, and none of these formed a lens (Table 2). This shows that, at the open-neural-plate stage, the PLE is not yet specified to form a lens, suggesting that, to form a lens, the ectoderm must receive signals after this stage. These results are consistent with the results of transplant experiments, which indicate that signals are required between the open neural plate stage and the end of neurulation. Subsequent experiments were performed to determine whether these signals might be generated from mesodermal or neural tissues in contact with the presumptive lens area.

To assay the ability of the mesoderm underlying the PLE to induce a lens, the PLE was removed at the early neural plate stage

along with its underlying mesoderm, and the explant, consisting of the two tissues, was cultured until the larval stage, as above, when it was assayed for lens formation. Of 33 cases, none formed lenses (Table 2). These data suggest that, like the optic vesicle, the mesoderm underlying the presumptive lens region is not sufficient to induce a lens, even in ectoderm at the neural plate stage when the PLE is presumed to already have a lens-forming bias.

Previous experiments (Henry and Grainger, 1990) have suggested that the early lens-inducing signals might originate from the neural plate medial to the PLE and be transmitted as planar signals through the ectoderm to the PLE. To test this possibility, we removed a portion of ectoderm consisting of the PLE along with the anterior neural plate at the open neural plate stage. As summarized in Table 2, of 29 explants, four formed a lens. Of the four that gave positive responses, two formed morphologically recognizable eye tissue. Since contact with the optic vesicle subsequent to neural tube closure is insufficient to induce a lens from ectoderm, these positive cases support the hypothesis that signals from the anterior neural plate are involved in lens induction. However, the number of positive cases is small, and it is therefore still possible that other tissue interactions play important roles in lens induction in the axolotl.

In the series described above, tissues were removed from the embryo at the neural plate stage when contact of the PLE with both neural and non-neural tissues has already taken place. To minimize the extent of contact of the PLE with non-neural tissues anterior neural tissue and the presumptive lens ectoderm were removed as a contiguous sheet at mid-gastrula stages and cultured as above. Of 20 such explants, two showed lens formation (Table 2). Again, these results are suggestive, but do not eliminate the possibility of non-neural inductive signals.

As a more stringent test of whether neural signals are sufficient to induce lenses, we combined anterior neural plate (from the open-neural-plate stage) with gastrula ectoderm at several different stages. Of 40 cases, no lenses were observed (Table 2).

In summary, these experiments have not yet revealed unequivocally the source(s) of the early lens-inducing signals in the neurula embryo. It is clear that neither the optic vesicle, nor the mesoderm underlying the PLE, is sufficient to induce a lens. Our results suggest that signals from the anterior neural plate, transmitted through the plane of the ectoderm, are likely to be important in lens induction in the axolotl, but we have observed such induced lenses only in a small proportion of explants.

In studies of *Xenopus* lens induction (Henry and Grainger, 1990) a larger fraction of explants and recombinants of the type described above yielded a lens-forming response than in our axolotl experiments. There are a number of possible explanations for this difference. It is possible that culture conditions which are satisfactory for differentiation of *Xenopus* tissues are not adequate for the axolotl. Growth or differentiation factors may be present at sub-threshold levels in isolated tissues grown in saline solution and thus a strong lens-forming response might only be seen in whole embryos. Thus, gastrula ectoderm may form lenses when transplanted to neural plate stage embryos, but not when cultured as an explant in combination with neural plate stage tissues.

An alternative explanation is that a combination of factors is required to elicit lens formation from the ectoderm. Evidence from studies on *Xenopus* supports this proposal, though in *Xenopus* there appears to be less of a requirement for multiple inductive interactions than there would seem to be in the axolotl. In *Xenopus*,

it is known that the optic vesicle, while insufficient to induce a lens from gastrula ectoderm, can induce a lens from ectoderm that has been exposed to earlier lens-inducing signals. Thus, the optic vesicle has some inducing ability, but it is too weak on its own to induce a lens. If a similar situation exists in the axolotl, then it is possible that the primary lens-inducing signal is transmitted by the anterior neural plate to the presumptive lens ectoderm through the plane of the ectoderm. This anterior neural plate signal is still insufficient, by itself, to elicit lens specification from the ectoderm in most cases, but in combination with signals from either the mesoderm or optic vesicle would lead to lens induction. Consistent with this, Henry and Grainger (1990) showed that, in *Xenopus*, the presence of mesoderm potentiates the inducing ability of the anterior neural plate. Thus, the lens forms in the region where two tissues act in concert, and this might be a means used by the embryo to restrict the lens to a small, and accurately placed, region of ectoderm. This hypothesis remains to be tested in the axolotl.

Summary of axolotl experiments

While substantial gaps remain in our understanding of the mechanisms of lens induction both in urodeles and anurans, the data that we have obtained to date nevertheless allow us to draw a number of conclusions about lens induction in axolotls. First, the optic vesicle is not sufficient to induce a lens from competent ectoderm. Second, early signals, sent to the presumptive lens ectoderm during neurula stages of development, are required for lens induction. While the source of these signals has not yet been determined unambiguously, our observations in axolotls are consistent with the current model of lens induction in *Xenopus* (Henry

TABLE 2

EXPLANT/RECOMBINANT EXPERIMENTS: TESTS OF THE ABILITY OF TISSUES TO FORM LENSES IN CULTURE

Explanted or recombined tissue(s)	Number of cases	Number of lenses formed
PLE[1]		
neural plate stage (st. 14)	19	0
PLE + underlying mesoderm		
neural plate stage (st. 14)	33	0
PLE + neural plate		
both from st. 14	29	4[2]
both from st. 111/2-12	20	2
Gastrula ectoderm + st. 14 neural plate		
st. 10 ectoderm	13	0
st. 11 ectoderm	7	0
st. 12 ectoderm	20	0

[1]Presumptive lens ectoderm. [2]of the four cases forming lenses, two contained eye tissue that formed from the explanted neural plate. Ectoderm, alone or in combination with the tissues indicated, was explanted from the stage and embryonic region indicated, and cultured *in vitro* to the equivalent of stage 39, at which time the explant was fixed, sectioned, and stained with antibodies to *Xenopus* lens (Henry and Grainger, 1990). Lens formation was scored on the basis of immunofluorescence (to verify that the induced structure is a lens). In recombinants the origin of lenses was monitored by observing FDA lineage labeling (only one of the two tissues was labeled in each case). The presumptive lens region from stage 14 embryos was verified by fate mapping using Nile Blue Sulfate.

and Grainger, 1990; Grainger, 1992), which proposes that the primary signal comes from the anterior neural plate that may be enhanced by a signal from the mesoderm underlying the presumptive lens ectoderm. Third, the period of ectodermal competence for lens induction appears to be somewhat different in the two species. Early gastrula ectoderm is competent to form lenses (supporting the idea that lens-inducing signals are transmitted long before the optic vesicle forms), and the ectoderm remains competent to respond to lens induction longer in *Ambystoma* than it does in *Xenopus*. However, the apparently protracted period of lens competence in the axolotl may be due to inductive signals biasing ectoderm *in vivo*.

Questions for the future

A number of questions about the mechanisms of lens induction in axolotls remain unresolved, though the data described here comprise a first step toward a obtaining a more generalized view of amphibian lens induction. One question that remains is to determine exactly when lens-inducing signals are transmitted. While we know that signals are required prior to formation of the optic vesicle, it is unclear whether the signals are restricted to a particular period during neurulation, whether they are required throughout the entire period, or perhaps even longer. If signals come primarily from a particular subset of mesodermal cells, for example, then the signals would presumably be restricted to the time during which those cells are in contact with head ectoderm.

Related to this question is the issue of when lens specification occurs. In *Xenopus*, the lens is specified prior to contact by the optic vesicle (Henry and Grainger, 1990), again supporting a limited role for the optic vesicle in lens specification [though the optic vesicle may restrict the formation of the lens to a small region of a larger domain of biased head ectoderm (Grainger, 1992)]. We have not yet tested this point in the axolotl, though it is clearly an important piece of the puzzle. If the lens ectoderm is specified prior to contact by the optic vesicle, this would confirm that the optic vesicle plays at most a minor role in lens specification, and would provide a further parallel with lens induction in *Xenopus*. If, however, the lens is not specified until after contact with the optic vesicle, this might suggest a more active role for the optic vesicle in the axolotl (though still insufficient to induce a lens on its own), and might provide an explanation for why simple explant cultures that do not contain presumptive eye tissue are unable to produce lenses.

What tissue(s) transmit early lens-inducing signals? Our results to date are consistent with results in *Xenopus*. In *Xenopus*, the neural plate, the mesoderm, and the optic vesicle may all have a role in lens induction, and the relative importance of these tissues remains to be elaborated (Henry and Grainger, 1990; Grainger, 1992). Our experiments suggest that in the axolotl, neither the mesoderm nor the optic vesicle is sufficient, in itself, to induce a lens, and the neural plate alone is unable to induce lenses. However, these results must be interpreted with caution in the absence of consistent differentiation of lenses in explant cultures. Nevertheless, our data are consistent with the observations in *Xenopus* that more than one inducing tissue is involved in lens induction.

A surprising observation is the apparent difference in the period of competence for lens induction between the axolotl and *Xenopus*. Before we can be certain there is a difference in competence, however, further experiments must be performed. The competence of ectoderm isolated and cultured from the embryo before lens induction commences must be tested to eliminate the possibility that the older ectoderm in the embryo is exposed to lens-inducing signals. If a difference is still found one might then speculate about an adaptive purpose for this difference in competence. The change in the timing of lens competence may have evolved with no particular purpose being served by the change. Alternatively, the difference in competence may be associated with a subtle difference in signaling mechanisms in the two species, such that a short period of competence in axolotls might be insufficient to allow a lens to form, or a long period of competence in *Xenopus* might produce a lens that is too large, or is incorrectly positioned. It is unlikely that we will be able to resolve this question until we are able to manipulate the period of competence, perhaps by overexpression of genes that act as signaling molecules, receptors, or in the signal transduction pathway leading to lens specification. The recent results of Coffman *et al.* (1993) suggest that modulation of levels of members of the *Notch* gene family may afford such an opportunity.

An interesting sidelight to this question is the observation that lens formation in the absence of the optic vesicle varies according to the temperature at which embryos are reared. Both Ten Cate (1953), using *Rana esculenta*, and Jacobson (1958), using *Taricha torosa* (=*Triturus torosus*) showed that there are substantial temperature effects on the formation of lenses in embryos from which the eye rudiment had been excised at neural plate stages. In both studies, lens induction appeared to be enhanced at low temperatures, that is, under conditions in which embryonic development was slower. By analogy, tissue interactions might somehow differ in the slowly-developing axolotl embryos relative to the rapidly-developing *Xenopus* embryos, and the difference in lens-forming competence might reflect some underlying difference in the rate at which tissues can transmit or respond to inductive signals.

As molecular biological data are garnered that apply to the problem of retina and lens specification and patterning, it has become clear that these tissues are initially specified as part of a field during early development. *Pax-6* is expressed during early development in a domain that includes both the retinal and lens rudiments, and, consistent with this expression pattern, both the retina and lens are disrupted in *Pax-6* mutants (reviewed in Saha *et al.*, 1992). Experiments in *Drosophila* have implicated the *Drosophila* homologue of *Pax-6*, *eyeless*, as a master switch controlling eye specification. If *Pax-6* serves a similar role in vertebrate embryos, its mode of action must be somewhat more complex, because the anterior neural plate is specified to form retina by the neural plate stage (Saha and Grainger, 1992), while the lens is not yet specified at that stage (Henry and Grainger, 1990; this paper). Presumably, regulation within the field leads initially to specification of the retina, followed later by specification of the lens, perhaps as a result of signals from the retinal region. Thus *Pax-6* is likely to regulate only part of the eye determination program, and it remains important to untangle the signaling systems in different organisms, as we have begun to do with different amphibians, to understand what provides the differences that account for the divergent forms of eyes in different organisms.

Conclusions

While the axolotl and *Xenopus* have not shared a common ancestor for at least 250 million years, and, despite the differences

in the rate of development in the two species, the mechanism of lens induction in the axolotl appears very similar to that in *Xenopus*. In both forms, the optic vesicle appears to play at most a minor role in lens induction, and signals transmitted during neurulation are required for lens specification. Given the strong evolutionary conservation of many developmental mechanisms, it would perhaps have been surprising if, upon reexamination, the mechanism of lens induction had differed substantially between *Xenopus* and *Ambystoma*. However, given the historical importance of the lens as a model system for the study of embryonic induction (Spemann, 1938; Saha *et al.*, 1989), and given the controversy over the relative roles of the optic vesicle and early inducers in amphibian embryos, it is important to reevaluate the mechanism of lens induction in urodeles as well as amphibians. While it remains possible that *Ambystoma mexicanum* simply represents an example of an amphibian in which early inducers predominate over the optic vesicle, the experiments of Grainger *et al.* (1988) argue that the model of lens induction developed in *Xenopus* is likely to be applicable to a broad range of amphibians, and perhaps to other vertebrates as well.

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