Reinvestigation of the role of the optic vesicle in embryonic lens induction

ROBERT M. GRAINGER, JONATHAN J. HENRY and ROBERT A. HENDERSON

Department of Biology, University of Virginia, Charlottesville, VA 22901, USA

Summary

The induction of the lens by the optic vesicle in amphibians is often cited as support for the view that a single inductive event can lead to determination in a multipotent tissue. This conclusion is based on transplantation experiments whose results indicate that many regions of embryonic ectoderm which would normally form epidermis can form a lens if brought into contact with the optic vesicle. Although additional evidence argues that during normal development other tissues, acting before the optic vesicle, also contribute to lens induction, it is still widely held, on the basis of these transplantation experiments, that the optic vesicle alone can elicit lens formation in ectoderm. While testing this conclusion by transplanting optic vesicles beneath ventral ectoderm in *Xenopus laevis* embryos, it became apparent that contamination of optic vesicles by presumptive lens ectoderm cells can generate lenses in these experiments, illustrating the need for adequate host and donor marking procedures. Since previous studies rarely used host and donor marking, it was not clear whether they actually demonstrated that the optic vesicle can induce lenses. Using careful host and donor marking procedures with horseradish peroxidase as a lineage tracer, we show that the optic vesicle cannot stimulate lens formation in neurula- or gastrula-stage ectoderm of *Xenopus laevis*. Since the general conclusion that the optic vesicle is sufficient for lens induction rests on studies in many organisms, we felt it was important to begin to test this conclusion in other amphibians as well. Similar experiments were therefore performed with *Rana palustris* embryos, since it was in this organism that optic vesicle transplant studies had originally argued that this tissue alone can cause lens induction. Under conditions similar to those used in the original report, but with careful controls to assess the origin of lenses in transplants, we found that the optic vesicle alone cannot elicit lens formation. Our data lead us to propose that the optic vesicle in amphibians is not generally sufficient for lens induction. Instead, we argue that lens induction occurs by a multistep process in which an essential phase in lens determination occurs as a result of inductive interactions preceding contact of ectoderm with the optic vesicle.

Key words: embryonic induction, cell determination, lens development, optic vesicle, *Xenopus laevis*.

Introduction

The first experimental demonstration that the formation of one embryonic tissue depends on its interaction with an adjacent tissue was performed by Spemann (1901), who showed that lens formation is dependent on an interaction with the eye rudiment. Normally the presumptive lens ectoderm first comes in contact with eye tissue when the latter is formed as an outgrowth of the forebrain just after neural tube closure. Lens differentiation commences shortly after this stage. The cells in the presumptive lens region first become elongated, then invaginate and finally pinch off to form a lens vesicle inside the eye cup. Spemann showed that the optic rudiment was necessary for lens development in the amphibian *Rana temporaria* (known originally as *Rana fusca*) since ablation of this rudiment blocked lens formation. Lewis (1904, 1907) reported shortly thereafter that in *Rana palustris* and *Rana sylvatica* one could move the optic vesicle beneath nonlens ectoderm and stimulate lens formation in this tissue. The experiments of Spemann and Lewis, and numerous similar studies that followed (reviewed by Reyer, 1958a), introduced
what has now become a widely cited idea: that the specification of cell fate in a multipotent tissue can be controlled by a single inductive interaction.

There is considerable evidence that, besides the optic vesicle, other inductive effects are also important in lens formation. King (1905) and many others subsequently (Reyer, 1958a) have shown that small lenses or lens-like structures (lentoids) can form in some amphibian species even when the optic rudiment is removed from young embryos. Arguments have been presented (Liedke, 1942, 1951; Jacobson, 1958, 1966) that the formation of these ‘free’ lenses is the result of interactions of head ectoderm with endoderm and mesoderm underlying it during gastrulation and early neurulation. Thus, there is evidence that important inductive effects are caused by these tissues as well as by the eye vesicle. All of these studies taken together suggest that inductive effects from either source can be sufficient to elicit some degree of lens formation and that the relative effects may vary from species to species.

In this paper, we reexamine the view that the optic vesicle alone can cause lens induction in ectoderm and consider whether such a single-step model for induction accurately represents a mechanism for determination in this system. Our first experiments involved optic vesicle transplants in *Xenopus* embryos. It became clear that an unambiguous method for distinguishing host and donor tissues was required to determine whether lenses found in association with eye tissue in these experiments were induced from host ectoderm or were derived from presumptive lens ectoderm cells which are difficult to remove from donor optic vesicles. Our results indicated that optic vesicles from which all presumptive lens ectoderm is removed cannot elicit lens formation in neurula flank or belly ectoderm of *Xenopus laevis*. This finding stimulated two directions of investigation. It raised concern about the lens-forming potential of ectoderm in *Xenopus* at various developmental stages and led to a systematic analysis of this problem (Henry & Grainger, 1987). It also led us to question the sufficiency of the optic vesicle for lens induction among amphibians in general since careful host and donor marking has rarely been used in such studies (Spemann, 1908, 1912; Stone & Dinnean, 1943; Liedke, 1951). We therefore undertook a more extensive series of transplantation experiments, presented here, with *Xenopus* and, in addition, with *Rana palustris* since this was the organism used in the first such transplantation experiments. Under conditions similar to those used in the original studies using *Xenopus* and *Rana palustris* (Brahma, 1959; Lewis, 1904), we find, contrary to these reports, that the optic vesicle cannot elicit lens formation in ventral ectoderm. Based on this data, we propose that the optic vesicle is generally not sufficient for lens induction and that, therefore, early inductive effects are an essential part of a multistep process for lens cell determination in amphibians.

**Materials and methods**

**Embryos**

Embryos were obtained from *Xenopus laevis* as described by Henry & Grainger (1987). *Rana palustris* embryos were collected from local ponds. *Xenopus* and *Rana* embryos were raised in 20% Steinberg’s solution (Rugh, 1962) containing 100 units ml⁻¹ streptomycin, 100 units ml⁻¹ penicillin and 25 μg ml⁻¹ fungizone (Gibco).

**Surgical procedures and histology**

Operations were generally performed by dissecting tissues with glass needles in 100% Steinberg’s solution. In some cases, removal of the optic vesicle from overlying presumptive lens ectoderm was accomplished by brief treatment (15–20 min) of embryos in 2 x 100% Steinberg’s solution, since this procedure facilitated clean removal of the eye rudiment from overlying ectoderm. These treatments do not appear to have any effects on lens or eye development, nor on the inductive capacity of the eye or responsiveness of the ectoderm (R. M. Grainger & J. E. Mannion, unpublished data). After healing of transplants in 100% Steinberg’s solution (approximately 1 h), embryos were transferred to 70% Steinberg’s (1 h) then 50% Steinberg’s (1 h), and finally returned to 20% Steinberg’s solution. In experiments involving ectoderm transplants over the optic vesicle, the transplanted ectoderm was held in place with a siliconized glass bridge until healing occurred (approximately 1 h). In experiments with *Xenopus laevis* embryos, either host or donor tissues were labelled with horseradish peroxidase (HRP) as described by Henry & Grainger (1987).

The temperature at which embryos were raised both before and after surgery was carefully controlled to obviate possible effects of temperature on lens cell determination. In *Xenopus* transplants involving gastrula ectoderm, all embryos were raised at 16°C from gastrula stages onward since this gave a greater fraction of positive lens-forming responses in ventral ectoderm than tissues from embryos raised at higher temperature: This result may be due to the stronger effects of early inductors at lower temperatures, as suggested by other studies (Jacobson, 1958). *Rana* embryos were raised at 16, 20 or 23°C until the time that operations were performed. Afterwards these embryos were raised at 20°C.

*Xenopus* embryos were harvested at stage 40 of Nieuwkoop & Faber (1967), and those embryos labelled with HRP were fixed, sectioned and stained as described by Henry & Grainger (1987). In some experiments, embryos were fixed in Bouin’s solution, embedded in paraffin, sectioned at a thickness of 8 μm, and stained with haematoxylin and eosin. 5 days after transplants were performed, *Rana* embryos were also fixed in Bouin’s solution and embedded and sectioned as for *Xenopus* embryos.
Results

Transplantation experiments in *Xenopus laevis* embryos

In order to test whether the optic vesicle can stimulate lens formation in nonlens ectoderm, we performed a series of optic vesicle transplants in *Xenopus laevis* because of a report (Brahma, 1959) that concluded that early or late gastrula ectoderm from *Xenopus* embryos can form a lens when exposed to the optic vesicle of early-tailbud-stage embryos [stage 24 of Nieuwkoop & Faber (1967)]. We took optic vesicles shortly after their formation (stages 22-23) and transplanted them beneath flank or belly ectoderm of neurula (stages 14-16) embryos. The transplants often formed well-differentiated eye cups, which were associated with lenses (Fig. 1A). As controls, some transplanted optic vesicles were rotated to point inwards, since one would not expect to find induced lenses within the eye cup in such cases. In these control experiments, however, optic tissue was often associated with lenses (Fig. 1B). At the stages used in these transplants, the optic vesicle was in contact with the presumptive lens ectoderm and we were concerned that some presumptive lens cells had remained adherent to the eye tissue when it was transplanted. Histological examination of isolated optic vesicles confirmed the presence of patches of adhering ectoderm.

To demonstrate more clearly whether lenses formed in this kind of transplantation experiment were induced from host tissue or derived from contaminating donor lens cells, either host or donor embryos were labelled with the cell-autonomous lineage tracer horseradish peroxidase (HRP) (Jacobson & Hirose, 1978; Weisblat et al. 1978). Embryos were labelled by injection at the 1-cell stage. All lenses formed in these experiments were found to be derived from contaminating donor tissue (Fig. 2A). In 17 transplants, 16 had lenses, all of which were of donor origin. It is exceedingly difficult to remove all presumptive lens ectoderm cells from optic vesicles at this stage. We found, however, that clean optic vesicles could often be isolated at slightly earlier stages (just after the closure of the neural folds; stages 18–19), when the forebrain is just approaching the presumptive lens ectoderm. When the forebrain from this stage, or from the presumptive eye region of earlier neural-plate-stage embryos (stage 14), was transplanted beneath neurula (stages 14–16) belly ectoderm, no induced lenses were observed (Fig. 2B). In ten cases of such transplants, two samples had lenses both of which were of donor origin. Because of the obvious importance in determining whether lenses were derived from host or donor tissue, in all subsequent experiments with *Xenopus* either host or donor tissues were labelled with HRP.

While these experiments demonstrate that lenses cannot be induced in neurula belly ectoderm by the optic vesicle, we were not certain whether this was the result of a weak inductive capacity of the eye rudiment or inability of the ectoderm at this stage to respond to the stimulus from the eye tissue. This question was addressed as part of a comprehensive analysis of the lens-forming potential of ectoderm in *Xenopus* embryos (Henry & Grainger, 1987). The ability of belly ectoderm to form lenses was assessed by transplanting it to the presumptive lens region of neural-plate-stage hosts (stage 14) so that it would be exposed not only to the optic vesicle but to early lens inducers as well. Ectoderm from late neurulae (stage 18) does not form lenses under these circumstances. However, lenses are induced in gastrula (stages 18–20) when placed in host embryos at these stages.

**Fig. 1.** Transplantation of optic vesicles beneath flank or belly ectoderm in *Xenopus laevis*. Optic vesicles were surgically excised from stage 22–23 embryos after removal of the ectoderm overlying the eye rudiment. Eye tissue was then implanted beneath flank or belly ectoderm of stage-14 to -16 embryos by making a slit in the ectoderm and inserting the optic vesicle. Embryos in this series were raised at either 16 or 23°C until approximately stage 40. (A) Example of whole embryo with transplanted eye facing the ectoderm and containing a well-developed lens. Bar, 1 mm. (B) Section through a transplanted eye cup (ec) facing away from flank ectoderm (fe) but associated with a well-developed lens (l). Bar, 100 μm.
Fig. 2. Transplantation of HRP-labelled eye rudiments beneath unlabelled flank or belly ectoderm in *Xenopus laevis*. Donor embryos at the 1-cell stage were injected with HRP. Eye tissues from labelled embryos were isolated and transplanted into unlabelled stage-14 to -16 hosts which were raised until stage 40 when they were fixed, sectioned and stained with diaminobenzidine to detect HRP. (A) Donor optic vesicle was transplanted from a stage-22 to -23 embryo. Both the eye tissue and lens are labelled, indicating that the latter is derived from donor tissue. (B) Here the optic rudiment (as well as most of the forebrain) was taken from a stage-14 embryo. No lens is found associated with the eye tissue, which in this case is collapsed. Tissues are labelled as in Fig. 1. Bar, 100 μm.

10–11) presumptive ventral ectoderm. There is a gradual decrease in the ability of this ectoderm to be induced to form lenses during gastrulation and neurulation, presumably reflecting its gradual determination for epidermis formation.

From the experiments of Henry & Grainger (1987), it is clear that ventral ectoderm from neurulae no longer has the potential to form lenses, and therefore one would not expect that lenses could be induced when optic vesicles are transplanted beneath neurula ectoderm. At earlier stages, however, presumptive ventral ectoderm can still form lenses; these results prompted us to test whether exposure of this ectoderm to the optic vesicle alone was sufficient to induce lens formation. This inductive capacity was tested in two ways. First, HRP-labelled gastrula presumptive ventral ectoderm (stages 10.5–11.5) was transplanted over the forebrain of unlabelled host embryos at the stage just after the closure of the neural folds (stages 18–19). In most cases, no lens-like response was detected (Fig. 3A; Table 1, *X. laevis* series 1). Two transplants (8% of the total) developed very small lentoids. One was composed solely of donor cells (Fig. 3B) and the other, approximately the same size, of both donor and host cells. The fraction of cases with a lens-like response is small and the response is quite weak when compared to transplants of stage-10.5 to -11 gastrula presumptive ventral ectoderm that is transplanted to the presumptive lens area of neural-plate-stage hosts (stage 14) and thus is exposed to both early lens inductors and the optic vesicle as mentioned above. In these cases induced lenses were much larger and constituted a higher fraction (29%) of transplants (Henry & Grainger, 1987). The importance of the HRP labeling of gastrula ectoderm in this series of experiments is underscored in Fig. 3C, a case in which a lens is present, but clearly derived from presumptive lens ectoderm cells contaminating the host eye tissue.

While these results suggest that the optic vesicle may be able to induce lentoids in a small fraction of the cases in ectoderm not exposed to early inductors, there are other possible inductive influences from tissues adjacent to the optic vesicle in this kind of experiment. At the stage when the neural folds are closing, the optic vesicle is not yet completely in contact with the presumptive lens ectoderm (Holt, 1980). The ventral part of the eye rudiment does not yet touch the overlying ectoderm, which is thus exposed to adjacent mesoderm that may also act as an inductor. In addition, when the presumptive lens ectoderm is removed during the transplantation procedure, mesoderm surrounding the eye has a tendency to migrate over it, and may, at least transiently, have an effect on the transplanted ectoderm. There is evidence that head mesoderm at these stages does act as a lens inductor (Mikami, 1938, 1939; Okada, 1949). It has also been argued that there may be inductive influences from ectoderm adjacent to the presumptive lens region (Reyer, 1962). To eliminate these potential inductive effects, a second kind of transplantation experiment was performed. Forebrain tissue (including the presumptive optic region) from embryos at the stage just after neural fold closure (stages 18–19) was isolated and combined with gastrula (stages 10.5–11.5) presumptive ventral ectoderm. These recombinants were implanted in the flank region of host neurulae. This direct recombination of eye tissue and ectoderm obviates any potential effects of tissues in the region of the optic vesicle. In these transplants, no lens-forming response was detected (Table 1, *X. laevis* series 2).
Fig. 3. Tests of the ability of the optic vesicle to induce lenses in presumptive belly ectoderm from *Xenopus* gastrulae. (A) HRP-labelled stage-10-5 presumptive ventral ectoderm was transplanted over the forebrain of an unlabelled stage-18 host after removal of the presumptive lens ectoderm. The labelled ectoderm (le) shows no lens-forming response as in most of these transplants. The unlabelled host neural retina (nr) and pigmented retina (pr) are also denoted. (B) Same operation as in A; the only clear case that was seen of a small lentoid completely of donor origin. (C) Operation as in A and B shows a case in which a lens (denoted hl) associated with the host eye tissue must have arisen from contaminating presumptive lens ectoderm cells. Some labelled cells (le) have detached from the donor ectodermal transplant adjacent to the optic vesicle. (D) HRP-labelled stage-10-5 presumptive ventral ectoderm was transplanted over the isolated forebrain of an unlabelled stage-18 host. These two tissues were allowed to adhere to one another for approximately 1 h and then placed in the flank of an unlabelled stage-15 embryo from which a piece of ectoderm had been removed. No lens-forming response was seen in the labelled ectoderm. The neural retina in this case remains uninvaginated. Photographs are labelled as in previous figures. Bar, 100 \(\mu m\).

Since this experimental protocol involves several manipulations, it is possible that the optic vesicle is not as potent an inductor as it might be *in situ*. In these recombinants, however, eye tissue was still well differentiated, forming pigmented and neural layers. In the absence of lens formation, however, we often see that the optic vesicle does not invaginate to form an optic cup (Fig. 3D). This finding is similar to that observed during the previous series in which gastrula ectoderm was placed over the forebrain.

Together these results argue that in *Xenopus* the optic vesicle is not sufficient to elicit lens formation in embryonic ectoderm even though it has the potential for lens formation.

**Eye vesicle transplants in *Rana palustris* embryos**

As mentioned earlier, similar experiments of Lewis (1904, 1907) using *Rana palustris* and *Rana sylvatica* concluded that the optic vesicle alone can stimulate lens formation in nonlens ectoderm from embryos at the stage of neural fold closure. In most of these experiments, however, the optic vesicle was moved beneath nonlens ectoderm in the head region. Since large regions of head ectoderm are exposed to early lens inductors (Jacobson, 1966; R. M. Grainger & J. E. Mannion, unpublished data), this ectoderm may have already obtained a predisposition for lens formation. In addition, Lewis did not use a host and donor marking scheme and so there is at least the possibility that some lenses may have arisen from presumptive lens ectoderm adhering to transplanted optic vesicles. These concerns are supported by those cases, similar to our Fig. 1B, of optic vesicles, implanted facing away from the ectoderm, that are associated with lenses (Lewis, 1907).

Because of these concerns, we repeated optic vesicle transplants in *Rana palustris*, at the same
In many (as in our experiments with *Xenopus*), there were other responses from ventral ectoderm, which were scored as well.

Table 1. Ability of optic vesicle to induce lenses in ventral or lateral ectoderm

<table>
<thead>
<tr>
<th>Experimental series</th>
<th>Ectoderm stage</th>
<th>Optic vesicle stage</th>
<th>Number examined</th>
<th>Induced lens or lens-like response</th>
<th>Cellular aggregate or ectodermal thickening</th>
<th>No response</th>
<th>Lens derived from cell contamination</th>
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</thead>
<tbody>
<tr>
<td><em>X. laevis</em> 1*</td>
<td>gastrula (st. 10.5–11.5)</td>
<td>closed neural fold (st. 18–19)</td>
<td>24</td>
<td>2</td>
<td>13</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td><em>X. laevis</em> 2†</td>
<td>gastrula (st. 10.5–11.5)</td>
<td>closed neural fold (st. 18–19)</td>
<td>17</td>
<td>0</td>
<td>6</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td><em>R. palustris</em> (eye outward)</td>
<td>closed neural fold</td>
<td>closed neural fold</td>
<td>22</td>
<td>0</td>
<td>13</td>
<td>9</td>
<td>–</td>
</tr>
<tr>
<td><em>R. palustris</em> (eye inward)</td>
<td>closed neural fold</td>
<td>closed neural fold</td>
<td>13</td>
<td>–</td>
<td>8</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
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Transplants were scored according to several criteria. The number examined comprises those cases in which an interpretable response was discerned. Transplants with *Xenopus laevis* embryos were not included if HRP staining of host or donor tissues was ambiguous, if the graft did not completely cover the eye tissue, or if the optic vesicle did not differentiate into clearly delineated pigmented and neural retina. Approximately 50% of the cases had to be discarded for one of these reasons. In transplants with *Rana palustris*, approximately 10% of the cases were not counted because eye tissue was not well differentiated. The category 'induced lens or lens-like response' includes all cases in which either a lens or lentoid was formed that had characteristic features of lens tissue. In the frozen sections used to assay lens differentiation in *Xenopus*, lens cells have a characteristic translucent appearance and completely lack pigment granules found in the outer layer of ectoderm. Cellular aggregates or ectodermal thickenings were cases that were not scored as lens-like responses because the cells involved lacked these features. In general, ectodermal thickenings involved both inner and outer layers of ectoderm and were pigmented, often very extensively, sometimes exhibiting features characteristic of the cement gland. Occasionally, these thickenings had characteristics of neural tissue. Cellular aggregates and ectodermal thickenings were often not located directly overlying eye tissue, but adjacent to it, consistent with an interpretation that some of these formations might be incidental to effects of the eye vesicle. Some had features characteristic of healing artifacts. The category 'lenses derived from cell contamination' comprised cases in which HRP labelling indicated that lenses resulted from presumptive lens ectodermal contamination of the optic vesicle. In many such embryos, there were other responses from ventral ectoderm, which were scored as well.

* In this series, presumptive ventral ectoderm was placed over an optic vesicle from which the overlying presumptive lens ectoderm had been removed. Either host or donor tissue was labelled with HRP. One of the two lens-like responses in this series was a very small lentoid which was composed of both host and donor cells.

† In this series, HRP-labelled presumptive ventral ectoderm was placed over an isolated piece of unlabelled forebrain, in contact with the presumptive optic region and this recombinant placed into the flank of an unlabelled neurula host embryo (stages 14–17) after removal of the ectoderm from that site.

Stages used by Lewis, but placed the transplanted tissues some distance away from head ectoderm, either beneath lateral or ventral ectoderm. Although we attempted to use HRP as a lineage tracer here, it was found to be toxic in preliminary experiments. We discovered, however, that it was consistently possible to isolate optic vesicles free of adhering presumptive lens ectoderm, but only if the surgery was done in a relatively high-ionic-strength medium [in twice-full-strength Steinberg’s solution]. This treatment during surgery appears to have no effect on eye or lens development in sham operations in which the presumptive lens ectoderm is removed and then replaced on the optic vesicle. To ensure that contamination was not a problem, optic vesicles were also inserted facing away from ectoderm to test for the presence of selfdifferentiating presumptive lens cells. In no cases with eye vesicles facing the ectoderm were any definitive lenses or lentoids observed (Fig. 4A; Table 1). The optic rudiment consistently differentiated into pigmented and neural retina, though invagination of the optic vesicle was often incomplete (as in our experiments with *Xenopus*). In many embryos, there were substantial thickenings in the ectoderm overlying the transplanted eye tissue (Fig. 4B; Table 1). These had none of the properties characteristic of early lens differentiation, which is typified by thickening in the ectoderm, but which also involves an elongation and parallel orientation of cells of the inner, sensorial ectodermal layer (from which the lens is derived) as well as a loss of pigment granules from this layer (Mangold, 1931). In contrast to normal lens differentiation, here the thickenings were always composed of randomly oriented pigmented cells. In addition, these thickenings appeared to involve the outermost layer of ectoderm and often resulted in a bulge extending out from the ectoderm. We also saw some ectodermal thickenings even when the eye was facing away from ectoderm (Fig. 4C; Table 1). No definitive lenses or lentoids were seen in association with eye tissue facing away from the ectoderm in these experiments. It may be that eye or brain tissue elicits hyperplasia in overlying ectoderm. It is also possible that some thickenings were a result of healing artifacts due to damage of the ectoderm during the transplantation procedure. There is no evidence, however, of a lens-forming response.
Embryonic lens induction

Fig. 4. Transplantation of the forebrain beneath belly ectoderm of *Rana palustris* embryos. Both host and donor embryos were at stages shortly after closure of the neural folds. To remove all of the presumptive lens ectoderm from forebrain tissue it was necessary to treat embryos briefly in 2× 100% Steinberg’s solution (see Materials and methods). A small slit was made in the belly ectoderm of the host and the forebrain then implanted beneath belly ectoderm. (A) No response is discerned in the ectoderm overlying the optic cup, which in this case is facing the ectoderm. (B) In this transplant, a highly pigmented thickening (*pt*) is seen in the ectoderm overlying the optic cup, which again faces the ectoderm. (C) Here the optic cup is turned away from the ectoderm. There is no evidence of lens cells associated with the eye tissue. A small thickening (*t*) is seen, however, in the ectoderm adjacent to the eye. Photographs are labelled as in previous figures. Bar, 100 μm.

Discussion

Reevaluation of earlier transplant studies

Our results with *Xenopus laevis* and *Rana palustris* question earlier studies suggesting that the optic vesicle is sufficient to induce lens formation in lateral or ventral ectoderm. Our first major concern is with possible artifacts in these earlier studies and other similar experiments, very few of which used careful host and donor marking schemes. This problem has also been raised by other investigators (Spemann, 1908, 1912; Stone & Dinnean, 1943; Liedke, 1951). In some reports (von Ubisch, 1927), tissues have been labelled with vital dyes to ensure that a transplanted piece of ectoderm covered the eye, but it could not be determined whether lenses that formed subsequently arose from this labelled tissue. As is clear from our studies (e.g. Fig. 3C), the ectoderm may cover the eye, yet a lens can still form from residual presumptive lens ectoderm which had previously been incompletely removed. Many authors also have concluded that contact of the developing lens with overlying ectoderm implies that the lens is derived from the ectoderm (von Ubisch, 1927; Adelmann, 1928). Again Fig. 3C shows that this can be a misleading criterion. In numerous reports, host and donor have been from different species and could be identified in theory by unique pigmentation, yolk-granule-staining properties, and cell or nuclear size. There are severe limitations, however, in using this approach. Differences in cell or nuclear size are only useful in this regard when the differences are substantial, as they can be, for example, if transplants are done between urodeles and anurans (Hewitt, 1932). Even with these combinations, however, size classes overlap and ambiguous cases can arise (Liedke, 1955). Pigmentation markers are often lost during lens differentiation and staining properties may also change; these are therefore not necessarily reliable indices of species origin (Liedke, 1955; Reyer, 1958a).

One might argue that different experimental conditions, particularly the temperature at which embryos are raised, could possibly account for differences in the outcome of our lens induction studies and other published studies; however, we believe this is unlike. Discrepancies in several studies of the role of early lens inductors have been traced to differences in the temperature at which embryos were allowed to develop (discussed by Jacobson, 1958). At lower temperatures, embryos from some species can form lenses in the absence of the optic vesicle, while at higher temperatures they may not. This is apparently because at lower temperatures early inductors have a longer time to act and ectoderm a longer time to respond, an effect which appears not to be mitigated by a lower metabolic rate. Whether temperature
similarly affects the response of ectoderm to the optic vesicle is not clear. In previous work on *Xenopus* (Brahma, 1959) where it was argued that the optic vesicle can induce lenses, the temperature of development was 18–22°C. In our studies, donor ectoderm came from embryos raised at 16°C; after transplantation, host embryos were also raised at 16°C. This temperature was chosen because gastrula presumptive ventral ectoderm was found to give a stronger lens-forming response to lens inductors at lower temperatures. In our early experiments, done at neurula stages (e.g. Fig. 2B), embryos were raised at 16 or 23°C with no effect on induction of lenses. In published studies on *R. palustris* (Lewis, 1904), temperatures of development were not reported. In *R. palustris* our results were the same whether embryos were raised at 16, 20 or 23°C. While we have not completed an exhaustive analysis, our data do not suggest that temperature differences account for the differences between our results and those of previous workers.

**Lens induction by the optic vesicle and models of cell determination**

The results presented here support the conclusion that in *Xenopus laevis* embryos the optic vesicle is at most a very weak lens inductor in ventral ectoderm at stages in which this ectoderm can be shown to have the potential for lens formation (Henry & Grainger, 1987). The only other organism in which similar studies have been done, using host and donor marking, is *Ambystoma punctatum*; these reports all reached essentially the same conclusion (Stone & Dinnean, 1943; Liedke, 1951; Reyer, 1958a). Although these studies were done with vital dye marking to identify host and donor tissues in histological sections, there were many specimens in which the authors questioned the reliability of such dye markers because they were often lost during lens differentiation. The clarity of HRP labelling in our experiments eliminates this possible source of ambiguity.

Our eye vesicle transplants with *Rana palustris* were done on the same organism and during the same stages in which it had been argued that the optic vesicle alone could elicit lens formation in nonlens ectoderm (Lewis, 1904). Since this study was done at neural tube stages when ventral ectoderm may have lost its lens-forming potential (as it has in *Xenopus*) it is possible that the optic vesicle would be sufficient to elicit lens formation from ventral ectoderm at earlier stages. In preliminary results with *Ambystoma mexicanum* embryos (R. Grainger, unpublished data), we have also been unable to confirm published results that the optic vesicle can induce lenses in ventral ectoderm at neural tube stages (Woerdeman, 1938).

While we do not know whether our conclusions about the inductive capacity of the optic vesicle extend to all amphibians, there is at least some uncertainty about most studies on other organisms since virtually none of them utilized an unambiguous host and donor marking procedure. There may, however, be a stronger inductive capacity of the eye vesicle or differences in responsiveness of belly or flank ectoderm in organisms we have not examined. The results from the organisms we have studied, together with the data from *Ambystoma punctatum* (Stone & Dinnean, 1943; Liedke, 1951; Reyer, 1958a), nevertheless constitute the most unambiguous data available about whether the optic vesicle alone can cause lens induction and support a model of amphibian lens induction in which the optic vesicle is not generally sufficient for lens formation. These experiments focus attention on the role of early inductive interactions in the lens induction process. Recent models for lens induction (Jacobson, 1966) stress the significance of early inductive interactions in lens formation. Jacobson (1958) argues that these early inductive effects are essential for lens development in the salamander *Taricha torosa*. The data discussed here lead us to expand this argument and propose that early inductive effects become an essential part of the lens determination process in other amphibians as well if the optic vesicle alone cannot generally elicit lens formation. While these early inductive interactions are not well studied, their effects have been clearly documented, giving rise to what might be described as a lens-forming bias in head ectoderm (Jacobson, 1966; Karkinen-Jääskeläinen, 1978; Henry & Grainger, 1987). The evidence for free lens formation, discussed earlier, provides support for the strong influence of early inductors. The acquisition of a lens-forming bias in head ectoderm appears to be a discrete step in the lens induction process occurring after it can be demonstrated that ectoderm has the potential for lens formation at the gastrula stage (Henry & Grainger, 1987).

While the role of the optic vesicle may depend on earlier inductive effects, it nonetheless could have a critical function in the determination process. Which region of head ectoderm will form lenses would appear to be the result of where the optic vesicle contacts this ectoderm. This is clear from the formation of lenses in the large, aberrantly placed eyes of cyclopean embryos (Stone & Dinnean, 1943) and from transplants of nonlens head ectoderm over optic vesicles in experiments using careful host and donor marking (Reyer, 1958b; R. M. Grainger & J. E. Mannion, unpublished data). In addition, the optic vesicle may provide important factors for lens differentiation (Beebe et al. 1980).
Our conclusions about the lens induction process bear on other more general models that are derived, at least in part, from analyses of lens induction. One of these is Spemann’s principle of double assurance (Spemann, 1938), which proposes that there is a natural redundancy in developmental mechanisms. He argues that determination of the lens is a primary example, since experiments had concluded that it could form either as a result of early factors or the late inductive influence of the optic vesicle. Since our work and that on Ambystoma punctatum (Reyer, 1958a) argue that the optic vesicle is generally not sufficient for lens induction there is therefore not likely to be a simple redundancy in the process, but two separate contributing elements.

Experiments arguing that the optic vesicle alone can elicit lens formation in ectoderm have contributed significantly to the view that vertebrate cell determination may be controlled by single inductive events. Our data certainly suggest that this model may not be an accurate one for lens induction. Induction of several other tissues appears to involve multiple steps; this is especially clear in nose and ear induction. In fact, the early phases of induction of lens, nose and ear tissues appear to be occurring simultaneously in head ectoderm (Jacobson, 1966). Other determination events are often cited as single-step processes, notably mesoderm induction and neural induction, though in both cases these events are in actuality quite complex (Gurdon, 1987). Further studies are required to ascertain whether multiple steps are essential for induction of these tissues, as we argue they are for lens induction.

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R. M. Grainger, J. J. Henry and R. A. Henderson


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