New Perspectives on Embryonic Lens Induction

Robert M. Grainger[1]
and
Jonathan J. Henry[2]*

[1]Dept. of Biology
University of Virginia
Charlottesville VA 22901
and
[2]Dept. of Biology
Indiana University
Bloomington IN. 47405

The earliest experiments defining the concept of embryonic induction, done at the turn of the century, were those concerning induction of the lens by the optic vesicle. By this time it was already clear that the lens formed from ectoderm overlying the optic vesicle (the spatial relationship of the presumptive lens ectoderm and optic rudiment in the late neurula can be seen in Fig. 1D). Observations on many kinds of defective embryos had suggested to embryologists that the lens was dependent on the eye rudiment for its formation. For example, in cyclopean embryos the large median eye was always seen to be associated with a single lens, also derived from medial ectoderm, not the lateral ectoderm that normally gives rise to the lens.

Two kinds of experiments from this period defined the basic conceptual framework for lens induction. First, it was found that if the optic rudiment is removed from neural-plate-stage embryos, not only is eye tissue missing from these embryos, but so is the lens. Thus it was argued that the eye was necessary for lens formation. In the second kind of experiment, the optic rudiment was transplanted beneath non-lens ectoderm, and a lens was found in the ectopic location. These classic experiments, as well as others (reviewed by Saha et al., 1989) supported the conclusion that the optic vesicle was not only necessary, but sufficient for lens induction. This view of lens induction has been widely cited since this time, and has provided a framework for thinking about induction in general: that such simple interactions can lead to determination of vertebrate tissues.

**Authors listed in alphabetical order*

Over the past several years we have reinvestigated many of the older experiments, and we have arrived at a somewhat more complex view of the lens induction process. We believe that there are at least three steps to the process. First ectoderm becomes competent to respond to lens-inducing signals during mid-gastrulation. During this competent period the second phase of the process is initiated: large regions of presumptive head ectoderm begin to acquire a lens-forming bias. Finally, determination of the lens in a particular region of this ectoderm occurs by the time of neural tube closure. Although this view of lens induction as a multiple-step process is quite different from the classical view, it probably still serves as an excellent model for many vertebrate inductive interactions, many of which are now being shown to involve multiple steps (see discussion by Saha et al., 1989).

Our proposal that lens induction is a multiple-step process resulted from experiments done several years ago in which we attempted to repeat the classic experiments indicating that the optic vesicle was sufficient for lens induction. Our conclusion was that, at least in some amphibian species, the optic vesicle is not sufficient (Henry and Grainger, 1987; Grainger, Henry and Henderson, 1988). When we attempted to transplant the optic vesicle beneath flank ectoderm of host embryos as in earlier reports, we found that lenses were often found associated with the transplant, but host and donor labeling procedures showed that these lenses were derived from donor tissue. We established that presumptive lens cells often adhered to the optic vesicle when we attempted to isolate the optic rudiment for transplantation. By using slightly earlier developmental stages, we were able to remove the optic vesicle cleanly and to show that it was unable to induce flank ectoderm to form lenses. In fact we were unable to obtain lens induction by the optic vesicle from ectoderm of any stage tested (from early gastrula to late neurula). Although very few earlier studies used host and donor marking to assess the contamination problem, the few that did concurred with our findings. We concluded from these studies that lens induction was likely to require at least two steps, since the optic vesicle alone could not induce lenses.

Our studies (as well as others published earlier) argued that important inductive signals affecting head ectoderm precede its contact with the optic vesicle. While gastrula ectoderm transplanted to the presumptive

lens area of neural-tube-stage hosts (thereby being exposed to the optic vesicle as its only inducer) did not form lenses, we found that the same ectoderm transplanted to the presumptive lens area of neural-plate-stage hosts did form lenses. This result implied that an essential inductive influence was being imparted to this ectoderm between the neural plate and neural tube stage. The source of this early inductive signal was the subject of a recent study (Henry and Grainger, 1990). There are two obvious possibilities. Either the early signal comes from tissues underlying the presumptive lens area at these early stages, or it is generated from the ectoderm surrounding the presumptive lens area, for example, from the adjacent neural plate. These spatial relationships can be seen in the neural-plate-stage embryo shown in Fig. 1C. Although earlier reports had argued that the underlying tissues were the source of important early inductive signals, we found that, at least in *Xenopus*, the neural plate is a more potent early lens inductor. The underlying tissues do potentiate the effect of the neural plate, however.

If the early signal in lens induction comes from the neural plate, when does it begin to act? Does lens induction commence only after neural plate formation? Experiments defining the period during which ectoderm is able to initiate the lens induction process (its competent period) were quite suprising (Servetnick and Grainger, 1991). There is a very narrow window (only a few hours) when it is possible to initiate lens induction, during mid to late gastrula stages. Prior to these stages ectoderm has a strong neural competence, but no lens-forming competence. After these stages, ectoderm is competent to form some placodal structures (e.g., ear vesicles) but not lens. Thus the lens induction process must begin during gastrulation, and therefore the initial signal must reach the presumptive lens ectoderm, probably from the presumptive neural area, at this stage.

Our current model for lens induction is summarized in Figure 1. At the late blastula stage (Figure 1A) ectoderm is not yet competent for lens formation. At the mid gastrula stage (Figure 1B) ectoderm has a brief period of lens competence, and we believe that the initial lens-inducing signals are generated from presumptive neural tissue at this time. The presumptive foregut endoderm, which underlies the presumptive lens area, may contribute to the lens induction signal as well. By the neural plate stage (Figure 1C) lens induction is well underway. A large region of head ectoderm has a lens-forming bias, again, at least in large part due to signals from the neural plate. By this stage the presumptive lens ectoderm is underlain by presumptive heart mesoderm, which probably contributes to the lens-forming response. By the time the embryo reaches the neural tube stage, when the optic vesicle first reaches the presumptive lens ectoderm (Figure 1D), lens determination is largely complete. The optic vesicle is likely to contribute to the final stages of lens determination by pinpointing the exact site of lens formation within a larger region of induced head ectoderm. The presence of the eye cup certainly is important for proper lens differentiation.

The elucidation of these three stages in the lens determination process will permit investigation of several important mechanisms in induction. For example, the changes in gene expression in ectoderm causing the appearance and disappearance of competence, and gain of a lens-forming bias can now be studied. These embryological studies should also make it feasible to begin to identify inducing factors produced by specific tissues which lead to the lens-forming responses.

Literature Cited

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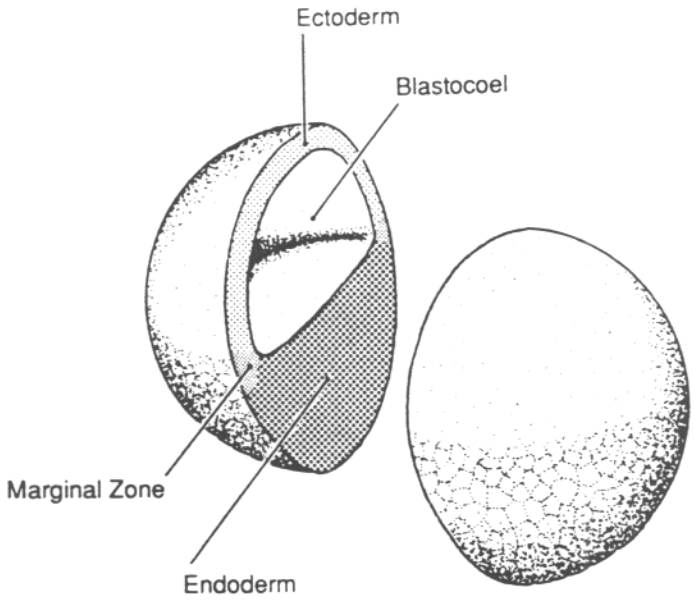
Figure 1. Overview of the process of lens induction.

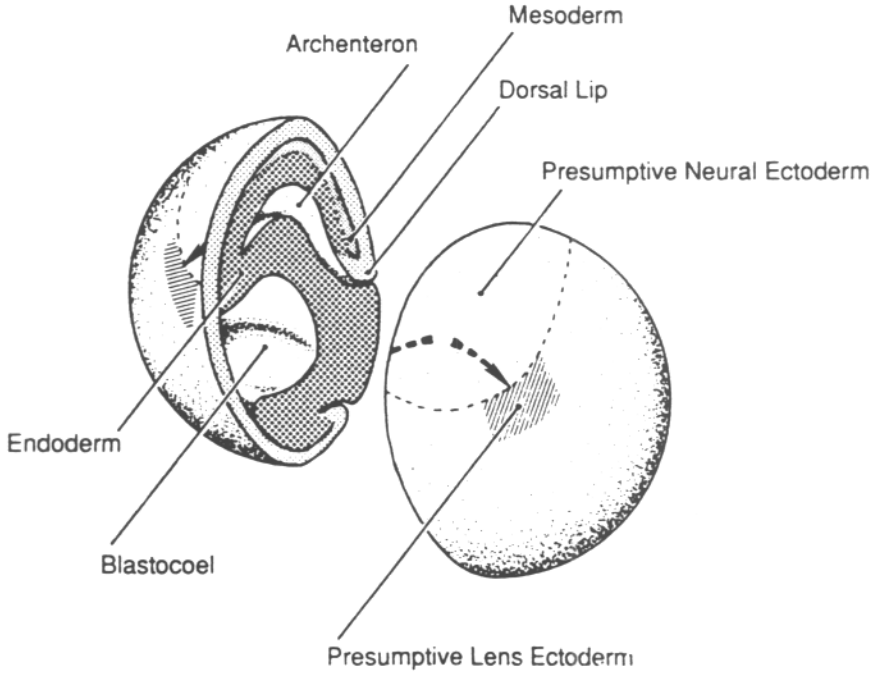
A. Late blastula stage. All body wall epidermis, neural tissue, and placodal tissues such as the lens are derived from the ectoderm. At this stage ectoderm is not yet competent for lens formation, however.

B. Mid-gastrula stage. This is the stage at which lens induction commences, since it is the only stage during which ectoderm is competent to respond to lens inductive interactions. During this period the presumptive lens ectoderm first comes into contact with tissue which has involuted during gastrulation, namely the presumptive foregut endoderm. The latter tissue may play a role in lens determination. Studies in *Xenopus* show that an interaction, which is essential for lens induction, occurs between presumptive lens ectoderm and the anterior neural plate during this period (illustrated by the arrows).

A


Ectoderm
Blastocoel
Marginal Zone
Endoderm

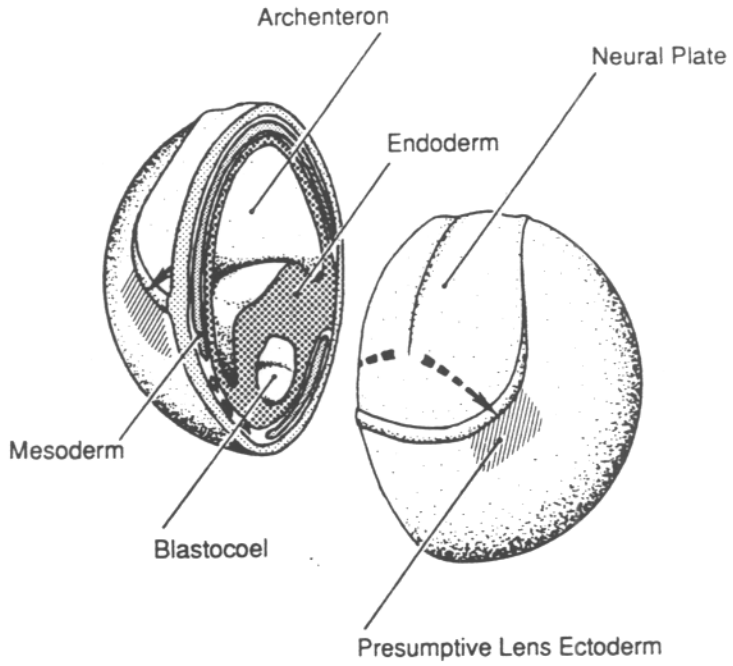
B


Mesoderm
Archenteron
Dorsal Lip
Presumptive Neural Ectoderm
Endoderm
Blastocoel
Presumptive Lens Ectoderm

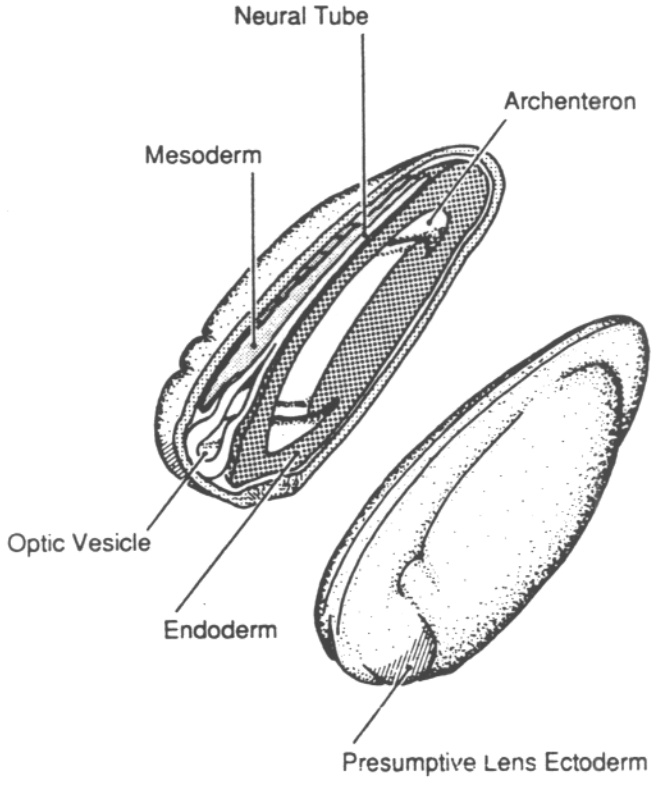
C. Neural plate stage. At this time the presumptive lens ectoderm is underlain by presumptive heart mesoderm, which appears to enhance inductive effects from the anterior neural plate. A lens-forming bias is being established throughout head ectoderm at this stage.

D. Neural tube stage. At this time the presumptive lens ectoderm first comes in contact with the newly formed optic vesicles. Inductive interactions between these tissues, while not sufficient for lens formation, pinpoint the exact location of lens formation in head ectoderm, which has a very strong lens-forming bias by this stage. The eye cup also enhances lens cell differentiation, and its presence is required for the continued development of the lens.

C


Archenteron
Neural Plate
Endoderm
Mesoderm
Blastocoel
Presumptive Lens Ectoderm

D


Neural Tube
Archenteron
Mesoderm
Optic Vesicle
Endoderm
Presumptive Lens Ectoderm