Changes in neural and lens competence in *Xenopus* ectoderm: evidence for an autonomous developmental timer

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Summary

The ability of a tissue to respond to induction, termed its *competence*, is often critical in determining both the timing of inductive interactions and the extent of induced tissue. We have examined the lens-forming competence of *Xenopus* embryonic ectoderm by transplanting it into the presumptive lens region of open neural plate stage embryos. We find that early gastrula ectoderm has little lens-forming competence, but instead forms neural tissue, despite its location outside the neural plate; we believe that the transplants are being neuralized by a signal originating in the host neural plate. This neural competence is not localized to a particular region within the ectoderm since both dorsal and ventral portions of early gastrula ectoderm show the same response. As ectoderm is taken from gastrulae of increasing age, its neural competence is gradually lost, while lens competence appears and then rapidly disappears during later gastrula stages. To determine whether these developmental changes in competence result from tissue interactions during gastrulation, or are due to autonomous changes within the ectoderm itself, ectoderm was removed from early gastrulae and cultured for various periods of time before transplantation. The loss of neural competence, and the gain and loss of lens competence, all occur in ectoderm cultured *in vitro* with approximately the same time course as seen in ectoderm *in vivo*. Thus, at least from the beginning of gastrulation onwards, changes in competence occur autonomously within ectoderm. We propose that there is a developmental timing mechanism in embryonic ectoderm that specifies a sequence of competences solely on the basis of the age of the ectoderm.

Key words. competence, induction, neural, lens, *Xenopus*, developmental timer

Introduction

Embryonic induction is often assumed to be regulated by the temporally or spatially restricted presentation of inducer molecules. However, it is clear that competence, the ability of a tissue to respond to an inductive signal (Lehmann, 1929; Mangold, 1929; Waddington, 1932), often plays a critical role in regulating induction (see Jacobson and Sater, 1988). Although progress has been made in understanding the nature of inducing signals, particularly in the induction of mesoderm (reviewed by Smith, 1989), very little is understood about what defines the state of competence in any inductive system. It has been hypothesized that competence corresponds to the presence of receptors for inducing molecules (for example, Gillespie *et al.*, 1989); however, while the control of competence is likely to involve some aspect of signal transduction, changes in competence could also be effected by alterations in any of a number of downstream events in the signal transduction process.

The role of competence in regulating induction has been argued in a number of inductive systems. In amphibian mesoderm induction, the loss of mesodermal competence may regulate the number of animal cells that form mesoderm (Gurdon, 1989). Similarly, neural inductive signals are present in the embryo long after the ectoderm has lost neural competence (Holtfreter, 1933; Jones and Woodland, 1989), suggesting that the amount of tissue that is neuralized may be limited solely by the loss of competence within the responding ectoderm (Albers, 1987).

Holtfreter (1938) was also the first to suggest that changes in competence might occur autonomously in ectoderm. He cultured ectoderm *in vitro* for various periods of time, then exposed it to the same inductor tissue. The ectoderm responded in different ways depending on its age: young ectoderm formed predominantly neural tissue, while older tissue lost its neural competence but continued to form other ectodermal derivatives, showing that the fate of the responding tissue is apparently dependent on its state of competence, not on the inducing tissue. The loss of mesodermal competence occurs autonomously in cultured ectoderm as well (Leikola, 1963; Grainger and Gurdon, 1989).

Our purpose in this study was to examine the role of lens-forming competence of ectoderm in the process of...
lens induction. We have shown that lens induction is a multistep process in which the ectoderm in the head region becomes predisposed, or biased, to form a lens, after which actual lens determination and differentiation occur (Henry and Grainger, 1987; Grainger et al. 1988; reviewed by Jacobson and Sater, 1988; Saha et al. 1989). Lens induction in *Xenopus* begins during late gastrula and neurula stages and involves an inductive signal that comes from the newly forming anterior neural plate, possibly from the presumptive eye region (Henry and Grainger, 1990). This signal travels through the plane of the ectoderm to the lens-forming region, which lies just outside the anterior neural plate, resulting in a lens-forming bias in this ectoderm. Although the anterior neural plate appears to be the principal early inducer of the lens, the mesoderm underlying the presumptive lens region at these stages enhances the inductive effect of the neural plate (Henry and Grainger, 1990). The lens ectoderm is (according to the terminology of Slack [1983]) specified by the time that it is contacted by the evaginating optic vesicle at the time of neural tube closure (Henry and Grainger, 1990). Contact with the optic vesicle, which has previously been thought to be the primary inducer of the lens, does not appear to be necessary for the induction of a rudimentary lens, though it does play a role in the final stages of lens determination, and enhances lens differentiation. The optic vesicle can elicit lens formation in non-lens head ectoderm which has already acquired a lens-forming bias (Grainger and Mannion, unpublished).

Because lens induction begins during gastrula stages, ectoderm must be competent at this stage to respond to the initial lens-forming signals. We wished to learn when the initial state of lens competence appears in ectoderm, when it is lost, and whether this competence plays a role in regulating the induction of the lens, as it does in the case of neural induction. In addition, we wished to establish whether the gain and loss of lens competence is an intrinsic property of ectoderm, as it appears to be for mesoderm and neural induction, or arises as a result of tissue interactions within the embryo.

Although our experiments initially focused on lens competence, we frequently observed induced neural tissue, reflecting the high level of neural competence of early gastrula ectoderm. Consequently, we have examined changes in both neural and lens competence of *Xenopus* ectoderm. Based on the current view of the timing of lens induction, we have assayed the ability of ectoderm to form lens by transplanting tissue to the presumptive lens region of early neurula embryos. In such a transplant, the tissue will be exposed to early lens inducers, as well as to any influence from the optic vesicle later in development; this procedure therefore assays the ability of ectoderm to respond to the entire sequence of interactions leading to lens formation. Our experiments have used host and donor marking to verify that induced structures are made by transplanted tissue, and experiments were scored with neural- or lens-specific antibodies to ascertain the identity of induced structures. We find that the loss of neural competence and the gain of lens competence both occur during mid-gastrula stages, and that lens competence is then lost after only a few hours. These changes in competence all occur autonomously in ectoderm, independently of tissue interactions, suggesting that a timing mechanism operates in ectoderm to control its competence to respond to various inductive interactions.

**Materials and methods**

**Embryos**

Adult *Xenopus laevis* were mated, and embryos collected and dejellled as described in Henry and Grainger (1987). Embryos were reared in 20% Steinberg’s solution (Rugh, 1962) containing either 10 μg ml⁻¹ penicillin and 10 μg ml⁻¹ streptomycin, or 50 μg ml⁻¹ gentamycin sulfate.

**FLDx injection**

Dejelled 1-cell embryos were placed in 20% Steinberg’s solution with 5% Ficoll, injected with approximately 5 nl of a 50mg ml⁻¹ solution of fluorescein–dextran (FLDs; Molecular Probes, Junction City, OR), and subsequently reared as described above.

**Transplantation of tissues**

Tissues were transplanted from FLDx-labelled donor embryos to unlabelled hosts, as described in Results. Transplants were performed in 100% Steinberg’s solution using glass microneedles, and tissues were held in place during heating with small curved glass bridges, as described by Henry and Grainger (1987). After heating of transplants, embryos were gradually returned to ~30% Steinberg’s solution over the course of several hours. Embryos were staged according to Nieuwkoop and Faber (1967) because of differences in staging criteria, embryos denoted as stages 10i–12i in this study were about half a stage older than the stages indicated in the studies of Henry and Grainger (1987, 1990).

**Culturing of ectoderm**

Animal cap tissue was removed from stage 10 FLDx-labelled embryos using either glass microneedles or sharpened forceps. The excised animal caps were placed on a base of 1% agarose in 100% Steinberg’s solution, with the outer layer of ectoderm facing the agarose, and covered with a small piece of coverslip glass (previously washed in 100% ethanol, followed by distilled water), to prevent the explants from rolling up (Granger and Gurdon, 1989). At the end of the culture period, the coverslips were inverted and the tissues were removed from the glass. Cultured tissues were trimmed to remove areas in which outer layer cells had migrated over the inner layer, and to provide a fresh edge for healing into the host embryos. The age of the cultured ectoderm was monitored by using unoperated sibling controls.

**Histology and immunofluorescence**

For staining of lenses, fixation, embedding and sectioning were as described by Henry and Grainger (1990). Sections were dewaxed in 100% acetone, rehydrated in 0.1 M PBS (0.1 M phosphate buffer, 0.15 M NaCl), incubated for 15 min in 5 μm urea, blocked for 30 min in 5% non-fat dry milk (Carnation), rinsed twice in PBS, 10 min per wash, and stained with a polyclonal rabbit antibody prepared against
adult Xenopus lenses (Henry and Grainger, 1990; 45 min at a dilution of 1:60), followed by rhodamine-conjugated goat anti-rabbit IgG (Sigma; 30 min at 1:100).

For staining of neural tissues, embryos were fixed in Romeis fixative (Romeis, 1968; Hausen et al. 1985) for 1–2 h at room temperature, washed 1 h in 100% ethanol, and stored at 4°C in 100% ethanol. Embryos were embedded in polyethylene glycol 400 diesterate (Ruger Chemical, Irvington NJ), sectioned and stained as described above, except that blocking was with PBS containing 2% bovine serum albumin (Sigma) and 2% normal goat serum (Gibco BRL), and sections were stained with a polyclonal rabbit antibody against adult Xenopus N-CAM (kindly provided by U. Rutishauser; 2 h at 1:25), followed by either rhodamine-conjugated goat anti-rabbit IgG (Sigma, 45 min at 1:100), or biotin-conjugated goat anti-rabbit IgG (Sigma; 1 h at 1:60) and TRITC-conjugated ExtrAvidin (Sigma, 1 h at 1:50).

Scoring of sections
Stained sections were scored for the presence of lenses, neural tissue and ear vesicles formed from donor tissue. Lenses were scored on the basis of immunofluorescence staining. Diffuse staining was sometimes seen in the cement gland, but cement gland staining was punctate under high magnification, clearly distinguishable from the even cytoplasmic staining observed in lens cells. Because operations were performed on only one side of each embryo, a control (host) lens was present in each embryo to serve as a positive control for the staining procedure.

Neural structures were scored either on the basis of morphology or immunofluorescence using an anti-N-CAM antibody, as noted. Morphologically, neural bodies could easily be distinguished from cement gland cells, and from mesenchyme cells, which were present as single cells, or as irregular cell groups. Ambiguous cases – those in which the identification of transplants as neural or non-neural was not clear – were not included, these constituted fewer than 10% of the total cases. Ear vesicles were scored on the basis of their distinctive morphology; that is, thin-walled vesicles, elongated in the dorsoventral direction, present posterior to the eye.

Results

Competence of early gastrula ectoderm
To determine whether ectoderm is competent for lens formation before the lens induction process begins, we asked whether the ectoderm of early gastrula (stage 10) embryos is competent to form lens tissue. To do this, pieces of animal cap tissue were removed and transplanted to the presumptive lens region of open neural plate (stage 14) hosts from which the presumptive lens ectoderm had been removed. In these experiments, ectoderm from dorsal, middle and ventral regions of the animal cap was tested, because animal cap tissue has been shown to be regionalized in that dorsal animal cap is already predisposed toward neural induction (Sharpe et al. 1987; Phillips and Doniach, unpublished data), as a result of early signals coming through the ectodermal layer from the dorsal blastopore lip (Savage and Phillips, 1989; Dixon and Kintner, 1989). This experiment is illustrated in Fig. 1A.

The results (Fig. 2) show that the stage 10 animal cap forms a lens in only a small fraction of cases (9 of 65

Fig. 1. Transplant and culture experiments to assay lens competence (A) Ectoderm was removed from FLDx-labelled early gastrula (stage 10) embryos, and the dorsal, middle or ventral portion of the animal cap was transplanted to the presumptive lens region (PLR, shown in gray) of a neural plate stage (stage 14) embryo from which the presumptive lens ectoderm had been removed. (B) Presumptive ventral ectoderm was removed from FLDx-labelled embryos at different stages of gastrulation and transplanted to the stage 14 PLR (shown in gray). (C) Ectoderm was removed from FLDx-labelled early gastrula (stage 10) embryos and placed in culture for various periods of time, after which it was transplanted to the PLR of a stage 14 embryo. The developmental age of the ectoderm was monitored using unoperated sibling embryos. In all experiments, host embryos were cultured to swimming tadpole stage, fixed, sectioned and stained by immunofluorescence with antibodies that recognize either lens proteins or N-CAM.
Neuralization appears to be occurring in response to a N-CAM with an antibody directed against Xenopus.

In the second series of transplants and staining tissue sections, induced structures were indeed neural by performing a region do not form neural structures (data not shown).

Neuralization was unexpected, because these transplants are not a result of the surgical manipulation of the tissue; transplants of ectoderm to the presumptive ventral region do not form neural structures (data not shown). Neuralization appears to be occurring in response to a signal that comes from the host neural plate (homeo-genetic induction) and travels through the ectoderm (as suggested by Albers, 1987), since neural tissue alone, but not the underlying mesoderm, can neuralize ectoderm (Servetnick and Grainger, unpublished data).

These experiments clearly show that gastrula ectoderm is competent to be neuralized. We use the term neural competence here to refer to the ability of ectoderm to form neural tissue when transplanted to the presumptive lens region. However, it is important to emphasize that the timing of this competence may differ from competence to respond to other neuralizing signals (e.g. dorsal mesoderm; see Discussion).

We observe no regional differences in the ability of stage 10 animal cap ectoderm to form either lenses or neural tissue (Fig. 2). All ectoderm shows a uniformly low level of lens-forming competence, and a uniformly high level of neural competence, regardless of its original position in the animal cap. Thus while there may be a neural predisposition in dorsal animal cap ectoderm, ventral ectoderm still remains competent to be neuralized.

Changes in competence occur during gastrulation

To determine when ectoderm loses neural competence, and when it gains lens-forming competence, ectoderm from gastrulae of different stages was transplanted to the stage 14 presumptive lens region (illustrated in Fig. 1B). For these experiments, presumptive ventral ectoderm was used, because this tissue is the last to become underlain by endomesoderm during gastrulation, and is therefore least likely to have been influenced by inductive signals from the underlying tissues. Results of these experiments are shown in Figs 4 and 5.

Neural competence (scored on the basis of morphology) remains high during early gastrula stages (stage 10–11), then drops sharply. The neural structures induced from later gastrula ectoderm (stages 11–12) are not only less frequent than those formed from early gastrula ectoderm, but are also substantially smaller in size. These observations on neural competence have been confirmed with N-CAM staining of a second series of transplants (Fig. 5A–C; of 10 cases at stages 11–11\(\uparrow\), 5 formed distinct neural structures, which all stained for N-CAM; of 12 cases at stages 12–12\(\uparrow\), only 1 formed a distinct neural structure, which was N-CAM-positive). Interestingly, of the transplants of stage 12–12\(\uparrow\) ectoderm, 7 of the 12 cases showed tiny groups of induced, N-CAM-positive nerve cells which would not have been scored as neural inductions solely on the basis of morphology. These nerve cell groups are very different from any of the neural-tube-like inductions seen in younger ectoderm. Based on their morphology as well as their location (just ventral to the eye), these small inductions are likely to be induced cephalic neural placodes, which give rise to portions of the sensory cranial nerves (Stone, 1922; Knouff, 1927), part of the peripheral nervous system. A particularly striking example of such an induction, in which cranial nerves are clearly visible, is shown in Fig. 5G–I. This
Fig. 3. Structures induced from transplanted early gastrula ectoderm Each row shows a case of a transplant of early gastrula ectoderm to the neural plate stage presumptive lens region (as shown in Fig 1A). In each row, left column shows section viewed with differential interference contrast (DIC); middle column shows FLDx fluorescence, and right column shows immunofluorescence (E stained with anti-N-CAM antibody, H stained with anti-lens protein antibody).

Abbreviations: he, host eye; ie, induced eye; in, induced neural tissue, il, induced lens; pr, pigmented retina.

(A,B) Section through host eye (he; note pigmented retina [pr] at far right). Transplanted tissue (labelled tissue in B) has formed an induced neural tube-like structure (labelled in, however, the neural nature of this tissue has not been confirmed by antibody staining in this case) and eye tissue (labelled ie), as judged by the presence of pigmented retina, which has fused to the host eye. (C–E) Induced neural tissue The transplant (labelled tissue in D) has made large structures (labelled in), which stain brightly with anti-N-CAM antibody (E), showing that they are neural. (The host eye tissue also stains with N-CAM.) Part of the transplant has also made induced eye tissue (ie), as judged by the appearance of pigmented retina in this portion of the transplant, which is fused to the host eye (he). (F–H) Induced eye and lens. The transplant (labelled tissue in G) has made what is apparently induced eye tissue (ie), as judged by the appearance of pigmented retina, and a small induced lens (il), as shown by anti-lens antibody staining (H). Magnifications are ×110 (A,B), ×95 (C–E), ×120 (F–H).
Changes in ectodermal competence occur autonomously

The above experiments show that changes in competence occur in ectoderm during gastrulation. These changes could result from interaction of the ectoderm with mesoderm (either underlying mesoderm, or mesoderm which is at a distance [Sive et al. 1989]), or with presumptive neural tissues during gastrulation. Alternatively, changes in competence could result from events intrinsic to the ectoderm itself.

To distinguish between these alternatives, we removed animal cap ectoderm at stage 10, cultured it for various periods of time in vitro, then transplanted this aged ectoderm into the stage 14 presumptive lens region (see Fig. 1C). The results of these experiments are shown in Figs 6 and 7.

As ectoderm is cultured for longer periods of time, a progressively smaller proportion of transplants forms neural structures (Fig. 6; scored morphologically). In addition, as observed in vivo, the neural structures induced from older ectoderm are not as striking as those formed by stage 10 ectoderm (data not shown), and by late gastrula stages the only neural structures that form are small balls of neuriod cells. Although the loss of neural competence is not quite as rapid as in vivo, by late gastrula stages ectoderm has lost neural competence.

The lens competence of cultured ectoderm increases during culture, peaking at mid-gastrula stages (Fig. 6). The percentage of lenses formed at these stages is similar to that observed when ectoderm is transplanted directly from gastrula embryos (cf. Fig. 4); thus ectoderm autonomously acquires the levels of lens competence observed in mid-gastrula embryos. Ectoderm cultured beyond this stage rapidly loses lens-forming competence; therefore both the gain and loss of lens competence in cultured ectoderm mirror changes observed in ectoderm in vivo. The lenses formed from ectoderm cultured to mid-gastrula stages stain clearly with the anti-lens antibody (Fig. 7A–C) and sometimes contain lens fibers, but are generally not as well developed as lenses induced in ectoderm transplanted directly from mid-gastrula embryos. The small rise in lens competence observed at stages 12–13 in vivo does not occur in ectoderm cultured in vitro (cf. Figs 4 and 6), supporting the hypothesis that the small increase may be due to tissue interactions of ventral ectoderm with either the endomesoderm that underlies it at these stages, or signals originating in the neural plate which reach presumptive ventral ectoderm only at late gastrula stages.

Although the losses of neural and lens competence in vitro parallel those in vivo, it remains possible that these losses in competence in vitro result from damage to the ectodermal cells during culture under glass. To address this concern, we have examined cultured ectoderm to determine whether it is competent to form ear vesicles, since ectoderm remains competent for ear induction through early neurula stages (Gallagher et al. unpublished) Cultured ectoderm continues to form ear vesicles at a very high rate, well after the loss of both lens and neural competences. Fig. 7D,E shows an ear induced from ectoderm cultured to the equivalent of stage 13; such ectoderm has essentially lost its lens-forming competence (Fig. 6). The continued ability of ectoderm to respond to ear induction indicates that its
Fig. 5. Structures induced from transplanted mid-gastrula ectoderm. Each row shows a case of a transplant of mid-gastrula ectoderm to the neural plate stage presumptive lens region (as shown in Fig. 1B). In each row, figure at left shows section viewed with DIC; middle figure shows FLDx fluorescence; right figure shows immunofluorescence (C, I stained with anti-N-CAM antibody; F stained with anti-lens protein antibody). Abbreviations. e, eye; in, induced neural tissue; il, induced lens. (A–C) Induced neural tissue. Transplant (labelled tissue in B) has formed a small mass of induced neural tissue (in) near the host eye (e). The induced tissue has not detached from the ectoderm, and anti-N-CAM staining shows that the transplant stains in a patchy manner (arrow in C; cf Fig. 3E), indicating poor neural development. (D–F) Induced lens. Transplant (labelled tissue in E) has formed a large, morphologically well-developed lens (il), as shown by immunostaining (F). (G–I) Induced cranial nerve. Transplant (labelled tissue, marked by arrows in H), has the appearance of a nerve, which stains brightly for N-CAM (arrows in I). Magnifications are ×160 (A–C), ×140 (D–F), ×120 (G–I).

Responsiveness is not likely to be lost solely as a result of in vitro culture.

Competence of cultured animal cap regions
It is possible that the appearance of lens competence in mid-gastrula ectoderm is a consequence of early ectodermal signals which predispose dorsal animal cap to neural induction (discussed above), i.e. that lens competence is a later response of ectoderm to signals from the dorsal blastopore lip. To test this hypothesis, we isolated dorsal and ventral portions of animal cap
from early gastrulae, cultured these separately to mid-gastrula stages (when lens competence has appeared in cultured whole animal caps), then transplanted them into the presumptive lens region of a stage 14 host. If lens competence arises as a result of early signalling by the dorsal lip, then dorsal, but not ventral, animal cap regions should acquire competence at mid-gastrula stages. The results indicate that both dorsal and ventral animal cap regions form lenses with approximately equal frequency (Fig. 8). This result is consistent with

Fig. 6. Competence of ectoderm cultured \textit{in vitro}. Ectoderm was removed from FLDx-labelled embryos at the early gastrula stage (stage 10), cultured \textit{in vitro} for various periods of time, and was then transplanted to neural plate stage embryos, as shown in Fig. 1C. The age of the ectoderm was monitored with unoperated sibling embryos. Tissues were scored as described in Fig. 2. Bars show the proportion of transplants that formed lens or neural tissue at each of the stages tested. Data for stage 10 are from Fig 2. \textit{n=number of cases scored}

Interestingly, neural inductions (scored on the basis of morphology), occur with slightly higher frequency with dorsal ectoderm, although the observed difference between dorsal and ventral ectoderm is too small to allow us to conclude that it is significant. It is possible that this small difference reflects a neural predisposition of dorsal animal cap. No predisposition was detected when early gastrula ectoderm was used in these transplants (Fig. 2), perhaps because both the dorsal and ventral regions at the earlier stage are so highly responsive to neural induction (see Discussion). The predisposition may only become apparent in cultured ectoderm, in which overall responsiveness is lower.

Fig. 7. Structures induced from early gastrula ectoderm, which was cultured \textit{in vitro}, then transplanted to the presumptive lens region of a neural plate stage embryo (as shown in Fig. 1C). A,D are viewed with DIC; B,E show FLDx fluorescence; C shows immunofluorescence. Abbreviations: \textit{e}, eye; il, induced lens; io, induced otic vesicle; nt, neural tube \textit{(A–C)} Lens induced from ectoderm cultured to mid-gastrula (stage 11). Transplant (labelled tissue in B) has formed an induced lens (il), as judged by anti-lens antibody staining (C), showing that ectoderm has acquired lens competence during the period of culture. \textit{(D,E)} Ear vesicle induced from ectoderm cultured to the equivalent of early neurula (stage 13). Transplanted ectoderm has given rise to an otic vesicle (io), indicating that even after long periods of culture, ectoderm still has ear competence. Magnifications are \times 125 (A–C), \times 145 (D,E).
Competence of Xenopus ectoderm

Discussion

The major conclusions from our results are that the competence of ectoderm changes over developmental time, and that it does so autonomously. The critical point in these experiments is that transplanted ectoderm is being exposed to the same inductive environment – the presumptive lens region of a neural plate stage host – yet its response differs, in some cases forming neural tissue, in other cases forming lens, depending solely on the age of the ectoderm. Our results further demonstrate that no tissue interactions are required for these changes in competence. Precisely timed changes in competence therefore appear to be a part of the normal developmental program of ectoderm.

These results, taken together with those of many others, suggest that ectoderm goes through a series of stages, in which it is first competent to form mesoderm, then neural tissue and finally placodal tissue (e.g. lens; see Fig. 9). Previous studies have shown that competence to form mesoderm is acquired during cleavage stages (Jones and Woodland, 1987) and subsequently lost at early gastrula stages (Dale et al. 1985; Gurdon et al. 1985; Jones and Woodland, 1987; Grainger and Gurdon, 1989; Green et al. 1990). Following this period of mesoderm competence, ectoderm is competent to form neural, and then lens tissue. Our findings further suggest that, following the loss of lens competence, ectoderm becomes (or remains) competent to form other structures, specifically ear vesicles and cranial ganglion placodes. At least some of these changes in competence take place as a result of intrinsic processes occurring within the ectoderm. Loss of mesodermal competence (Grainger and Gurdon, 1989), loss of neural competence (this paper, and see below), and the gain and loss of lens competence (this paper) can all occur in isolated ectoderm.

It is formally possible that tissue interactions prior to the gastrula stage set in motion a timer, which then operates autonomously in ectoderm. However, the loss of mesodermal competence occurs in ectoderm isolated from early blastulae (Grainger and Gurdon, 1989), and neural and lens competence are lost autonomously in ectoderm isolated from late blastulae (Servetnick and Grainger, unpublished), suggesting that the autonomous timing mechanism is set in motion earlier in development, perhaps even at fertilization.

Fig. 8. Structures induced from early gastrula ectoderm, which was divided into dorsal and ventral halves, cultured in vitro until stage 11 (mid-gastrula), then transplanted to the presumptive lens region of a neural plate stage embryo. Tissues were scored as described in Fig. 2. Bars show the proportion of transplants that formed lens or neural tissue n=number of cases scored.

Fig. 9. Successive changes in ectodermal competence during development. Periods of competence which are well-established are shown as solid lines, those that are not unambiguously known are shown as dashed lines. The timing of gain and loss of mesodermal competence and loss of neural competence are well-documented (see text for references); gain and loss of lens competence are from data presented here. The gain of neural competence has not been extensively studied using natural inductor tissues (see Nieuwkoop et al. 1985). Ear competence is known to be present during neurula stages (Gallagher et al. in preparation) and has been lost from cultured ectoderm by stage 18 (M. Servetnick, unpublished observations), but gain and loss of ear competence have not been studied in detail. The period of competence for cranial neural placodes is based on observations made here, and by Nieuwkoop (1958). In general, the end of competence can be readily determined; gain of competence is more difficult to define experimentally, especially if the period during which the inductive signal is present is not known precisely, since the transplant may acquire competence some time after transplantation and still be exposed to inductive influences, which often persist for a greater period of time than the window of competence (see text for examples; see also Waddington, 1936). Although individual periods of competence are shown to overlap, it has not been clearly established whether individual cells have more than one competence at a given time. [Some of the features of this model were originally proposed by Nieuwkoop (1973).]
Neural competence

Because transplants of ectoderm to the presumptive lens region neutralized in a high proportion of cases, we were able to assay changes in neural competence as well as changes in lens competence. Competence to respond to neural induction in this region could differ from competence to respond to dorsal mesoderm, or to other neural inductors. However, there is striking agreement between our results and previous studies examining the competence of ectoderm to be neuralized in response to either dorsal mesoderm (Lehmann, 1929 [Triturus]; Machemer, 1932 [Triturus and Ambystoma]; Schechtman, 1938 [Hyla]), or to homeogenetic neural induction (Mangold, 1929 [Triturus]; Nieuwkoop, 1958 [Ambystoma]; Albers, 1987 [Ambystoma]).

Our results show not only that neural competence is gone by the end of gastrulation, but that cultured ectoderm loses this competence autonomously. These results are also in agreement with work on other species (Holtfreter, 1938 [Triturus]; Galler, 1952 [Triturus]; Ohara and Hama, 1979 [Cynops]) as well as Xenopus (Sive et al., 1989; Sharpe and Gurdon, 1990). In Holtfreter's experiments, ectoderm cultured to late gastrula and early neurula stages could no longer make neural plate, but did make scattered groups of ganglion-like nerve cells (see also Nieuwkoop, 1958); we have observed a similar transition from CNS-like to ganglion-like neural inductions in our experiments. We observe that all regions of the early gastrula animal cap respond equally well when transplanted to the presumptive lens region of a neural plate embryo. This finding contrasts with recent reports that dorsal animal cap is more easily induced to form neural tissue in response to anterior mesoderm (Sharpe et al., 1987), a tissue that is only a weak neural inducer (Mangold, 1933; Dixon and Kintner, 1989; Phillips and Doniach, unpublished). However, ventral ectoderm can be induced to form neural tissue when exposed to a sufficiently strong inducer, such as the early blastopore lip (e.g. Spemann and Mangold, 1924). Thus, the uniform response of ectoderm in our experiments may occur because the neural inducing signal(s) encountered by the transplant are sufficiently strong that no neural predisposition can be detected using transplants to the presumptive lens region. We find that there may be a weak neural predisposition in cultured dorsal ectoderm, in which overall neural responsiveness is lower.

The experiments reported here extend previous studies on neural competence in several ways. First, we have used both host and donor marking and a neural-specific molecular marker in our assays. We have also shown that the period of neural competence of ectoderm cultured in vitro corresponds closely to that observed in vivo, arguing that the loss of neural competence is unlikely to be an artifact of in vitro culture. The observation that cultured ectoderm gains lens competence after neural competence is lost, as occurs in vivo, also argues that ectoderm is undergoing normal developmental changes in culture.

Lens competence

Because the tissue interactions that induce the lens occur largely during neurula stages of development, we assayed lens-forming competence by transplanting ectoderm to the presumptive lens region of open neural plate stage embryos. To discuss our results in light of previous studies, it is helpful to review our current view of lens induction in Xenopus. Although many aspects of this model are likely to apply to lens induction in other amphibians, and other phyla as well (see Saha et al., 1989), it is possible that there are species differences, especially between anurans and urodèles, that may result in differences in the process of lens induction. In late gastrula and early neurula Xenopus embryos, head ectoderm begins to receive lens inductive stimuli from the anterior neural plate, and possibly from underlying tissues (Henry and Grainger, 1990). This initial, early phase of induction is not sufficient to cause the ectoderm to form a lens, but it does predispose the ectoderm toward lens formation, facilitating later stages of induction (Henry and Grainger, 1987). Lens induction continues during neurula stages, with the lens ectoderm becoming specified by the end of neurulation (Henry and Grainger, 1990); we do not know whether the early and late phases of lens induction (leading respectively to lens-forming bias and to lens specification) involve the same or different signals. The optic vesicle does not appear to be essential for normal lens induction (Henry and Grainger, 1987, 1990; Grainger et al., 1988), although it does possess at least some inductive capacity (Grainger and Mannion, unpublished).

We have here used the term competence to refer to the basal state of ectoderm, in which it is able to respond to all the interactions leading to lens formation. This is operationally defined as the ability to form a lens when transplanted to the neural plate stage (in which ectoderm is exposed to the principal lens inducer, the anterior neural plate during neurula stages). We use the term bias (or predisposition) to represent the intermediate state, in which the ectoderm has been partially induced (or biased) by early inductive interactions, but is not yet specified to form a lens. Bias is defined operationally as the ability to form a lens when transplanted to the presumptive lens region at the neural tube stage (in which ectoderm is exposed only to the inductive effects of the optic vesicle). Most previous studies assayed lens-forming ability by transplanting ectoderm over the optic vesicle of a late neurula embryo; these studies assayed bias, not competence to respond to early inductive interactions (Liedke, 1942, 1951, 1955; Reyer, 1958a,b; Jacobson, 1966).

Several studies have found that ventral ectoderm of gastrula stage embryos was able to form a lens when transplanted to early neurula embryos, but that this ability to respond was lost by neurula stages (Liedke, 1951, 1955; Reyer, 1958b; see also Jacobson and Sater, 1988). Our results also show that ectoderm from these stages is competent to form lens. We have further shown that this competence is not yet present in early gastrula ectoderm; lens competence is gained, and
subsequently lost, in a time-dependent manner, and these changes in competence occur independently of tissue interactions. These results underscore the importance of early interactions in lens induction: because lens competence is lost long before the time of ectoderm–optic vesicle contact, the optic vesicle cannot be the sole inducer of the lens during normal development.

One final important conclusion from our work is that the window of lens competence lasts only a few hours, and that this period of lens competence occurs quite early in development, during middle to late gastrula stages. The implication of this finding is that the initial interactions which ultimately lead to lens determination must occur during this competent period. Because ectoderm does not become specified to form a lens until the closure of the neural tube (stage 19; Henry and Grainger, 1990), this implies that the lens induction process spans about 9 h.

Given that lens competence is lost after only 2–3 h, how can lens induction continue over the course of some 9 h? Presumably, the initial signalling from the neural plate causes changes in the responding tissue that allow it to respond for many hours. Similar processes may be taking place in other inductive interactions in *Xenopus*. In mesoderm induction, inducing signals lead to the persistence of mRNA encoding the FGF-receptor past the time when it would otherwise have disappeared (Musci *et al.* 1990; Friesel and Dawd, unpublished). Also, during the specification of the anteroposterior axis, the initial signals that lead to specification of the cement gland enable ectoderm to respond to neural induction longer than it would otherwise (Sive *et al.* 1989).

Involvement of a developmental timer in changes in competence

Loss of mesodermal and neural competence have both been shown to occur in cultured ectoderm (mesodermal competence: Leikola, 1963; Grainger and Gurdon, 1989; neural competence: Holtfreter, 1938; Gallera, 1952). Our study, however, has demonstrated not only the loss of a previously existing competence, but also the acquisition of a new competence (lens) during the culture period. This result, which demonstrates that the ectoderm is in fact actively changing during development, challenges the commonly held view of the ectoderm as a passive tissue, which forms epidermis unless diverted to another developmental path. These active changes result in an orderly sequence of ectodermal competences—possibly changes in receptor or signal transduction systems within ectodermal cells. What controls these changes? Because they occur autonomously, apparently an internal timing mechanism within the ectoderm must be responsible. Such a developmental timer in ectoderm has been invoked to account for the timing of morphogenetic movements during gastrulation, and the timing of gene expression in response to mesoderm induction (reviewed by Cooke and Smith, 1990). Our results imply that an ectodermal timer is also responsible for changes in ectodermal competence. Whether a single timer controls all of these developmental changes, or whether there are separate timing mechanisms, awaits further dissection of these processes.

Mechanisms underlying changes in competence

The molecular basis of competence remains largely obscure. Clearly, competence may correspond to the presence of appropriate receptors on the cell surface (see, for example, Gillespie *et al.* 1989). Therefore, successive periods of competence may simply reflect the temporal appearance and disappearance of successive receptors.

Alternatively, changes in competence could be due to changes downstream in the signal transduction pathway through which the receptor operates. Such a mechanism is suggested by the model of Nieuwkoop and colleagues (Nieuwkoop, 1963, 1985; Albers, 1987; Nieuwkoop and Albers, 1990), in which a single signal leads to the induction of the nervous system as well as surrounding placodal structures; the response of ectoderm to the signal depends on its state of competence. Because the proposed signal—presumably acting through a single type of receptor—elicits distinct responses from ectoderm of different ages, this model implies that the receptor must be acting on different downstream targets in the signal transduction pathway.

Whether changes in the state of ectodermal competence are mediated at the level of receptors for inducing signals, or downstream in signal transduction pathways, the characterization of the timing and autonomy of these changes define biological parameters that should allow us to design experiments that distinguish among the various possible mechanisms that regulate competence.

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References
