

## Features of embryonic induction

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### Summary

The patterned distribution of different organs in the amphibian embryo begins with the establishment of two domains, the animal and vegetal regions, that differ in developmental potency. Differences amplify as inductive interactions occur across boundaries between areas of different potency. Embryonic induction establishes a temporally and spatially dynamic area of developmental potency – a morphogenetic field. The final arrangement and differentiation of cell types within the field emerge from subsequent interactions occurring primarily within the field. These principles are illustrated in a review of the induction of the lens and the heart. Recent studies show that the induction of the lens of the eye and the induction of the heart begin early in development. Most of lens induc-

tions occurs before the formation of the optic vesicle, and the heart appears to be part of a complex of dorsal structures whose formation is dependent upon the establishment of the dorsoventral axis. Suppressive as well as inductive tissue interactions occur during the determination of both of these organs, affecting their position and time of appearance. The complex processes of induction defined by the past nine decades of experimental work present many challenging questions that can now be addressed, especially in terms of the molecular events, cellular behaviour and regulatory physiology of the responding tissue.

Key words: embryonic induction, morphogenetic fields, competence, lens, heart.

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### Introduction

Embryonic induction consists of an interaction between inducing and responding tissues that brings about alterations in the developmental pathway of the responding tissue. This review and commentary uses the induction of the lens of the eye and induction of the heart as examples to illustrate some of the processes involved in embryonic induction. This review also directs more attention to the appearance of the regional heterogeneity that sets the stage for subsequent inductive interactions, and to the consequences of induction in the responding cells, in particular to the dynamic field of developmental capability that is established by induction.

Experimental analyses of inductive interactions have defined the inductive history of numerous organs and revealed many of the principles underlying the process of induction. Some aspects of embryonic induction have resisted analysis, however, because of the difficulties of assaying for a response to induction. In addition, a number of workers have shown that

some of the best-studied inductive interactions can be mimicked by the substitution of a variety of substances for the inductive signal itself, leading to the paradox of an apparently nonspecific stimulus eliciting a specific developmental response (see list of neural “inductors” in Brachet, 1950, pp. 394–395).

A recent monograph by Nieuwkoop *et al.* (1985) gives a comprehensive and critical review of the vast older literature on induction. Readers will find that book an excellent guide to the literature, as well as a source that gives a stimulating analysis of many topics, including those discussed below.

The present resurgence of interest in embryonic induction is largely a result of the incorporation of molecular techniques. A number of genes or gene products have been identified whose activity in a given tissue is dependent upon a specific inductive interaction; the products of some of these genes serve as molecular markers of the inductive interaction. The specifics of this work are amply discussed in an excellent review by Gurdon (1987) and will not be considered extensively here. The use of molecular

markers, instead of morphological or histological criteria, in assays of inductive events has two potential advantages. First, it allows one to examine the mechanisms by which an external (inductive) signal elicits the expression of specific genes. Second, the use of molecular markers that are expressed shortly after an inductive interaction, prior to the completion of large-scale morphogenetic and cytological events, permits an assay for *early* cellular events during the response to induction, which could not be examined using assays for morphological or functional differentiation events.

However, several aspects of embryonic induction that are critical to our understanding of induction as a developmental process have not yet been addressed using molecular approaches. The first is that embryonic induction brings about coordinated changes in cellular activities, including, though certainly not limited to, changes in gene expression. Alterations in cell motility and cell behaviour that result in morphogenetic movements are generally among the earliest responses to induction (Symes & Smith, 1987; Trinkaus, 1984). Changes in cell cycle activity and electrophysiological properties are also observed significantly earlier than terminal differentiation (Spitzer, 1979). Both the coordinated regulation of these changes and their role in determinative and differentiative processes remain largely unexplored.

Second and more important is that, according to the view of embryonic induction that has emerged from classical experimental embryology, inductive interactions establish a spatially and temporally dynamic region of developmental *potency*, referred to as a morphogenetic field. The term 'developmental potency' can be defined as the range of differentiative capabilities that can be expressed in a given tissue under a variety of experimental conditions, as well as *in vivo* (Slack, 1983). Initially, a morphogenetic field includes the area that is *fated* to participate in the formation of a specific organ, plus the surrounding area, which is capable of participating in organ formation in the event of experimental manipulation of the embryo. Over the course of subsequent development, this morphogenetic field becomes spatially restricted, as peripheral areas lose potency and become incorporated into other organs or structures. Normally, by the time a functional organ has formed, the surrounding areas have lost the potency to participate in formation of that organ under experimental conditions.

We discuss morphogenetic fields in order to point out that hypotheses regarding the cellular and molecular processes governing the response to an inductive signal should be consistent with the properties of the morphogenetic field established by the inductive interaction. For example, Ban-Holtfreter (1965),

among others, has shown that lateral and medial fragments of the dorsal lip of the urodele early gastrula are able to give rise to both notochord and somite mesoderm. This regulative ability of the isolated fragments demonstrates the existence of a notochord-somite field of potency. In amphibians, notochord does not contain muscle tissue, while somites give rise to the skeletal musculature. Tissue isolates from stage-14 (early neurula) *Xenopus* embryos that include notochord and somites will express muscle-specific actin transcripts (Mohun *et al.* 1984). Are muscle-specific actin transcripts expressed homogeneously throughout the prospective notochord-somite mesoderm? Presumably, the regulative ability of the dorsal axial mesoderm field is lost at some point following gastrulation. Are developmental changes in this regulative ability mirrored by developmental changes in the spatial pattern of muscle-specific actin expression in dorsal axial mesoderm during the same period? These questions illustrate the need for the application of molecular and cellular approaches to the aspects of embryonic induction that have proved especially challenging to experimental embryologists.

The establishment of a morphogenetic field depends upon one characteristic in particular, the ability of a group of cells to respond to a given inductive signal. This ability, referred to as competence (Waddington, 1932), is initially heterogeneous throughout the embryo and becomes altered during subsequent development. Some regional differences in competence are established during the earliest stages of embryonic development. For example, in an amphibian embryo, mesoderm can be elicited from the animal region, but not from the vegetal region (Nieuwkoop & Ubbels, 1972). Within an embryonic region, levels of responsive competence can increase or decrease with time. Increases in competence may be due to interactions with previously unrecognized inductors (Jacobson, 1966), as is the case for the presumptive lens epidermis, or to processes intrinsic to the responding tissue itself. Similarly, decreases in competence may result from intrinsic changes or from interaction with neighbouring tissues. Changes in competence during prolonged inductive interactions should alter the spatial extent of the resulting morphogenetic field.

It is useful to review several properties of morphogenetic fields. (1) Morphogenetic fields reflect developmental potency, as opposed to developmental fate; thus, these fields are initially larger than the area fated to give rise to a particular organ. (2) The spatial extent of a morphogenetic field changes over time. (3) Tissue within a morphogenetic field will give rise to several different cell types. (4) Morphogenetic fields possess polarity and axes are established sequentially within a field. (5) Morphogenetic fields

exhibit regulative development; *e.g.* a field may be cut in two and each half will form a whole organ, or two morphogenetic fields for the same organ may be combined experimentally and they will fuse and form a single larger organ. These properties suggest that morphogenetic fields represent the extent of communication within and across a tissue.

This conclusion should be emphasized. Much of the recent work on embryonic induction (*e.g.* Kimelman & Kirschner, 1987) refers to the role of inductive interactions in the specification of cell fate. Nieuwkoop *et al.* (1985, p. 7) have suggested that the term 'embryonic induction' should refer only to tissue interactions that *alter* developmental pathways. According to this definition, inductive interactions establish regions of developmental potency or morphogenetic fields. Since morphogenetic fields generally give rise to several cell types, the initial inductive interaction has little bearing on the specification of individual cell fates. Cell fates may be established by interactions *within* the morphogenetic field over the course of organogenesis.

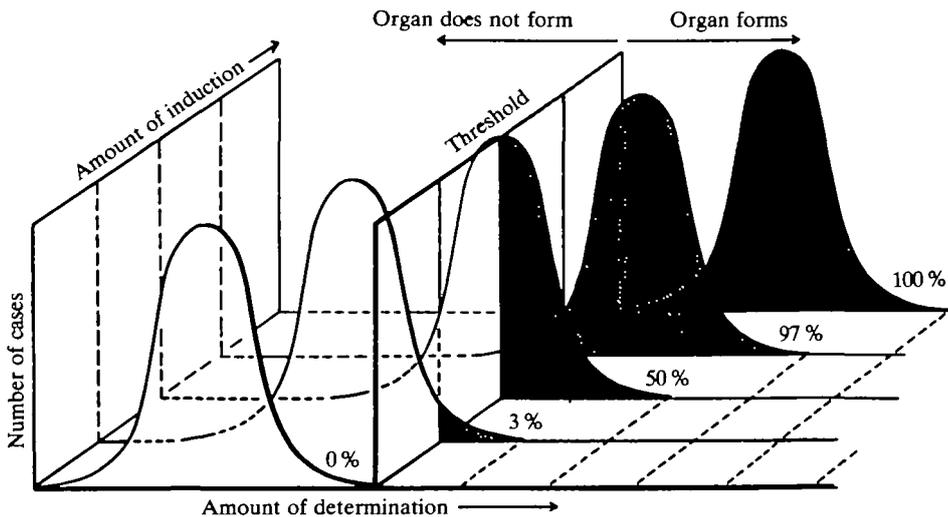
### Experimental criteria

We make a few general comments first for those who may not be familiar with the classical approaches to studies of induction. These approaches to the question of why an organ forms where and when it does involve experimental manipulation of the embryo in some way. Through marking experiments, one can locate in an early embryo the group of cells, referred to as the anlage, that will later form an organ. By microsurgical interventions, one can separate the organ anlage from its normal tissue environment to evaluate the state of developmental commitment of the organ anlage. These microsurgical interventions include explanting the tissue and culturing it in isolation, transplanting the tissue to a heterotopic site, removing putative inductor tissues from the vicinity of the anlage and removing the area fated to give rise to an organ to observe whether the surrounding tissue will regulate to form the organ. These different experimental approaches may give conflicting results, reflecting the degree of commitment of an embryonic region to form a particular structure. Slack (1983) reserves the terms 'specification' and 'determination' to distinguish between different levels of developmental commitment. According to Slack (1983), 'specification' is operationally defined as the level of commitment necessary for an explanted embryonic tissue to form a given structure when cultured in isolation, whereas 'determination' refers to the level of commitment necessary for formation of the structure in heterotopic transplants as well as in isolation.

These experiments also establish whether interactions with other tissues in the vicinity are essential for organ formation. In addition, these methods have been used to map morphogenetic fields, sometimes with differing results. Thus, any discussion of a morphogenetic field must indicate whether the field reflects a map of specification, determination or regulative ability. Interpreting these experiments can be difficult, since organs generally become morphologically recognizable well after the interactions that elicit them have occurred.

These experimental interventions generally give one of the following results. (1) The anlage may form the organ in every case, indicating that conditions that prevail from the time of the experiment, together with any determinative events that occurred prior to the experiment, are entirely sufficient for organ formation. Whether tissue interactions were involved at times prior to the experiment must be separately investigated. (2) The anlage may in no case form the organ. One can conclude that some events excluded by the experiment are essential to induce the organ. (3) A third possible result is that some experimental cases will form the organ and in other replicate experiments the organ will not form. This result indicates that the experiment was performed during a developmental period critical to the establishment of organ-forming potency. Such results are usually expressed as a percentage of cases exhibiting a positive response. What does such variable response mean?

One can expect variability in any biological system. Under identical experimental conditions with sibling embryos near the same stage of development, the response to the experimental conditions of the inductive interaction will be sufficient for organ formation in some cases and insufficient in others. One would expect that the threshold of response, *i.e.* the amount of inductive stimulation required to elicit organ formation, would exhibit a normal (Poisson) distribution. Increasing the amount of induction would push a greater percentage of cases through the response threshold, until finally all cases respond (Fig. 1). Since 'percent response' is a measure of the proportion of the population that has passed the threshold, the response is not linear, nor is it directly proportional to the amount of induction. Nevertheless, 'percent response' can be an accurate relative measure of the amount of developmental commitment to organ formation. We have found that groups of 30 embryos will consistently give the same response within plus or minus one case ( $\pm 3\%$ ). This explanatory model has three main components. (1) The processes that produce developmental commitment have cumulative effects over a prolonged time period. (2) There is variation among individuals treated equally so that one must deal with a distri-



**Fig. 1.** Graph of the relationship between induction and determination. When a population of cases experiences a certain amount of induction, the response is varied because of biological variation. If a portion of this distributed population (hatched portions) passes through a response threshold, then those cases form the organ.

bution of response levels. (3) An organ will form when the processes of developmental commitment reach some response threshold within the responding tissue in each case. These general considerations are critical to the design and correct interpretation of experiments on organ induction.

### Induction of the lens of the eye

Experimental work on induction of the lens since the turn of the century has provided many insights into induction. Recent reviews include McAvoy (1980) and Nieuwkoop *et al.* (1985). Work of the past few years, however, has considerably changed our view of how this organ is induced. The common text-book account that the optic vesicle induces the lens is incorrect. The inductive interactions responsible for lens formation occur primarily before the emergence of the optic vesicle, which has only a subsidiary role in the formation of the lens.

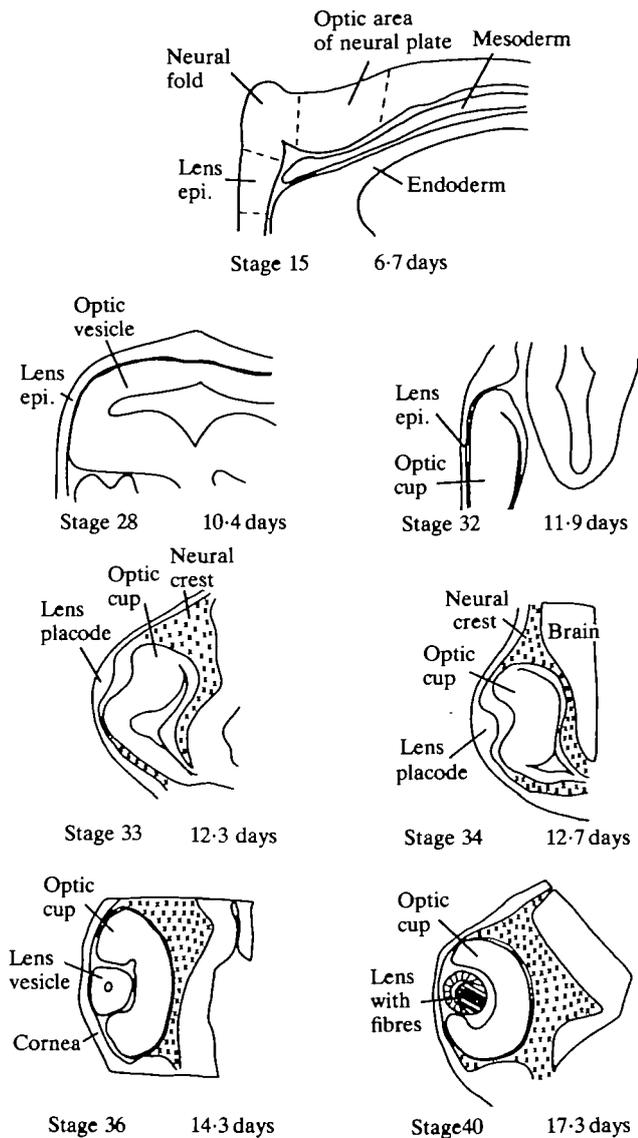
The retinal rudiments are part of the eye-forebrain field induced in the anterior neural plate by underlying pharyngeal endoderm and head mesoderm. The retinal rudiments emerge from this field as evaginating optic vesicles that protrude from the forebrain and contact the epidermis that will later form the lenses. Cranial neural crest cells surround the optic vesicle, but they do not enter between the lens epidermis and the optic vesicle. No sign of lens formation occurs until the optic vesicle begins to invaginate and form a cup. At that time the epidermis thickens, forming a lens placode. The placode then pinches off from the epidermis and differentiates into fibres and epithelium (Fig. 2).

Early work suggested that the optic vesicle or cup plays a critical determinative role in the formation of the lens. Herbst (1901), reporting that the number and position of lenses corresponds to the number and

position of abnormal optic cups in cases of cyclopia and near cyclopia, was among the first to propose this causal relationship. The first experimental evidence was provided by Spemann (1901), who cauterized the retinal rudiment in one side of the open neural plate of embryos of the frog *Rana fusca*. When these animals were sectioned at a larval stage, no lens was found on the operated side where the optic cup (the future retina) was absent. Spemann concluded from these results that 'lens formation is only possible under the influence of the optic cup' (Spemann, 1938).

Further experiments failed to support this conclusion. Mencl (1903) reported observations on a double-headed monster of the fish *Salmo salar* in which lenses had developed in the absence of optic cups in the abnormal head. Also, King (1905) reported that lenses developed in the frog *Rana palustris* after cautery of the retinal anlage in closing neural fold stages. These findings prompted Spemann (1912) to repeat his work, using a more gentle method involving surgical removal of the retinal rudiment. He again obtained negative results with *Rana fusca*. When he extended the experiments to two other anurans, however, he found that in the absence of the retina, *Rana esculenta* produced lenses and *Bombinator pachypus* produced poorly formed lenses.

Woerdeman (1939) repeated Spemann's experiments with *Rana esculenta* and obtained the opposite result. The difference between Spemann's and Woerdeman's results arises from differences in the temperature at which the embryos were kept prior to the operations. Spemann reared embryos at 10 to 15°C, while Woerdeman reared embryos at approximately 25°C. The temperature dependence of lens-forming ability in the absence of the optic cup was demonstrated by Ten Cate (1953), who reared *Rana esculenta* embryos at low or high temperatures before the operations, then kept both groups at room



**Fig. 2.** Positions of the retinal rudiments and the prospective lens epidermis are shown at different stages in the embryo of the newt, *Taricha torosa*. The figures are traced from sections. The stages and the time passed at 17°C since fertilization are given. Induction occurs mostly before and during neurulation, but the lens does not begin to form until much later.

temperature. In embryos reared at 12°C, 59% formed lenses in the absence of the retina, while the group reared at 25°C produced lenses in only 9% of the cases. These results were confirmed by Jacobson (1958), who reared embryos of the newt *Taricha torosa* (*Triturus torosus*) at temperatures ranging from 5 to 25°C before removing retinal rudiments. Optimal production of lenses (63%) in the absence of the retina occurred at 16°C in this species, though over 20% of the cases formed lenses at each of the different temperatures. These results are probably due to the dependence of developmental rate on

temperature. In *Taricha torosa*, development up through midneurula stages requires 3 days at 25°C, 6 days at 16°C, and 25 days at 5°C. The results suggest that early tissue interactions are enhanced at low temperatures.

Older experiments involving transplantation of the optic vesicle suggested that the optic vesicle is able to induce a lens in strange epidermis. Lewis (1904, 1907a,b) excised the optic vesicle from embryos of *Rana palustris* and *Rana sylvatica* and inserted it beneath the epidermis of the ear region. Lenses often formed in association with these transplanted optic vesicles. Lewis (1904) also obtained lenses in association with the optic vesicle when the presumptive lens epidermis was replaced by flank epidermis.

Despite the early conflicting results of the Spemann defect experiments, the transplantation experiments of Lewis and others led to a common perception that the lens is induced by the optic vesicle. However, new experimental results support the older body of work that suggests instead that the optic vesicle may have only a minor role in lens induction.

While the optic vesicle transplantation experiments (Lewis, 1904, 1907a,b; Brahma, 1959) indicated that the optic vesicle could induce a lens in strange epidermis, those experiments have proven to have been insufficiently controlled; specifically, it is unclear whether the lenses developed from donor or host tissue. Henry & Grainger (1987) and Grainger *et al.* (1988) have shown that prospective lens cells, adhering to the optic vesicle after removal of the epidermis, can give rise to a new lens when the optic vesicle is transplanted to an ectopic site, or when non-lens epidermis is grafted over the optic vesicle in *Xenopus laevis*, *Rana palustris* and *Ambystoma mexicanum*. These authors labelled donor or host embryos with cell lineage tracer (horseradish peroxidase) to determine the origins of the cells in the resulting lenses. When lenses were found in association with optic vesicles transplanted to other regions, the lenses had formed from donor tissue, and thus must have been carried along with the optic vesicle.

An alternative test for the role of the optic cup in lens formation, the ability of the prospective lens epidermis to form 'free' lenses in the absence of the retina, has been performed on the various species listed in Table 1. Of 37 species tested, 25 (68%) form lenses in the absence of the retina. In those species that do not form such free lenses, possible temperature effects have rarely been tested, so some of these species may be able to form free lenses when reared at some other temperature. In any event, the results of experiments with the majority of species tested do not support the idea that the optic vesicle is necessary for lens induction.

Several studies have indicated that the lens is

**Table 1.** List of species tested to determine whether the lens will (+) or will not (-) form in the absence of the retina

Species:	Reference
<b>Anurans:</b>	
1. <i>Bombina bombina</i>	- Popov <i>et al.</i> 1937
2. <i>Bombinator pachypus</i>	+ Spemann, 1912
3. <i>Bufo bufo japonicus</i>	+ Tahara, 1962a
4. <i>Bufo carens</i>	- Balinsky, 1951
5. <i>Bufo regularis</i>	- Balinsky, 1951
6. <i>Bufo vulgaris</i>	- Cotronei, 1922
7. <i>Pelobatus fuscus</i>	- Popov <i>et al.</i> 1937
8. <i>Phrynobatrachus natalensis</i>	- Balinsky, 1951
9. <i>Rana arvalis</i>	+ Filatow, 1925b
10. <i>Rana angolensis</i>	+ Balinsky, 1951
11. <i>Rana catesbiana</i>	+ Pasquini, 1931
12. <i>Rana esculenta</i>	+ Spemann, 1907; Ten Cate, 1953
13. <i>Rana fusca</i>	+ Von Ubisch, 1923
14. <i>Rana japonica</i>	+ Tahara, 1962a
15. <i>Rana limnocharis</i>	+ Tahara, 1962a
16. <i>Rana nigromaculata</i>	+ Tahara, 1962a
17. <i>Rana palustris</i>	+ King, 1905
18. <i>Rana pipiens</i>	+ Liedke, 1942
19. <i>Rana rugosa</i>	+ Tahara, 1962a
20. <i>Rana sylvatica</i>	- Lewis, 1907
21. <i>Rhachophorus schleglii</i>	+ Tahara, 1962b
22. <i>Xenopus laevis</i>	+ Balinsky, 1951; Henry & Grainger, 1987
23. <i>Pyricephalus</i> sp.	- Balinsky, 1951
<b>Urodeles:</b>	
24. <i>Ambystoma maculatum</i>	+ Harrison, 1920; Liedke, 1951
25. <i>Ambystoma mexicanum</i>	- Ten Cate, 1953
26. <i>Hynobius nebulosa</i>	+ Tahara, 1962a
27. <i>Pleurodeles waltlii</i>	- Pasquini, 1927
28. <i>Taricha torosa</i>	+ Jacobson, 1955
29. <i>Triton alpestris</i>	+ Von Woellwarth, 1961
30. <i>Triton cristatus</i>	- Popov <i>et al.</i> 1937
31. <i>Triton taeniatus</i>	- Mangold, 1931
32. <i>Triturus pyrrhogaster</i>	+ Tahara, 1962a
<b>Fishes:</b>	
33. <i>Fundulus heteroclitus</i>	+ Lewis, 1909; Oppenheimer, 1966
34. <i>Salmo</i> sp.	+ Mencl, 1903, 1908
35. "trout"	+ Demmill, 1906
<b>Bird:</b>	
36. Chick	+ Mizuno, 1970, 1972; Karkinen-Jääskeläinen, 1978
<b>Mammal:</b>	
37. Mouse	+ Karkinen-Jääskeläinen, 1978

Many of these species are listed by Tahara (1962a) with additional information about the percentage of cases forming free lenses and the quality of differentiation of the free lenses.

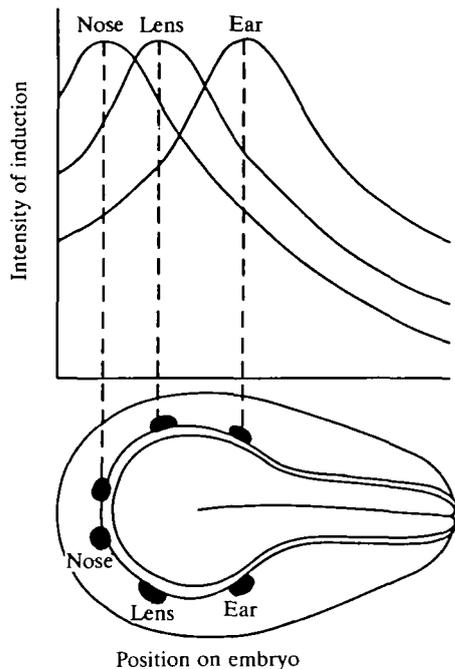
induced by a series of inductors (Liedke, 1951, 1955; Jacobson, 1955, 1958, 1963a,b,c, 1966; Reyer, 1958a,b). Jacobson did experiments involving explan-

tation or heterotopic transplantation of the prospective lens ectoderm on embryos of the newt *Taricha torosa* that led to the conclusion that lens induction is a long, cumulative process that begins in gastrulation, when the anterior dorsolateral endoderm first contacts the prospective lens epidermis. During neurulation, the prospective heart mesoderm is brought by morphogenetic movements into the region of the lens epidermis and the heart mesoderm also participates in lens induction. The period of strongest lens-inducing activity occurs during neurulation, when lens induction by the nearby heart mesoderm is added to lens induction by the subjacent endoderm; thus, the major part of lens induction occurs prior to the formation of the optic vesicle.

The neural plate may also be an inductor of lens at these early stages. Recent investigations by Henry & Grainger (personal communication) on embryos of *Xenopus laevis* implicate a possible contribution to lens induction by transfer of inductive signal from the contiguous neural plate through the plane of the ectoderm at early open-neural-plate stages. Hoper-skaya (1968) has made a similar suggestion.

Transplantation experiments in *Xenopus* embryos using donor tissues labelled with lineage tracer have demonstrated that the entire gastrula non-neural ectoderm can form lenses, and that this ability becomes restricted to the lens area during neurulation (Grainger *et al.* 1988; Henry & Grainger, 1987). Grainger and colleagues have also shown that the optic vesicle has only a minor role in lens induction, concluding that most of lens induction results from inductors encountered before the optic vesicle forms.

The retina does appear to influence the growth, differentiation and maintenance of the lens, perhaps in a manner similar to the 'formative effects' described by Holtfreter & Hamburger (1955, p. 253). It has been shown for a number of organs that the tissues that surround the emerging organ may affect its form, size and differentiation. Lenses that form independently of the retina do not achieve the same degree of growth and differentiation attained by lenses with a retinal association (Spemann, 1938; Ten Cate, 1953; Jacobson, 1966). There is a continuing influence of the retina on the development of the lens. Le Cron (1907) demonstrated this on *Amblystoma punctatum* by removing the retina at varying times during lens development and fixing and examining the animals at different intervals following the operation. After removal of the retinal influence, the lenses continued to differentiate to an extent that was determined by the amount of time they had previously been in contact with the retina. Eventually, however, these free lenses degenerated. Other authors have reported similar results with other species (Filatow, 1925a; Woerdeman, 1939, 1941;



**Fig. 3.** The spatial distribution of the capabilities to induce nose, lens or ear are shown along the axis of a newt embryo. Each organ arises at the region of greatest inductive intensity where the responding cells first pass a response threshold for that organ.

Popov, 1937; McKeehan, 1954).

Other placodal organs, such as the nose and the ear, are induced in a manner similar to that of the lens (Jacobson, 1963*a,b,c*; Michael & Nieuwkoop, 1967; Michael, 1968). The nose is induced primarily by underlying anterior endoderm and to some extent by the forebrain. The ear is induced by its underlying mesoderm and the nearby hindbrain. Experiments on the head ectoderm that forms the nose, lens and ear (Jacobson, 1963*a,b,c*) led to the following conclusions. (1) The endodermal and/or the mesodermal inductors have the dominant role in inducing and positioning nose, lens and ear. The neural structures that contribute to their induction have only a secondary importance. Each placodal structure can form in the absence of induction from the neural structures. (2) The separate capabilities to induce nose, lens and ear are spread in gradient fashion in the endoderm and mesoderm. That part of the head epidermis that sees the highest amount of induction for an organ forms that organ (Fig. 3). What the responding tissue 'sees' is complicated by the morphogenetic movements occurring in both inductors and responding tissue. (3) The head epidermis is simultaneously responding to the different inductors for nose, lens and ear (and probably other organs as well). When the responding tissue reaches the response threshold necessary to form one of the organs, that organ will form and the other organs are excluded.

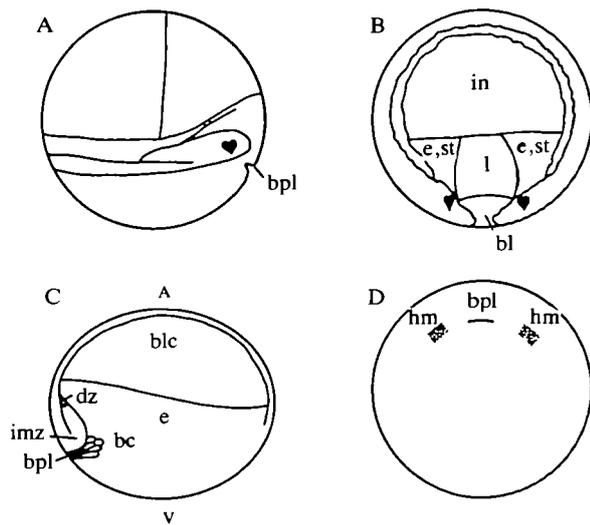
These results demonstrate that a responding tissue may at the same time be at some level of commitment for several organs. This concept has not been generally appreciated; however, it has considerable significance for the interpretation of molecular events and hypotheses for induction.

These studies have shown that lens formation is the culmination of a series of inductive interactions. Several tissues are early inductors of the lens and their effects appear to be cumulative and synergistic. As lens induction begins in the gastrula, the ability of the non-neural ectoderm to respond to lens induction (competence) becomes restricted to the head ectoderm, centring on the regions fated to be the lenses. The same head ectoderm is also being induced to form nose and ear and levels of commitment to each of these three organs can be measured in the same tissue. Which organ forms depends on which of the three levels of commitment reaches the response threshold. Through time, the morphogenetic field of the lens becomes limited to the cells that actually form the lens. This limitation may be due to suppressive interactions with neural crest, to be described below. The optic vesicle seems not to be involved in the elicitation of the lens, but it has a supportive role in maintaining appropriate growth and differentiation of the emerging lens structure.

### Induction and development of the heart

According to the classical view of heart induction in vertebrates, the heart mesoderm is determined during gastrulation and neurulation *via* induction by the pharyngeal endoderm. This view is supported by experimental analyses of heart formation in urodele and chick embryos, as well as teratological observations of mammalian embryos. Recent work on heart specification in *Xenopus* embryos, however, reflects some variation in this pattern.

In amphibians, the two mesodermal rudiments that are destined to give rise to the heart are first distinguishable immediately prior to gastrulation. In urodeles, most of the future mesoderm is on the surface of the early gastrula; the heart mesoderm, however, is located deep in the marginal zone near and lateral to the beginning dorsal lip of the blastopore (Fig. 4A,B). This location is based on specification maps, derived from the differentiation of explants in culture (Holtfreter, 1938), rather than on conventional fate maps. The heart rudiments lie next to the pharyngeal endoderm; therefore, induction may already be underway in blastula and gastrula stages. During gastrulation, the heart rudiments and the underlying pharyngeal endoderm advance together anteriorly just lateral to the future edge of



**Fig. 4.** The location of heart mesoderm is shown at early gastrula stages. (A) Lateral view of the surface of the left side of a urodele embryo. The heart mesoderm (dark heart shape) is located in the equatorial mesodermal band, but beneath the surface deep to the prospective chordamesoderm. bpl, blastoporal lip. (B) In a frontal section of a urodele gastrula, the heart mesoderm is in the deep layer of the marginal zone contiguous with anterior endoderm. The position of the heart mesoderm was determined by potency mapping by Holtfreter (1938); A and B are modified from this paper. bl, blastopore; e,st, esophageal and stomach endoderm; l, liver endoderm; in, intestinal endoderm. (C) Sagittal section of a *Xenopus* gastrula. Bottle cells (bc) define the blastoporal lip (bpl). At the tip of the involuting marginal zone (imz), or chordamesoderm, the head mesoderm is located within the deep zone (dz). The heart mesoderm is located in the deep zone lateral to the head mesoderm. (D) A vegetal view of the surface of a *Xenopus* gastrula. The heart mesoderm (hm) is located deep and lateral to the blastopore lip (bpl). Based on Keller, (1976).

the neural plate. At early to midneurula stages, the heart rudiments reside beneath the ear epidermis lateral to the future hindbrain and still overlie dorsolateral pharyngeal endoderm (Wilens, 1955). During later neurula stages, the heart rudiments migrate to the midventral site where they finally fuse and form the heart between tail-bud stages 28–31 in *Taricha torosa* (Jacobson, 1961). The heart begins to beat at larval stage 34.

Recent work on anurans has focused largely on *Xenopus laevis*. A fate map of the *Xenopus* embryo at the 32-cell stage produced by Dale & Slack (1987) demonstrates that the heart is derived primarily from the dorsal and dorsolateral blastomeres of the third tier, which also contribute much of their descendant tissue to the notochord and somites, respectively. Fate maps of the gastrula of *Xenopus* indicate that the paired prospective heart mesodermal rudiments are

located in the dorsolateral region of the deep zone above and adjacent to the preinvolved marginal zone (Fig. 4C,D) (Keller, 1976). The areas of prospective heart mesoderm are separated by the prospective head mesoderm, which is located at the dorsal midline.

During gastrulation in *Xenopus*, the prospective head and heart mesoderm move as a loose mass of crawling cells (Gerhart & Keller, 1986), in advance of the more cohesive chordamesoderm. By the onset of neurulation, the prospective heart rudiments begin to move laterally. In early neurulae, prospective heart mesoderm sits at the anterior lateral edge of the mesodermal mantle between the edge of the neural plate and the ventral midline. The regions of heart mesoderm migrate ventrally and fuse at the ventral midline immediately posterior to the cement gland during late neurula stages.

In *Xenopus*, clumps of cells that will give rise to the endocardium appear by the earliest tailbud stage. The dramatic mesodermal buckling that will form the myocardium is first visible immediately thereafter at stage 28 (Nieuwkoop & Faber, 1967); heartbeat is initiated at stage 33/34.

#### *Tissue interactions involved in heart formation*

The role of anterior, or pharyngeal, endoderm as an inducer of heart mesoderm has been demonstrated in amphibian and chick embryos, by explantation of the prospective heart mesoderm or by removal of the endoderm from the embryo. A summary of some of the earlier work on the establishment of heart mesoderm is given in Copenhaver (1955). Ekman (1921) demonstrated that prospective heart mesodermal rudiments isolated from postneurula stage *Bombinator* embryos are capable of differentiation in epidermal vesicles. These results were confirmed by Stohr (1924), who pointed out that heart differentiation in explants is correlated with the inclusion of endoderm in the vesicles. Bacon (1945), finding that explants of the lateral blastopore lip from midgastrula *Ambystoma* embryos would form beating hearts in culture, suggested that specification of the heart is complete by midgastrula stages. This interpretation is suspect, however, since the explants used for his experiments apparently included the prospective pharyngeal endoderm; thus, inductive interactions between the heart mesoderm and the pharyngeal endoderm presumably continued after explantation.

#### *Urodeles*

Removal of the entire endoderm from neurula-stage embryos prevents heart formation in several urodele species (Balinsky, 1939; Chuang & Tseng, 1957; Nieuwkoop, 1947; Jacobson, 1960, 1961; Jacobson & Duncan, 1968). Jacobson (1961) removed the entire

endoderm from embryos of *Taricha torosa* at various stages to find out when the mesoderm becomes capable of heart formation in the absence of the endoderm. No hearts are formed when the endoderm is removed at open neural plate stages (stage 14–16). When the entire endoderm is removed at early tail-bud stages 22–24, 17% of the cases form hearts. If the endoderm is not removed until later tail-bud stages 25–26, then 75% of the cases form hearts. The longer the endoderm is present, the more likely it is that hearts will form in these endoderm-deficient embryos. It was also noted that the quality of hearts that form is always correlated with how long the heart mesoderm has been in contact with the endoderm. This sort of 'formative effect' was also noted by Mangold (1956, 1957).

The heart-inducing activity of *Taricha torosa* embryos has been located within the endoderm. Embryos in which the anterior dorsolateral endoderm has been substituted for the entire endoderm form hearts in all cases. If the posterior fourth of the endoderm is substituted for the anterior endoderm, then a heart will not form in any case (Jacobson, 1961). Other experiments have shown that either the first or second anterior quarters of the endoderm will restore heart formation, but neither of the posterior two quarters are capable of doing so (Jacobson & Duncan, 1968). These and other experiments suggest that the ability to induce heart formation is restricted to the anterior endoderm.

Fullilove (1970) used explant recombinations to map the spatial distribution of heart-inducing capability in the endoderm of *Taricha torosa* embryos at early neural plate stages 14–15. The dorsolateral endoderm immediately underlying the prospective heart mesoderm itself was shown to cause increases in the rate of heart differentiation, as well as increases in the frequency of heart formation. To a lesser extent, the endoderm in the region of the future midventral heart site also increased frequency and rate of heart formation. The endoderm just anterior to the heart rudiments had the greatest ability to increase the frequency of heart formation, but the hearts were very slow to form. The endoderm immediately ventral or posterior to the heart rudiments also increased the frequency of heart formation, but had no effect upon the rate. Posterior-dorsal endoderm slowed the rate of heart formation. Fullilove also tested the mesoderm just posterior to the heart mesoderm in combination with the endoderm beneath it, or with that which normally underlies the heart rudiments. These explants formed hearts with a frequency greater than heart mesoderm explanted by itself, but the rate of formation was slower. These experiments illustrate how morphogenetic fields are established: both the ability of the endoderm to induce hearts and

the competence of the mesoderm to respond to heart induction extend in fields beyond the region that actually gives rise to the heart in the normal embryo.

The specification of heart mesoderm in the urodele *Taricha torosa* was examined by Jacobson & Duncan (1968), who recombined prospective heart mesoderm from early neurula embryos with anterior endoderm and dorsal epidermis, finding that (a) inclusion of anterior endoderm increased both the rate and frequency of heart differentiation in these explant recombinates; and (b) culture of heart mesoderm in epidermal vesicles increased the frequency of heart formation, but did not affect the rate of heart differentiation. These experiments were conducted using embryos at early- to mid-neurula stages (14–16), by which stages heart specification has progressed to such a degree that 10–14% of heart mesodermal explants cultured in isolation will form beating hearts.

Jacobson & Duncan (1968) also used hanging drop cultures to study heart determination in *Taricha*. Prospective heart mesoderm was tested in these cultures alone and in combination with other tissues, and with homogenates of various tissues. The effects of other tissues on heart mesoderm noted in vesicle cultures – increase in the frequency of heart formation and increase in rate of heart formation – were found also in the hanging drop cultures, both from the appropriate intact tissues, and from fractions of their homogenates.

A preliminary characterization of the heart-inducing activity from homogenates of anterior endoderm indicates that the activity is found at the aqueous–lipid interface after centrifugation (Jacobson, unpublished observations). It persists after boiling and can be detected in tissue samples stored at –10°C for over a year. The activity is restricted to the void volume of eluates from Sephadex G-100 gel filtration chromatography (Jacobson & Duncan, 1968). Further analysis was discontinued in the 1960s because analytical methods available at that time were somewhat primitive. The use of currently available analytical methods to characterize this heart-inducing activity might be considerably more fruitful.

Further insight into the role of anterior endoderm in amphibian heart induction comes from studies of the *cardiac lethal* (*cl*) mutation found in the axolotl, *Ambystoma mexicanum*. Embryos homozygous for *cl* are indistinguishable from wild-type embryos until the stage at which heartbeat is normally initiated; *cl* hearts do not beat, although slight regional contractions may be observed. This defect arises from a perturbation in the interactions between heart mesoderm and anterior endoderm.

Reciprocal transplants at stages 27–29 (early tail-bud) of mutant and wild-type heart mesoderm into

wild-type and mutant hosts demonstrate that the genotype of the host determines whether heartbeat is initiated: mutant hearts beat normally when transplanted into wild-type hosts (Humphrey, 1972). These results provide evidence that normal heart formation is prevented by expression of the *cl* mutation in a heart-inducing tissue, rather than the presumptive heart mesoderm itself. Explants of stage-34 (late tailbud) *cl* hearts will beat normally when cultured with explants of normal anterior endoderm (Lemanski *et al.* 1979). These workers have also excluded the possibility of inhibition by mutant pericardial tissue after observing that isolated *cl* hearts fail to beat in culture.

Recently, Davis & Lemanski (1987) demonstrated that RNA isolated from normal anterior endoderm at stage 29 will rescue the *cl* heart phenotype. Specifically, 90% of *cl* hearts cultured in isolation underwent myofibrillar assembly and began to beat after treatment with  $0.4 \text{ mg ml}^{-1}$  anterior endoderm RNA; this rescue activity is sensitive to RNase. Treatment with RNA isolated from liver or neural tube failed to rescue the *cl* hearts. These workers did not report the effects of RNA isolated from *cl* anterior endoderm on *cl* hearts, nor have they examined the mechanism by which their RNA preparations effect this rescue. It remains to be seen whether this rescue activity reflects events occurring in wild-type embryos *in vivo*.

Thus, expression of the *cl* mutation in anterior endoderm apparently results in deficiencies in tissue interactions that would normally support later stages of heart differentiation. Such later interactions, where continued association with the endoderm is essential for normal heart differentiation, have been observed in other species and referred to as 'formative influences' as noted above. Evidently the initial heart-inducing signals are normal, since *cl* mutants undergo normal early heart morphogenesis. This conclusion argues for a temporal pattern of qualitatively different signals mediating the induction and subsequent support of heart differentiation. Alternatively, the *cl* anterior endoderm may be unable to sustain the production of a heart-inducing signal beyond early heart morphogenesis.

#### Anurans

Our recent results indicate that in embryos of the anuran *Xenopus laevis* the heart mesoderm becomes specified considerably earlier than it does in urodeles. Explants of prospective heart mesoderm removed at stage 12.5 (late gastrula) and cultured in epidermal vesicles undergo heart formation in nearly 50% of cases (Sater, unpublished observations). Inclusion of pharyngeal endoderm in these vesicles increases the frequency of heart formation to approximately 70%.

Mesodermal explants removed at stage 14 (early neurula) form beating hearts in 100% of cases, whether or not they are cultured in the presence of pharyngeal endoderm. Thus, the acquisition of heart-forming potency is well underway by the end of gastrulation and the specification of heart mesoderm is complete by early neurula stages.

Clearly, the inductive interactions or other developmental events that participate in the specification of heart mesoderm occur during or prior to gastrulation. Unfortunately, the end of gastrulation at stage 12.5 is the earliest stage at which germ layers are clearly separable (Nieuwkoop & Faber, 1967); therefore, it is not possible to examine the development of prospective heart mesoderm isolated from younger embryos. Removal of regions of superficial endoderm that contribute to the pharyngeal endoderm from *Xenopus* stage-10 early gastrulae fails to prevent heart formation. In these experiments, bottle cells and suprablastoporal endoderm of the dorsal hemisphere, which give rise to the anterior archenteron lining and the archenteron roof, respectively (Keller, 1975), are removed at the onset of gastrulation, leaving only those regions of prospective pharyngeal endoderm that arise from the deep layer (Keller, 1976). The relative contributions of deep and superficial endoderm to the prospective pharyngeal endoderm are unknown.

In *Xenopus*, heart formation is inhibited by events that disrupt establishment of the dorsal-ventral axis. Embryos that have been treated with u.v.-irradiation, high pressure, nocodazole or low temperatures to inhibit the formation of axial structures (Scharf & Gerhart, 1980) have greatly reduced, and often non-functional, hearts; in the most severely abnormal of these embryos, heart formation does not occur (J. C. Gerhart, C. R. Phillips, personal communications). Conversely, embryos treated with lithium to enhance dorsoanterior determination often display large radial hearts (Kao & Elinson, 1988). Despite its eventual ventral location, during gastrulation the heart mesoderm behaves as a dorsal anterior character: in early gastrulae, heart-forming potency is restricted to paired mesodermal regions approximately  $30\text{--}45^\circ$  lateral to the dorsal midline; ventral and ventrolateral regions are incapable of heart formation when cultured in isolation (Sater, unpublished observations). The relationship between dorsoventral axis determination and heart formation may arise from any or all of the following alternatives. (1) Heart mesoderm may be induced directly by the dorsovegetal cells (the so-called Nieuwkoop Organizer) as part of the dorsal complex that includes the chordamesoderm and pharyngeal endoderm (Gerhart *et al.* 1984). (2) Heart mesoderm may be induced by the dorsalized pharyngeal endoderm. (3)

Heart mesoderm may arise as a result of the dorsaling activity of the chordamesoderm, the Spemann Organizer. These possibilities can be tested by transplanting grafts of tissue labelled with lineage tracers into u.v.-irradiated hosts. In any event, establishment of the dorsoventral axis creates regional heterogeneity that spatially restricts the acquisition of heart-forming potency later on.

The specification of heart mesoderm in *Xenopus* at a considerably earlier stage than that observed in urodeles is consistent with the notion that regional differences within the *Xenopus* embryo become apparent very early in development, as early as midcleavage stages in the case of dorsal mesodermal determination (Gimlich, 1986). Embryonic development in *Xenopus* is far more rapid than the development of the urodeles under discussion (*Ambystoma*, *Taricha*, *Triton*) when raised at identical temperatures (Schreckenberg & Jacobson, 1975; Nieuwkoop & Faber, 1967), and rapid embryonic development may be correlated with determination at younger developmental stages.

#### *Amniotes*

A fate map of prospective heart-forming regions of the chick embryo has been prepared by Rosenquist & DeHaan (1966), who have mapped the prospective heart mesoderm to paired lateral regions of the epiblast in the embryo at stage 3+ (forming primitive streak; Hamburger & Hamilton, 1951). Following gastrulation, the prospective heart mesoderm forms a large crescent in the lateral plate and prechordal mesoderm, which moves in a cephalic and medial direction to converge during the early stages of heart morphogenesis. Heart formation takes place during stages 6–12 (regressing primitive streak to 16-somite stages), and heartbeat is initiated at stage 11.

Heart determination in chick embryos has been subjected to a more limited experimental analysis, in keeping with the greater difficulty of the required surgery in these embryos. Orts-Llorca (1963) reported that removal of the endoderm underlying the paired regions of prospective heart mesoderm at stages 4–5 (regressing primitive streak) would prevent heart formation, concluding that endoderm induced heart formation in a manner analogous to that observed in amphibian embryos. DeHaan (1964) pointed out the heart mesoderm may have been removed with the underlying endoderm and demonstrated that culture of such 'endodermal' explants results in the formation of beating tissue, indicating that prospective heart mesoderm is included in such explants. At these stages, it is difficult to remove the endoderm and leave the mesoderm intact, using conventional microsurgery. In response to DeHaan's criticism, Orts-Llorca & Gil (1965) repeated their

experiments, using trypsin to help remove the endoderm as Le Douarin (1963) had done in similar experiments; illustrations of such dissections show that much of the mesoderm remains intact. Again, heart formation was not observed in the absence of the endoderm. They also report, though, that in some cases, the extirpated endoderm did form beating hearts in culture, so at least some heart mesoderm had been removed with it. The induction of the heart in the chick is thus not fully settled.

Studies of cardiac defects in human embryos reveal that defects in the anterior endoderm are correlated with the malformation or absence of the heart (Hommes, 1957). In addition, cardiac defects are generally associated with the loss of the cranial portion of the embryo, suggesting that, as in *Xenopus* embryos, disruption of the establishment of dorso-anterior axial characters leads to a loss of normal heart formation.

#### *The heart morphogenetic field*

Analysis of the heart morphogenetic field was initiated in the 1920s with Copenhaver's (1924) studies on *Ambystoma* and Ekman's (1925) work with *Bombinator*. Copenhaver extirpated presumptive heart mesoderm at postneurula stages 25–29 and found that the surrounding mesoderm would undergo heart formation, often resulting in the production of double hearts by the ventrolateral mesoderm to either side of the ventral midline. Removal of this ventrolateral mesoderm in addition to the presumptive heart mesoderm at the ventral midline prevented heart formation in 33% of cases. Replacement of the anterior ventral and ventrolateral mesoderm with the more dorsal and posterior flank mesoderm prevented heart formation. Ekman found that 'gill' (anterior lateral) mesoderm was capable of heart formation when transplanted into the anterior ventral region of postneurula embryos, whereas more posterior mesoderm was not.

These results may indicate that the heart morphogenetic field encompasses anterior ventral and ventrolateral mesoderm in postneurula stages. Alternatively, the early findings may reflect the spatial distribution of competence to respond to continuing heart induction, an interpretation apparently favoured by Ekman, rather than the extent of the already induced field. One can distinguish between these possibilities by culturing explants of ventral and ventrolateral mesoderm alone and in combination with anterior endoderm. Such experiments have provided support for both possibilities: at the end of neurulation in *Xenopus* embryos, the heart morphogenetic field encompasses both anterior ventral and anterior lateral mesoderm; ventral or lateral mesoderm posterior to the region fated to give rise to the

heart is not capable of heart formation in isolation. However, interaction with anterior endoderm does contribute to the maintenance of heart-forming potency in the anterior ventrolateral mesoderm that flanks the region of mesoderm destined to give rise to the heart (Sater, unpublished observations).

As mentioned earlier, morphogenetic fields have been mapped both by examining the state of specification of tissue contained within the morphogenetic field and by mapping the ability of the surrounding tissue to regulate to replace an organ following removal of the tissue fated to give rise to that organ. Studies of the heart morphogenetic field in chick embryos have been performed using both methods, producing different results. Rawles (1936) examined the developmental potencies of explants of the stage-5 (regressing primitive streak) chick blastoderm when grafted onto the chorioallantoic membrane, a relatively neutral site. She found that peripheral areas of the blastoderm, ranging from the level of the notochord to a point halfway down the primitive streak, expressed heart-forming potency under these conditions. This suggests that the heart morphogenetic field comprises the paired regions of lateral blastoderm over approximately half the length of the embryo. However, stage-5 embryos from which one of the paired regions of prospective heart mesoderm has been removed together with the underlying endoderm, do not undergo regulation to replace the prospective heart mesoderm (DeHaan, 1965). Instead, the heart arises solely from the contralateral unoperated side. If both regions of prospective heart mesoderm have been removed, the heart does not form. The difference between these results may arise from incomplete wound healing in DeHaan's tissue removal experiments. Alternatively, this difference may reflect the existence of some previously unrecognized inhibitory activity that suppresses heart formation in the operated embryos and is avoided in explants grafted to the chorioallantoic membrane.

These differences underscore the importance of the choice of experimental manipulation used to define the spatial extent of a morphogenetic field. In addition, analyses of changes in the spatial distribution of any field over time must examine the possibility that morphogenetic movements may also bring about such changes; thus an apparent restriction of the morphogenetic field in space may reflect cell movements, rather than a restriction of developmental potency.

Taken together, these studies of the establishment of heart mesoderm and the subsequent alterations of the heart morphogenetic field suggest that the acquisition of heart-forming potency occurs relatively early, during gastrula and neurula stages. However, regulation of the spatial distribution of heart-forming

potency, *i.e.* the heart morphogenetic field, continues into postneurula stages. Interactions between the prospective heart mesoderm and pharyngeal endoderm probably govern the establishment of heart-forming potency and participate in the spatial regulation of the heart morphogenetic field.

### Tissue interactions that suppress determination

The determination of an organ generally results from a pattern of tissue interactions that may include suppressive influences in addition to inductive interactions. These suppressive interactions affect the positioning and the time of appearance of the organ. Suppressive tissue interactions have been shown to occur during the determination of the lens, the heart, the mesonephric kidney and other organs; however, these phenomena have received little attention.

Von Woellwarth (1961) provided a clear demonstration of suppressive effects of the neural crest on lens determination by removing either the anterior neural plate (which includes the retinal rudiments), or the entire anterior neural plate and the associated neural folds (which include the prospective neural crest) from mid-neurula *Triton taeniatus* embryos. When the embryos were then reared at 14°C, the optimum temperature for lens formation, 26.5% of the cases produced lenses in the absence of the brain plate, while 95% produced lenses in the absence of the brain plate and the neural folds. Thus, more than three and a half times as many cases form lenses in the absence of the neural crest under these experimental conditions.

These embryos lack optic vesicles, so neural crest from the neural folds has direct access to the ectoderm that would normally form the lens. In intact embryos, the optic vesicle contacts that portion of the ectoderm that will form lens, and neural crest cells do not enter between the optic vesicle and presumptive lens epidermis until well after the lens has formed (Fig. 2). The ectoderm surrounding the lens-forming region is part of the lens field, but it becomes associated with neural crest cells that can suppress lens formation. Henry & Grainger (1987) suggest that neural crest cells suppress lens determination in all of the lens field except that part contacted by the optic vesicle, resulting in the positioning of the lens directly over the optic vesicle.

Heart determination is also inhibited by neural crest and neural plate. If the entire endoderm is removed at open neural plate stages in *Taricha*, no hearts will form; if the entire endoderm together with the neural plate and neural folds are removed, however, then heart formation will occur in as many as 71% of the cases (Jacobson, 1960). Fautrez &

Amano (1961) have achieved similar results with *Triturus pyrrhogaster*, but they also implicate notochord and somites as inhibitory to heart development.

The inhibitory effects of the neural plate and folds have been examined in *Taricha torosa*. When the endoderm and just the anterior neural plate were removed, leaving the anterior neural fold and posterior fold and plate, no cases formed hearts. If the endoderm and the entire neural plate were removed, leaving the neural folds, then hearts formed in 29% of the cases (Jacobson, 1961). This is less than the 71% of cases that form hearts if all neural plate and folds are removed, so it appears that both neural plate and neural folds are inhibitory. The entire presumptive heart mesoderm from midneurula (stages 14–16) embryos was combined in epidermal vesicles with various other tissues. The presumptive heart mesoderm in overlying epidermis formed hearts in 31% of the cases. The presumptive heart mesoderm in overlying epidermis with dorsolateral endoderm underwent heart formation in every case that did not give rise to neural tissue; the 36% of the cases that formed neural tissue did not undergo heart formation. Presumptive heart mesoderm in overlying epidermis with anterior neural plate and folds did not form hearts. Neither did the same combination together with anterior dorsolateral endoderm (Jacobson, 1960). Presumptive heart mesoderm in overlying epidermis with posterior neural plate and folds also did not form hearts, although similar cultures including anterior dorsolateral endoderm underwent heart formation in 67% of the cases (Jacobson, 1961). The experiments indicate that heart inhibition capabilities reside in both the neural plate and the neural folds, and that anterior plate and folds are more inhibitory than posterior plate and folds.

Jacobson & Duncan (1968) showed that fractions of homogenates of the cranial neural fold have a suppressive effect on heart formation in hanging drop cultures of presumptive heart mesoderm. The suppressive activity in these tissue homogenates is eluted in the void volume after Sephadex G-100 gel filtration chromatography. After centrifugation of the homogenate, the suppressive activity is located at the aqueous–lipid interface and it is destroyed by boiling (Jacobson, unpublished observations).

Jacobson & Duncan (1968) found that the earliest stage at which hearts will form in endoderm-free embryos, which still contain the inhibitory tissues, is considerably later than the earliest stage at which explants of heart mesoderm will form beating hearts when cultured in isolation. The differences in the acquisition of heart-forming capability through time in these two experimental situations illustrates the delaying effects that the presence of inhibitory tissues

must have on heart formation in normal development.

Suppressive interactions have been shown to occur during the determination of other organs. Mesonephric kidney tubules arise from mesonephrogenic intermediate mesoderm that is adjacent to the neural folds of amphibian embryos during stages with open neural plates. Etheridge (1968) analysed the determinative events that lead to mesonephric tubule formation in the newt *Taricha torosa*. He found that the neural crest strongly suppresses tubule formation, both while the neural crest is still present in the neural folds and after it has migrated into the mesonephrogenic mesoderm. Similar results were obtained with the anuran *Xenopus laevis* (Etheridge, 1972). Green (1970) suggested that neural crest may normally inhibit mesonephric tubule formation in mice, based on her observations of the *ch* mutant. The effect of this suppressive activity in normal development is to delay the time of appearance of the mesonephric kidney tubules.

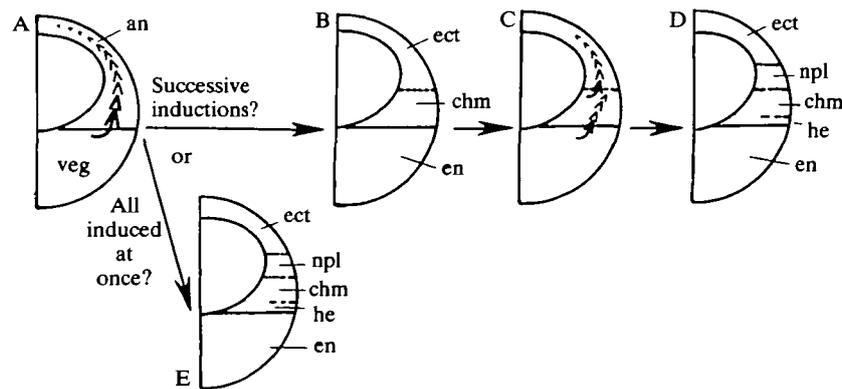
Most studies of induction do not look for suppressors; thus, hitherto-undiscovered suppressive interactions may occur during the determination of many other organs. Since the neural crest appears to have arisen very early during vertebrate evolution, its suppressive role in determination of some organs may have interesting evolutionary implications. Gans (1987) raises the question of whether, to some extent, the inhibitory capacity of the neural crest on heart and mesonephric kidney development reflects the posterior shift of these organs from a branchial position in protochordates to a visceral coelomic position in vertebrates.

It appears that in several induction systems, suppressive interactions between the organ anlage and nearby tissue are involved in regulating the temporal appearance and the spatial positioning of the organ.

### Some speculations about induction across boundaries

Induction occurs across boundaries between different domains of cells. Morphogenetic movements often bring inductor tissues into vertical apposition with responding tissues; indeed, induction events usually alternate with episodes of morphogenetic movements. Some of the more classical examples of induction, such as the inductions of neural plate, lens or heart by underlying tissues, occur mostly between tissues in vertical apposition.

Inductive interactions also occur across boundaries *within* the plane of a tissue. The processes of such planar inductions may establish some of the characteristics of morphogenetic fields and set the stage for



**Fig. 5.** Diagrammatic views of the dorsal portions of sagittal sections of *Xenopus* blastulae. (A) Across the boundary between vegetal (veg) and animal (an) materials, an inductive signal from the vegetal cells spreads and attenuates into the animal material. Either this results in the induction of a chordamesodermal field of potency (chm) that (B) becomes delimited from the ectoderm (ect) by a new boundary where the limit of response threshold is reached, and then inductions occur across both new boundaries (C) to induce (D) notoplate (npl) and anterior endoderm (he), or the original induction shown in A induces a large field of potency that segregates into notoplate, chordamesoderm and anterior endoderm. en, endoderm.

subsequent interactions within the field itself. As mentioned earlier, the inductive signal usually extends beyond the border that eventually delimits the organ being induced. During planar inductive interactions, attenuation of the inductive signal as it spreads through the plane of the tissue may lead to the establishment of two cell populations: one that has received levels of the inductive signal that are sufficient to elicit formation of the induced organ (*i.e.* threshold levels of the inductive signal), and one that has received insufficient, or subthreshold, levels of the inductive signal. The juxtaposition of tissues that have received threshold and subthreshold levels of an inductive signal may result in the formation of a new boundary between them, as well as in changes in the inductive ability and the responsive competence of both tissues. These changes could lead to additional inductive interactions.

Induction of the dorsal mesoderm in *Xenopus* is one example of a planar induction. The *Xenopus* blastula has two different domains of cells, the animal and the vegetal cells, that emerge from regional differences in the zygote that presumably originate during oogenesis. The early embryo thus has a single boundary between animal and vegetal regions, and the endoderm induces mesoderm in the animal region across this boundary (Nieuwkoop *et al.* 1985) (Fig. 5). During induction of the dorsal mesoderm, the inductive signal from the endoderm presumably attenuates as it spreads into the animal region (Fig. 5A). A new boundary becomes defined, possibly demarking those animal cells that have received enough of the inductive signal to pass through a response threshold. More distant areas of the animal cap receive levels of the inductive signal that are insufficient to elicit forma-

tion of chordamesoderm. The spatial distribution of attenuated levels of the inductive signal may be responsible for the delimitation of the dorsal mesodermal morphogenetic field. Peripheral regions of the dorsal mesodermal field that are eventually excluded from the presumptive chordamesoderm may display altered patterns of competence with respect to subsequent inductive interactions. For example, this subthreshold induction may impart dorsal characteristics to the dorsal ectoderm, making it more susceptible to neural induction (London *et al.* 1988; Sharp *et al.* 1987). The latter authors have used neural cDNA markers to demonstrate this phenomenon directly. Further Jones & Woodland (personal communication), using a monoclonal antibody specific for neural ectoderm, find that *Xenopus* stage-10 prospective dorsal ectoderm responds much faster to neural induction than does stage-10 prospective ventral ectoderm. London *et al.*'s report was based on a monoclonal antibody that recognizes epidermis.

The notoplate, an ectodermal region that is essential for the elongation of the neural plate (Jacobson, 1981), forms just beyond the border between the chordamesoderm and the ectoderm. The notoplate ultimately occupies the midline of the neural plate (Jacobson, 1987), and it undergoes convergent extension in the direction opposite to the convergent extension of the chordamesoderm (Keller *et al.* 1985) during gastrulation and induction of the neural plate. The notoplate must be induced before the neural plate, since it begins to function during neural induction (Jacobson, 1987). The notoplate may be induced in competent ectoderm by the contiguous chordamesoderm, or it may arise as part of a chordamesodermal-notoplate complex or field, *via* the inductive signal responsible for mesoderm formation.

Head mesoderm and pharyngeal endoderm are induced from the animal hemisphere between the chordamesoderm and the vegetal cells. Nieuwkoop *et al.* (1985) suggest that the anterior endoderm is induced simultaneously with the chordamesoderm. Alternatively, the anterior endoderm may be the result of induction between the vegetal cells and the chordamesodermal field. The latter possibility implies a change of competence in the region that had previously been induced to form the chordamesoderm.

In any event, the induction across the original animal-vegetal boundary leads to formation of four new boundaries (Fig. 5D,E). Inductive interactions presumably continue across these new boundaries. For example, the boundary between the notoplate and the chordamesoderm becomes the site of formation of the tail bud. Smith *et al.* (1985) propose that such sequential induction occurs within the mesoderm. Different vegetal cells induce the dorsal and the ventral mesoderm; then, inductive interactions between the dorsal and ventral mesoderm elicit the intermediate mesodermal structures.

In summary, an inductive signal may spread between two regions within the plane of the tissue, becoming attenuated within the responding tissue as it does so. A boundary will form between a region that has received sufficient induction to pass a response threshold to form the organ, and a contiguous region that has received some level of the inductive signal, but not enough to pass the threshold. The competence of both these regions may be changed by these events, and new inductions may then occur across the boundary, establishing in turn new boundaries where these processes may continue. Such a method would allow diversity to increase exponentially. Morphogenetic movements, which often alternate with inductive events, may also bring different regions of tissue into new appositions and allow additional inductions to occur.

## Conclusions

Recent investigations into the induction of the lens and heart have brought about a substantial modification in our view of the inductive interactions responsible for the formation of these organs. Results of Henry & Grainger (1987) and Grainger *et al.* (1988) stress the importance of lens induction that occurs before there is an optic vesicle, and their work indicates that the optic cup does not play a significant determinative role in lens formation in any of the species studied. Our work demonstrates that the establishment of heart-forming potency in the *Xenopus* embryo occurs during gastrulation and the

earliest stages of neurulation, resulting in part from the specification of the dorsoventral axis during earlier stages. In each case, determinative events are shown to begin earlier in development than was previously supposed.

These findings point toward a clearer elucidation of the relationship between global patterning events occurring relatively early in development and specific tissue interactions responsible for the establishment of organ-forming potency. Many of the earlier experimental analyses of embryonic induction viewed inductive interactions without regard for the processes of regional specification that generally precede the inductive events themselves. More recently, Gerhart and his colleagues (1984) have examined the relationship between events that initiate the formation of the dorsoventral axis during the first cell cycle and dorsal mesoderm induction during late cleavage and blastula stages. These processes are in turn connected with the timing of the initiation of gastrulation movements around the dorsoventral circumference of the embryo. Presumably, early specification events such as these produce regional differences that later manifest themselves as spatial differences in developmental potency or competence. This relationship warrants further investigation.

A central question that emerges from this consideration of inductive interactions involves the cellular and molecular events underlying the response to induction. How is the acquisition of developmental potency reflected at a cellular level, in terms of gene expression and the regulation of cellular activity? What is the nature of the intercellular communication that must coordinate changes in field characteristics? Warner & Gurdon (1987) have suggested that gap junctions may mediate communication within the mesoderm following induction. In addition, fibroblast growth factor alone (Stack *et al.* 1987) and in combination with transforming growth factor- $\beta$  (Kimelman & Kirschner, 1987) can elicit mesodermal characteristics from the animal cap cells of *Xenopus* blastulae. Medium conditioned by the *Xenopus* cell line XTC induces mesoderm formation in isolated animal cap explants (Smith, 1987). Recently, the XTC-conditioned medium has been shown to induce changes in cellular activity characteristic of those induced by TGF- $\beta$  (Rosa *et al.* 1988). The inducing activity has been purified and has biochemical characteristics that closely resemble those of TGF- $\beta$  (Smith *et al.* 1988). These findings may provide an excellent opportunity to address some of these questions. The mechanisms by which these growth factors act upon differentiated cells are still poorly understood; further studies elucidating their mechanisms of action should suggest explanations for their effects upon embryonic cells, which in turn may shed light on the

process by which the endogenous signal induces mesoderm formation *in vivo*.

The vast array of work in classical experimental embryology suggests that the change in developmental potency that occurs in response to induction is initially labile, gradually becoming fixed as a result of the cumulative effect of the continuing inductive signal. This notion is analogous to the model for the conversion of short-term memory to long-term memory proposed by Kandel and his colleagues (Goelet *et al.* 1986), which claims that the acquisition of short-term memory is dependent upon the covalent modification of pre-existing proteins in the presynaptic terminal in response to a stimulus. Continued exposure to the stimulus results in the activation of regulatory pathways that reversibly activate the expression of specific genes, whose products mediate the relatively irreversible expression of genes responsible for the acquisition of long-term memory. Kandel's model of the cellular processes responsible for the acquisition of long-term memory may offer hypotheses regarding the cellular basis for the acquisition of developmental potency in response to extracellular signals, namely, embryonic induction.

Aspects of induction such as the establishment of dynamic regions of developmental potency, the gradual commitment to a developmental pathway, and the role of regional specification in these processes are critical to embryonic development. Ultimately, these complex phenomena must be addressed by future models of the cellular mechanisms underlying inductive interactions.

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## References

- BACON, R. L. (1945). Self differentiation and induction in the heart of *Amblystoma*. *J. exp. Zool.* **98**, 87–125.
- BALINSKY, B. I. (1939). Experiments on total extirpation of the whole endoderm in *Triton* embryos. *C.r. Acad. Sci. URSS (Dokl Akad. Nauk. SSSR)* **23**, 196–198.
- BALINSKY, B. I. (1951). On the eye cup-lens correlation in some South African amphibians. *Experientia* **7**, 180–186.
- BAN-HOLTFRETER, H. (1965). Differentiation capacities of Spemann's organizer investigated in explants of diminishing size. Ph.D. dissertation, University of Rochester. Ann Arbor: University Microfilms.
- BRACHET, J. (1950). *Chemical Embryology*, New York: Interscience.
- BRADDA, S. K. (1959). Studies on the process of lens induction in *Xenopus laevis* (Daudin). *Wilhelm Roux Arch. EntwMech. Org.* **151**, 181–187.
- CHUANG, H. H. & TSENG, M. P. (1957). An experimental analysis of the determination and differentiation of the mesodermal structures of neurula in urodeles. *Scientia Sinica* **6**, 669–708.
- COPENHAVER, W. M. (1924). Experiments on the development of the heart of *Amblystoma punctatum*. *J. exp. Zool.* **43**, 321–371.
- COPENHAVER, W. M. (1955). Heart, Blood Vessels, Blood, and Entodermal Derivatives. In *Analysis of Development*. (ed. B. H. Willier, P. A. Weiss & V. Hamburger), pp 440–461. Philadelphia: W. B. Saunders.
- CONTRONEI, G. (1922). Corrélations et différenciations. Essai de Morphologie causale sur la tête des Amphibies. *Archs Ital. Biol.* **71**, 83–111.
- DALE, L. & SLACK, J. M. W. (1987). Fate map for the 32-cell stage of *Xenopus laevis*. *Development* **99**, 527–551.
- DAVIS, L. A. & LEMANSKI, L. F. (1987). Induction and myofibrillogenesis in cardiac lethal mutant axolotl hearts rescued by RNA derived from normal endoderm. *Development* **99**, 145–154.
- DEHAAN, R. L. (1964). Cell interactions and oriented movements during development. *J. exp. Zool.* **157**, 127–138.
- DEHAAN, R. L. (1965). Morphogenesis of the vertebrate heart. In *Organogenesis*, (ed. R. L. DeHaan & H. Ursprung), pp. 377–419. New York: Holt, Rinehart & Winston.
- DESMILL, J. F. (1906). Notes on supernumerary eyes, and local deficiency and reduplication of the notochord in trout embryos. *Proc. zool. Soc. Lond.* **1**, 449–452.
- EKMAN, G. (1921). Experimentelle Beiträge zur Entwicklung des Bombinatorherzens. *Oversikt. av. Finska Vetenskaps-societätens Forhandlingar* **63**, 1–37.
- EKMAN, G. (1925). Experimentelle Beiträge zur Herzentwicklung der Amphibien. *Wilhelm Roux Arch. EntwMech. Org.* **106**, 320–352.
- ETHERIDGE, A. L. (1968). Determination of the mesonephric kidney. *J. exp. Zool.* **169**, 357–370.
- ETHERIDGE, A. L. (1972). Suppression of kidney formation by neural crest cells. *Wilhelm Roux Arch. EntwMech. Org.* **169**, 268–270.
- FAUTREZ, J. & AMANO, H. (1961). Pourquoi le coeur ne se développe-t-il pas après extirpation de l'entoblaste dans une larve d'Urodèle? *C.r. Séanc. Soc. Biol.* **155**, 2219–2220.
- FILATOW, D. (1925a). Ersatz des linsenbildenden Epithels von *Rana esculenta* durch Bauchepithel von *Bufo vulgaris*. *Wilhelm Roux' Arch. EntwMech. Org.* **105**, 475–482.
- FILATOW, D. (1925b). Über die unabhängige Entstehen (Selbstdifferenzierung) der Linse bei *Rana esculenta*. *Wilhelm Roux Arch. EntwMech. Org.* **104**, 50–71.
- FULLILOVE, S. L. (1970). Heart induction: distribution of active factors in newt endoderm. *J. exp. Zool.* **175**, 323–326.

- GANS, C. (1987). The neural crest: a spectacular invention. In *Developmental and Evolutionary Aspects of the Neural Crest*, (ed. P. F. A. Maderson), pp. 361–379. New York: John Wiley & Sons.
- GERHART, J. & KELLER, R. (1986). Region-specific cell activities in amphibian gastrulation. *A. Rev. Cell Biol.* **2**, 201–229.
- GERHART, J. C., VINCENT, J.-P., SCHARF, S. R., BLACK, S. D., GIMLICH, R. L. & DANILCHIK, M. V. (1984). Localization and induction in early development of *Xenopus*. *Phil. Trans. R. Soc. Lond. B* **307**, 319–330.
- GIMLICH, R. L. (1986). Acquisition of developmental autonomy in the equatorial region of the *Xenopus* embryo. *Devl Biol.* **115**, 340–352.
- GOELET, P., CASTELLUCCI, V. F., SCHACHER, S. & KANDEL, E. R. (1986). The long and short of long-term memory – a molecular framework. *Nature, Lond.* **322**, 419–422.
- GRAINGER, R. M., HENRY, J. J. & HENDERSON, R. A. (1988). Reinvestigation of the role of the optic vesicle in embryonic lens induction. *Development* **102**, 517–526.
- GREEN, M. C. (1970). The developmental effects of congenital hydrocephalus (ch) in the mouse. *Devl Biol.* **23**, 585–608.
- GURDON, J. B. (1987). Embryonic induction – molecular prospects. *Development* **99**, 285–306.
- HAMBURGER, V. & HAMILTON, H. L. (1951). A series of normal stages in the development of the chick embryo. *J. Morph.* **88**, 49–92.
- HARRISON, R. G. (1920). Experiments on the lens in *Amblystoma*. *Proc. Soc. exp. Biol. Med.* **17**, 199–200.
- HENRY, J. J. & GRAINGER, R. M. (1987). Inductive interactions in the spatial and temporal restriction of lens-forming potential in embryonic ectoderm of *Xenopus laevis*. *Devl Biol.* **124**, 200–214.
- HERBST, C. (1901). *Formative Teize in der tierischen Ontogenese*, Leipzig: Arth. Georgi.
- HOLTFRETER, J. (1938). Differenzierungspotenzen isolierter Teile der Urodelengastrula. *Wilhelm Roux Arch. EntwMech. Org.* **138**, 522–656.
- HOLTFRETER, J. & HAMBURGER, V. (1955). Amphibians. In *Analysis of Development*, (ed. B. H. Willier, P. A. Weiss & V. Hamburger), pp. 230–296. Philadelphia: W. B. Saunders.
- HOMMES, O. R. (1957). *Primary Entodermal Defects, Development of Body Form and Genital Organs of Acardii in Uni-vitelline Twins*. Amsterdam: Jacob van Campen.
- HOPERSKAYA, O. A. (1968). Differentiation of eye area of the neural plate of early neurula of frog embryos without subsequent inductive interactions. *C.r. Acad. Sci. USSR (Dokl. Akad. Nauk SSSR)* **180**, 1012–1015.
- HUMPHREY, R. R. (1972). Genetic and experimental studies on a mutant gene (c) determining absence of heart action in embryos of the Mexican Axolotl (*Ambystoma mexicanum*). *Devl Biol.* **27**, 365–375.
- JACOBSON, A. G. (1955). The roles of the optic vesicle and other head tissues in lens induction. *Proc. natn. Acad. Sci. U.S.A.* **41**, 522–525.
- JACOBSON, A. G. (1958). The roles of neural and non-neural tissues in lens induction. *J. exp. Zool.* **139**, 525–557.
- JACOBSON, A. G. (1960). The influences of ectoderm and endoderm on heart differentiation in the newt. *Devl Biol.* **2**, 138–154.
- JACOBSON, A. G. (1961). Heart determination in the newt. *J. exp. Zool.* **146**, 139–152.
- JACOBSON, A. G. (1963a). The determination and positioning of the nose lens and ear. I. Interaction within the ectoderm and between the ectoderm and underlying tissues. *J. exp. Zool.* **154**, 273–284.
- JACOBSON, A. G. (1963b). The determination and positioning of the nose lens and ear. II. The role of the endoderm. *J. exp. Zool.* **154**, 285–292.
- JACOBSON, A. G. (1963c). The determination and positioning of the nose lens and ear. III. Effects of reversing the antero-posterior axis of epidermis, neural plate, and neural folds. *J. exp. Zool.* **154**, 293–304.
- JACOBSON, A. G. (1966). Inductive processes in embryonic development. *Science* **152**, 25–34.
- JACOBSON, A. G. (1981). Morphogenesis of the neural plate and tube. In *Morphogenesis and Pattern Formation* (ed. T. G. Connelly, L. L. Brinkley and B. M. Carlson), pp. 233–263. New York: Raven Press.
- JACOBSON, A. G. (1987). Determination and morphogenesis of axial structures: Mesodermal metamerism, shaping of the neural plate and tube, and segregation and functions of the neural crest. In *Developmental and Evolutionary Aspects of the Neural Crest*, pp. 147–180. (ed. P. F. A. Maderson). New York: John Wiley & Sons.
- JACOBSON, A. G. & DUNCAN, J. T. (1968). Heart induction in salamanders. *J. exp. Zool.* **167**, 79–103.
- KAO, K. R. & ELINSON, R. P. (1988). The entire mesodermal mantle behaves as Spemann's organizer in dorsoanterior enhanced *Xenopus laevis* embryos. *Devl Biol.* **127**, 64–77.
- KARKINEN-JÄÄSKELÄINEN, M. (1978). Permissive and directive interactions in lens induction. *J. Embryol. exp. Morph.* **44**, 167–179.
- KELLER, R. E. (1975). Vital dye mapping of the gastrula and neurula of *Xenopus laevis*. I. Prospective areas and morphogenetic movements of the superficial layer. *Devl Biol.* **42**, 222–241.
- KELLER, R. E. (1976). Vital dye mapping of the gastrula and neurula of *Xenopus laevis*. II. Prospective areas and morphogenetic movements of the deep layer. *Devl Biol.* **51**, 118–137.
- KELLER, R. E., DANILCHIK, M., GIMLICH, R. L. & SHIH, J. (1985). The function and mechanism of convergent extension during gastrulation of *Xenopus laevis*. *J. Embryol. exp. Morph.* **89 Supplement**, 185–209.
- KIMELMAN, D. & KIRSCHNER, M. (1987). Synergistic induction of mesoderm by FGF and TGF- $\beta$  and the identification of an mRNA coding for FGF in the early *Xenopus* embryo. *Cell* **51**, 869–877.
- KING, H. D. (1905). Experimental studies on the eye of the frog embryo. *Arch. EntwMech. Org.* **19**, 85–107.
- LE CRON, W. L. (1907). Experiments on the origin and differentiation of the lens in *Amblystoma*. *Am. J. Anat.* **6**, 245–258.

- LE DOUARIN, N. (1963). Action inductrice précocose du mésoderme de l'aire cardiaque sur l'endoderme hépatique de l'embryon de poulet. *C.r. hebd. Séanc. Acad. Sci. (Paris)* **257**, 1357–1360.
- LEMANSKI, L. F., PAULSON, D. J. & HILL, C. S. (1979). Normal anterior endoderm corrects the heart defect in cardiac mutant salamanders (*Ambystoma mexicanum*). *Science* **204**, 860–862.
- LEWIS, W. H. (1904). Experimental studies on the development of the eye in Amphibia. I. On the origin of the lens, *Rana palustris*. *Am. J. Anat.* **3**, 505–536.
- LEWIS, W. H. (1907a). Experimental studies on the development of the eye in Amphibia. III. On the origin and differentiation of the lens. *Am. J. Anat.* **6**, 473–509.
- LEWIS, W. H. (1907b). Lens formation in strange ectoderm in *Rana sylvatica*. *Am. J. Anat.* **7**, 145–169.
- LEWIS, W. H. (1909). The experimental production of cyclopia in the fish embryo (*Fundulus heteroclitus*). *Anat. Rec.* **3**, 175–181.
- LIEDKE, K. B. (1942). Lens competence in *Rana pipiens*. *J. exp. Zool.* **90**, 331–351.
- LIEDKE, K. B. (1951). Lens competence in *Amblystoma punctatum*. *J. exp. Zool.* **117**, 573–591.
- LIEDKE, K. B. (1955). Studies on lens induction in *Amblystoma punctatum*. *J. exp. Zool.* **130**, 353–379.
- LONDON, C., AKERS, R. & PHILLIPS, C. (1988). Expression of Epi 1, an epidermis-specific marker in *Xenopus laevis* embryos, is specified prior to gastrulation. *Devl Biol.* (in press).
- MANGOLD, O. (1931). Das Determinationsproblem. III Teil. Das Wirbeltierauge in der Entwicklung und regeneration. *Ergebn. Biol.* **7**, 193–403.
- MANGOLD, O. (1956). Experimente zur Analyse der Herzentwicklung bei *Triton*. *Naturwiss.* **43**, 287.
- MANGOLD, O. (1957). Zur Analyse der Induktionsleistung des Entoderms der Neurula von Urodelen (Herz, Kiemen, Geschlechtszellen, Mundöffnung). *Naturwiss.* **44**, 289–290.
- MCAVOY, J. W. (1980). Induction of the eye lens. *Differentiation* **17**, 137–149.
- MCKEEHAN, M. S. (1954). A quantitative study of self-differentiation of transplanted lens primordia in the chick. *J. exp. Zool.* **126**, 157–175.
- MENCL, E. (1903). Ein Fall von beiderseitiger Augenlinsenausbildung während der Abwesenheit von Augenblasen. *Arch. EntwMech. Org.* **16**, 328–339.
- MENCL, E. (1908). Neue Tatsachen zur Selbstdifferenzierung der Augenlinse. *Wilhelm Roux Arch. EntwMech. Org.* **25**, 431–450.
- MICHAEL, M. I. (1968). The induction of the head sense placodes in the axolotl (*Ambystoma mexicanum*). Effect of the removal of the entire prospective brain area S.S., or including the inner half or the entire neural fold. *Alex. med. Jour.* **14**, 53–64.
- MICHAEL, M. I. & NIEUWKOOP, P. D. (1967). Experimental analysis of the development of the head sense placodes of *Ambystoma mexicanum*. *Vers. Kon. Nedl. Akad. Wet.*, serie C, **70**, 272–279.
- MIZUNO, T. (1970). Induction de cristallin *in vitro*, chez le poulet, en absence de la vésicule optique. *C.r. hebd. Séanc. Acad. Sci. Paris* **271**, 2190–2192.
- MIZUNO, T. (1972). Lens differentiation *in vitro* in the absence of optic vesicle in the epiblast of chick blastoderm under the influence of skin dermis. *J. Embryol. exp. Morph.* **28**, 117–132.
- MOHUN, T. J., BRENNAN, S., DATHAN, N., FAIRMAN, S. & GURDON, J. B. (1984). Cell type-specific activation of actin genes in the early amphibian embryo. *Nature, Lond.* **311**, 716–721.
- NIEUWKOOP, P. D. (1947). Experimental investigations on the origin and determination of the germ cells, and on the development of the lateral plates and germ ridges in Urodeles. *Archs Neerl. Zool.* **8**, 1–205.
- NIEUWKOOP, P. D. & FABER, J. (1967). *Normal Table of Xenopus laevis (Daudin)*. Amsterdam: North-Holland.
- NIEUWKOOP, P. D., JOHNEN, A. G. & ALBERS, B. (1985). *The Epigenetic Nature of Early Chordate Development*. Cambridge: Cambridge University Press.
- NIEUWKOOP, P. D. & UBBELS, G. A. (1972). The formation of mesoderm in urodelean amphibians. IV. Quantitative evidence for the purely "ectodermal" origin of the entire mesoderm and of the pharyngeal endoderm. *Wilhelm Roux Arch. EntwMech. Org.* **169**, 185–199.
- OPPENHEIMER, J. M. (1966). Dependent and independent differentiation of the lens in *Fundulus* embryos. *Archs zool. Ital.* **51**, 667–685.
- ORTS-LLORCA, F. (1963). Influence of the endoderm on heart differentiation during the early stages of development of the chicken embryo. *Wilhelm Roux Arch. EntwMech. Org.* **154**, 533–551.
- ORTS-LLORCA, F. & GIL, D. R. (1965). Influence of the endoderm on heart differentiation. *Wilhelm Roux Arch. EntwMech. Org.* **156**, 368–370.
- PASQUINI, P. (1927). Ricerche di embriologia sperimentale sui trapianti omeoplastici della vesicola ottica primaria in *Pleurodeles waltlii*. *Boll. Ist. Zool., Univ. Roma*, **5**, 1–83.
- PASQUINI, P. (1931). Sul differenziamento correlative della lente cristallina e della cornea nello sviluppo di Anfibi ed Urodeli. *Rend. R. Acc. Naz. die Lincei* **14**, 56–61.
- POPOV, W. W. (1937). Über den morphogenen Einfluss des Augenbeckens auf verschiedene embryonale Gewebe und die Anlage einiger Organe. *Zool. Jahrb. Abt. Allg. Zool. Physiol.* **58**, 23–56.
- POPOV, W. W., KISLOV, NIKITENKO & CANTURISVILI (1937). Über die linsenbildende Fähigkeit des Epithels bei Embryonen von *Pelobates fuscus*, *Bufo viridis*, *Bombina bombina* und *Triton cristatus*. *C.r. Acad. Sci. USSR (Dokl. Akad. Nauk. SSSR)* **16**, 245–248.
- RAWLES, M. E. (1936). A study in the localization of organ-forming areas in the chick blastoderm of the head process stage. *J. exp. Zool.* **72**, 271–315.
- REYER, R. W. (1958a). Studies on lens induction in *Amblystoma punctatum* and *Triturus viridescens viridescens*. I. Transplants of prospective belly ectoderm. *J. exp. Zool.* **138**, 505–555.
- REYER, R. W. (1958b). Studies on lens induction in *Amblystoma punctatum* and *Triturus viridescens viridescens*. II. Transplants of prospective lateral head

- ectoderm. *J. exp. Zool.* **139**, 137–180.
- ROSA, F., ROBERTS, A. B., DANIELPOUR, D., DART, L. L., SPORN, M. B. & DAWID, I. B. (1988). Mesoderm induction in amphibians: The role of TGF- $\beta$ 2-like factors. *Science* **239**, 783–785.
- ROSENQUIST, G. C. & DEHAAN, R. L. (1966). Migration of precardiac cells in the chick embryo: a radioautographic study. *Carnegie Inst. Wash. Publ.* **625**, *Contributions to Embryology* **263**, 113–121.
- SHARP, C. R., FRITZ, A., DE ROBERTIS, E. M. & GURDON, J. B. (1987). A homeobox-containing marker of posterior neural differentiation shows the importance of predetermination in neural induction. *Cell* **50**, 749–758.
- SCHARF, S. R. & GERHART, J. C. (1980). Determination of the dorsal-ventral axis in eggs of *Xenopus laevis*. Rescue of UV-impaired eggs by oblique orientation. *Devl Biol.* **79**, 181–198.
- SCHRECKENBERG, G. M. & JACOBSON, A. G. (1975). Normal stages of development of the axolotl, *Ambystoma mexicanum*. *Devl Biol.* **42**, 391–400.
- SLACK, J. M. W. (1983). *From Egg to Embryo. Determinative Events in Early Development*. Cambridge: Cambridge University Press.
- SLACK, J. M. W., DARLINGTON, B. G., HEATH, J. K. & GODSAVE, S. F. (1987). Mesoderm induction in early *Xenopus* embryos by heparin-binding growth factors. *Nature, Lond.* **326**, 197–200.
- SMITH, J. C. (1987). A mesoderm-inducing factor is produced by a *Xenopus* cell line. *Development* **99**, 3–14.
- SMITH, J. C., DALE, L. & SLACK, J. M. W. (1985). Cell lineage labels and region-specific markers in the analysis of inductive interactions. *J. Embryol. exp. Morph.* **89 Supplement** 317–331.
- SMITH, J. C., YAQOUB, M. & SYMES, K. (1988). Purification, partial characterization and biological effects of the XTC mesoderm-inducing factor. *Development* **103**, 591–600.
- SPEMANN, H. (1901). Über Korrelationen in der Entwicklung des Auges. *Verh. Anat. Ges.* **15 Vers** Bonn, 61–79.
- SPEMANN, H. (1907). Neue Tatsachen zum Linsenproblem. *Zool. Anz.* **31**, 379–386.
- SPEMANN, H. (1912). Zur Entwicklung des Wirbeltierauges. *Zool. Jahrb. Abt. allg. Zool. Phys. Tiere*, **32**, 1–98.
- SPEMANN, H. (1938). *Embryonic Development and Induction*, New Haven: Yale University Press.
- SPITZER, N. C. (1979). Ion channels in development. *A. Rev. Neurosci.* **2**, 363–397.
- STÖHR, PH. JR (1924). Experimentelle studien an embryonalen Amphibienherzen. I. Über Explantation embryonaler Amphibienherzen. *Wilhelm Roux Arch. EntwMech. Org.* **102**, 426–451.
- SYMES, K. & SMITH, J. C. (1987). Gastrulation movements provide an early marker for mesoderm induction in *Xenopus laevis*. *Development* **101**, 339–350.
- TAHARA, Y. (1962a). Formation of the independent lens in Japanese amphibians. *Embryologia* **7**, 127–149.
- TAHARA, Y. (1962b). Determination of the independent lens rudiment in *Rhacophorus schlegelii arboreus*. *Embryologia* **7**, 150–168.
- TEN CATE, G. (1953). *The Intrinsic Development of Amphibian Embryos*. Amsterdam: North-Holland.
- TRINKAUS, J. P. (1984). *Cell into Organs*, second edition. Englewood Cliffs, New Jersey: Prentice-Hall.
- VON UBISCH, L. (1923). Linsenbildung bei *Rana fusca* trotz Entfernung des Augenbechers. *Verh. d. D. Zool. Ges.* **28 Vers. Leipzig**, 35–36.
- VON WOELLWARTH, C. (1961). Die Rolle des neuralleistenmaterials und der Temperatur bei der Determination der Augenlinse. *Embryologia* **6**, 219–242.
- WADDINGTON, C. H. (1932). Experiments on the development of chick and duck embryos, cultivated *in vitro*. *Phil. Trans. R. Soc. Lond.*, **B. 221**, 179–230.
- WARNER, A. & GURDON, J. B. (1987). Functional gap junctions are not required for muscle gene activation by induction in *Xenopus* embryos. *J. Cell Biol.* **104**, 557–564.
- WOERDEMAN, M. W. (1939). On lens induction. *Proc. Kon. Ned. Akad. Wetensch.* **42**, 290–292.
- WOERDEMAN, M. W. (1941). Self-differentiation of the lens anlage of *Rana esculenta* after transplantation. *Acta Neerl. Morph.* **4**, 91–94.
- WILENS, S. (1955). The migration of heart mesoderm and associated areas in *Amblystoma punctatum*. *J. exp. Zool.* **129**, 579–606.

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