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Embryonic induction – molecular prospects

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Synopsis

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(1) Introduction

Embryonic induction is an interaction between one (inducing) tissue and another (responding) tissue, as a result of which the responding tissue undergoes a change in its direction of differentiation. This is probably the single most important mechanism in vertebrate development leading to differences between cells and to the organization of cells into tissues and organs.

The phenomenon is considered to have been discovered by Spemann in 1901 and by Lewis (1904) who both established that in certain species of *Rana* the formation of a lens from ectoderm is dependent on an influence of the underlying optic lobe of the brain. Interest in induction was enhanced by the famous Spemann & Mangold (1924) experiment in which the dorsal lip of the unpigmented *Triturus cristatus* embryo was transplanted to the ventral region of a pigmented *Triturus taeniatus* gastrula, where it induced the pigmented host cells to form a secondary axis (Spemann, 1938). Within the secondary axis is the neural tube which is induced in ectoderm by the underlying mesoderm, a process referred to as primary induction. It was soon discovered that a variety of easily obtainable materials could substitute for mesoderm in inducing ectoderm to undergo neural differentiation, and it was expected in the 1930s that these could serve as sources for purifying a neural inducing substance (excellent review by Witkowski, 1985). This optimism disappeared when it was found that newt embryo ectoderm was so far predisposed to neural differentiation that almost any substance could act as an inducer (Holtfreter, 1934, 1947; Waddington, Needham & Brachet, 1936; Barth, 1968). Although it is now nearly a century since embryonic induction was discovered, the molecular basis of inducers and the inductive response remains almost totally obscure.

Embryonic induction has been most fully analysed in Amphibia, on account of the large size and easy manipulation of their eggs and embryos, and amphibian work is emphasized in this review. Cell interactions having the characteristics of induction probably constitute the single most prevalent mechanism in the development of the vertebrates. Though much less studied, inductive cell interactions certainly take place in the development of invertebrates. For example, in sea urchins, vegetal pole micromeres transplanted to the animal half of an embryo induce secondary vegetal differentiation (Horstadius, 1973). In the nematode *C. elegans*, a signal from an adjacent gonadal anchor cell induces hypodermal precursor cells to adopt one of two types of vulval cell lineage, as opposed to nonvulval development in the absence of a gonadal cell (Sternberg & Horvitz, 1986).

The purpose of this review is to summarize some common properties of embryonic inductions and to consider ways whereby current methods of molecular biology, which are proving of such enormous value to fields as diverse as taxonomy and membrane physiology, may be usefully applied to the extraordinarily recalcitrant problem of embryonic induction.

(2) Molecular assays

For analysis at the molecular level, the single most desirable characteristic in any experimental induction system is a rapid and quantitative assay for an early molecular event. The importance of having an assay for an *early* inductive response should be strongly emphasized. It has become increasingly clear that most, if not all, inductions consist of several separate steps. For example, liver induction in the chick requires first an induction by heart mesenchyme and then by liver mesenchyme, neither alone being sufficient (Wessells, 1977). *Triturus* lens induction normally involves three sequential inductions by endoderm, heart mesoderm and retina (Jacobson, 1966). If an induction can only be recognized after several sequential steps have taken place, this greatly complicates attempts to resolve the process into its component parts. Therefore to simplify the problem, it is almost essential to use a direct assay for an early response. For example, the first synthesis of a single gene's transcripts can be detected using sensitive biochemical probes that depend on the *in vitro* formation of nuclease-resistant hybrids. However, such assays do not give localization at the cellular level. For a complete analysis, it is essential to be able to apply either *in situ* hybridization with nucleic acid probes or a specific antibody, to distinguish induced from uninduced cells at the cellular level. Antibodies have the great advantage of providing single cell resolution, a situation very hard to attain by *in situ* hybridization in embryos.

The following paragraphs give examples of molecular markers that can be used in some of the most widely studied inductions. In normal amphibian development, cells of the ectodermal lineage are subjected to at least three sequential inductions. As seen in Fig. 1, some of the animal (future ectodermal) cells of a blastula are induced by vegetal cells to form mesoderm; this includes muscle for which several excellent markers are available (Figs 2–4), either as muscle actin-specific probes (Mohun, Brennan, Dathan, Fairman & Gurdon, 1984; Gurdon, Fairman, Mohun & Brennan, 1985), or as antibodies (Kintner & Brockes, 1984; Smith, Dale & Slack, 1985). An antibody that recognizes keratan sulphate in the notochord (an embryonic form of vertebral column also formed by mesoderm induction) has been described by Smith & Watt (1985), but this appears to bind only extracellular material and may not be diagnostic for single cells. The next major induction in amphibian development takes place during gastrulation when mesodermal cells at the dorsal blastopore lip and inside the embryo induce the overlying ectoderm to form nerve (neural or primary induction). Most molecular markers of neural induction appear rather late in development (Takata, Yamamoto & Ozawa, 1981, 1984; Duprat, Kan, Gualandris, Foulquier & Marty, 1985; Godsave, Anderton & Wylie, 1986). However, two early molecular markers have recently been described for neural induction in *Xenopus*. One is the extracellular glycoprotein N-CAM, a neural cell adhesion molecule. This can be detected at the neurula stage by an antibody (Jacobson & Rutishauser, 1986), or at the late gastrula stage by a cDNA probe as seen in Fig. 5 (Kintner & Melton, 1987). The other is a *Xenopus* homeobox-containing gene XlHbox6, part of which serves as a probe for spinal cord induction as early as the late gastrula (Sharpe, Fritz, DeRobertis & Gurdon, unpublished data). It is also worth noting that sodium pump activation can be detected by electrophysiological means in neural cells a few hours after they have been induced (Blackshaw & Warner, 1976). Fortunately there are excellent cytokeratin or other markers for epidermal differentiation (Jonas, Sargent & Dawid, 1985); these are expressed in the absence of induction but are suppressed by neural differentiation (Slack, 1985; Jones & Woodland, 1986; Akers, Phillips & Wessells, 1986). *In situ* hybridization has been used successfully in relatively late larval stages of *Xenopus* (stage 42) to detect muscle, epidermal or neural differentiation (Dworkin-Rastl, Kelley & Dworkin, 1986; Fig. 3). The third much-studied induction in amphibian development is lens induction, which results mainly from the optic lobes of the embryonic brain inducing overlying ectoderm to invaginate and form a lens vesicle (Jacobson, 1966). Nucleic acid probes and antibodies are available for lens-specific crystallins (e.g. Grainger, Hazard-Leonards, Samaha, Hougan, Lesk & Thomsen, 1983; Kondoh & Okada, 1986).

In fact early amphibian inductions are more complex than just suggested. Each major inductive step, such as mesodermal induction or neural induction, includes more precise regional information. Thus vegetal cells on the dorsal side of an embryo induce notochord and muscle, whereas vegetal cells on the ventral side induce blood and mesenchyme, considered to be lower grades of mesodermal induction (Boterenbrood & Nieuwkoop, 1973). Similarly, Mangold (1933) and Horst (1948) concluded, in respect of neural induction in newts, that anterior mesoderm induces brain, while posterior mesoderm induces

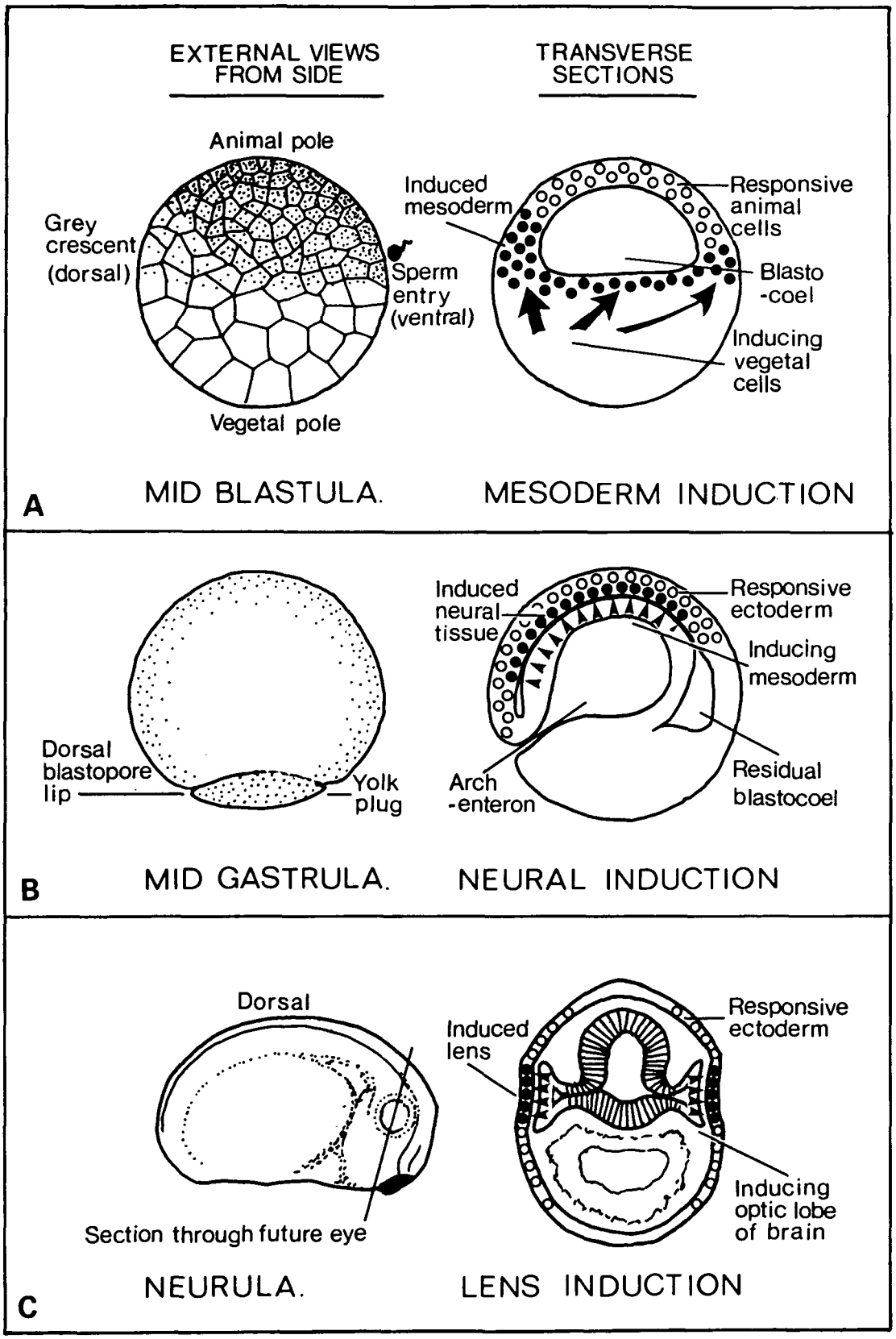


Fig. 1. Sequential inductions in amphibian development. Inducing tissues are indicated by arrows, cells competent to respond to induction by open circles, and cells which actually respond by filled in circles.

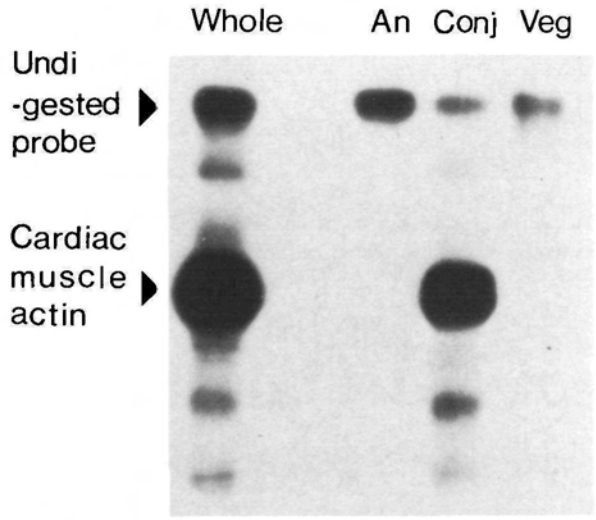


Fig. 2. A molecular probe for an early inductive response. A single stranded 380-nucleotide length of DNA, complementary to part of the 3′ region of *Xenopus laevis* cardiac actin mRNA, is ^{32}P-labelled by *in vitro* synthesis from an M13 vector. After hybridization to RNA, S1 nuclease digestion shortens the probe to a 250-nucleotide length protected by the complementary mRNA. Animal (An), vegetal (Veg), or animal–vegetal conjugates (Conj) were prepared from blastulae and cultured to the neural folds stage when RNA was extracted and analysed. Animal (responding) or vegetal (inducing) tissues are negative for muscle actin gene transcription when cultured in isolation, but strongly positive, as are normal whole embryos, when placed in contact. Similar results can be obtained using anti-sense RNA probes synthesized with SP6 or T7 RNA polymerase (from Gurdon *et al.* 1985).

spinal cord. The regional nature of early inductions has been strongly emphasized and explored in detail by Slack & Forman (1980), Dale, Smith & Slack (1985), and Smith *et al.* (1985), who believe that an independent dorsalizing induction spreads from the dorsal equatorial region during the early gastrula stage, causing some ventral equatorial cells to become muscle. There appear to be two possible interpretations of these events. One is that each major induction in reality consists of two or more separate processes, such that vegetal and dorsal inducers differ from each other, as would anterior and posterior neural inducers. Indeed, there is no reason to limit the presumed diversity of inducers; perhaps there are separate mesoderm inducers and receptors for notochord, muscle, blood, mesenchyme, etc. The other interpretation is that dorsal vegetal cells of a blastula and early gastrula emit only one kind of inducer, which in high concentration (dorsal side) would induce notochord and muscle, but in lower concentration (ventral side) would induce blood and mesenchyme. The same argument could apply to neural induction, one inducer having different effects in anterior and posterior regions. There seems to be no decisive experiment that distinguishes these interpretations. However, for the immediate prospects of molecular analysis, the difference is not crucial; what matters most is the availability of early expressed quantitative markers, as discussed above for muscle. It should be mentioned that globin gene transcription, as a marker for blood, first occurs rather late, at stage 30, in amphibian development (Banville & Williams, 1985*a*,*b*), and a molecular marker has not been described for mesenchyme.

In avian development, cartilage formation in the future vertebral column is induced in somite cells by spinal cord; chondroitin sulphate serves as a quantitative measure of inductive effect (Kosher, Lash & Minor, 1973), though this is synthesized at a significant but much lower level in noninduced tissue. Similarly, collagen synthesis serves as a marker for the enhanced differentiation of chick cornea by lens (Meier & Hay, 1975). Epidermal differentiation into keratinized, mucous or ciliated epidermis is dependent on induction by the underlying dermis. Although molecular markers have not been used in such experiments, it would not appear difficult to develop markers for products characteristic of these kinds of differentiation.

In mammals, pancreas differentiation (Fig. 6) is accompanied by the synthesis of many well-defined gene products (Rutter, Wessells & Grobstein, 1964). Specialized cells of the pancreas are derived from the endodermal (gut) epithelium, after induction by the adjacent mesodermal mesenchyme. Exocrine cells secrete such enzymes as trypsin, ribonuclease and carboxypeptidase; the endocrine cells synthesize glucagon and insulin, the assays for insulin being exceedingly sensitive.

A huge amount of very careful work has been done on many other inductive systems (excellent summary by Wessells, 1977, where references can be found). These include kidney (formed from mesoderm induced by other mesoderm of the ureteric bud), mouse mammary gland (formed from mammary epithelium induced by mammary or other sources of mesenchyme), and avian feathers and scales (formed in the epidermis induced by mesodermal dermis). In all these cases, the emphasis has been on the morphogenetic patterns formed by populations of cells. Pattern formation is very hard to analyse by molecular means, though recent work suggests that cell adhesion molecules may be involved in the morphogenetic response to induction (Gallin, Chuong, Finkel & Edelman, 1986).

(3) Instructive and permissive inductions

A key question in the analysis of any induction is whether the kind of response is determined by the inducing or responding tissue. The distinction emphasized by Saxen (1977) between a permissive and an instructive induction is particularly helpful. A permissive induction is one in which the responding tissue is already so far committed to its final state of differentiation that any, often unspecific, influence will serve to complete the process; in such a case the nature of the response is almost entirely determined

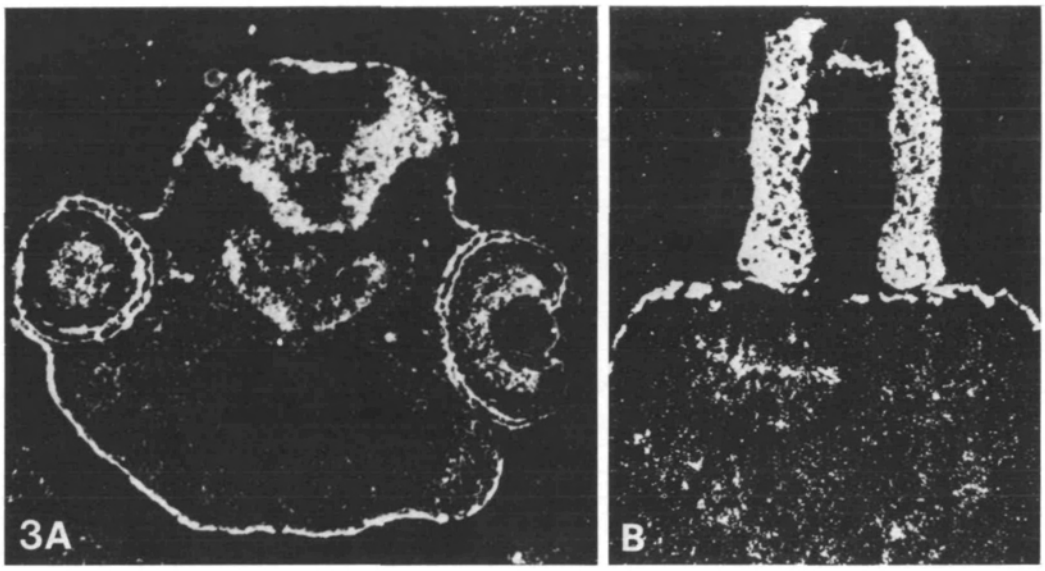


Fig. 3. The use of *in situ* hybridization to recognize gene activation in induced tissues. Single-stranded DNA probes, 50–250 nucleotides long, were ^{3}H-labelled by nick-translation of plasmids containing cDNAs complementary to tadpole mRNAs. The probes were hybridized to sections of fixed embryos, which were subsequently autoradiographed; autoradiographic grains, representing hybridized probe, are seen as white areas in the figures shown. (A) This probe hybridizes preferentially to the nervous system, the brain and neural retina being clearly labelled; it also labels epidermis. (B) This probe labels muscle very strongly, but not epidermis or nerve. Both sections are of normal hatched tadpoles. *In situ* hybridization nearly always generates more background labelling than antibodies: compare Fig. 3B with 4A (from Dworkin-Rastl *et al.* 1986).

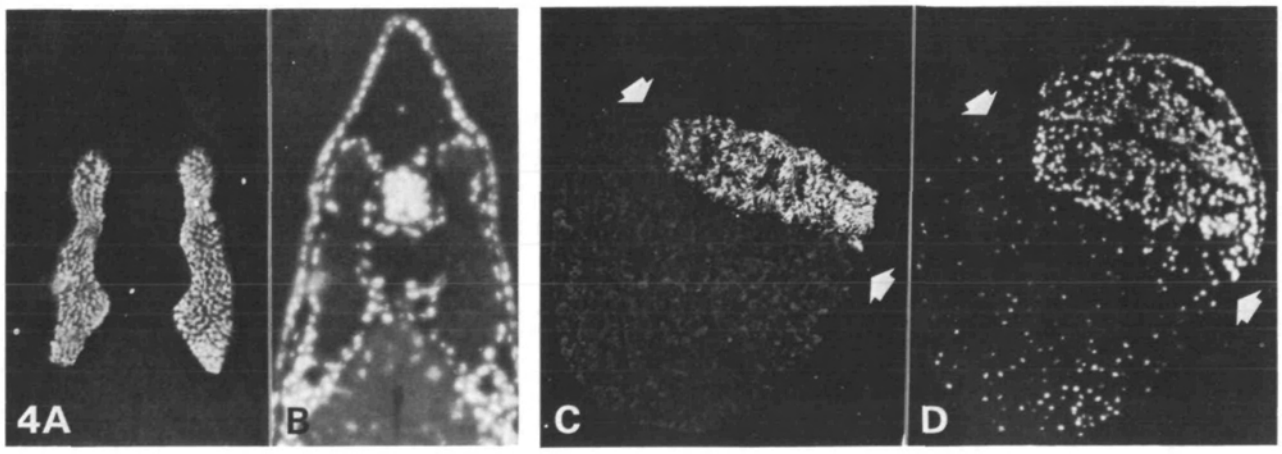


Fig. 4. The use of an antibody to recognize muscle induction, showing low background and single cell resolution. The 12/101 monoclonal antibody of Kintner & Brockes (1984), against an as yet unidentified muscle antigen, has been applied to sections of *Xenopus* embryos, and visualized by a secondary fluorescent rhodamine-labelled antibody. (A,B) Whole embryo, hatching stage. (C,D) An animal–vegetal conjugate, cultured until controls reached the muscular response stage. (A and C) Stained with antibody; note the absence of nonmuscle background in A. (B and D) Similar sections stained with Hoechst to show the distribution of cells. In C and D, the junction between animal and vegetal tissues is indicated by white arrows.

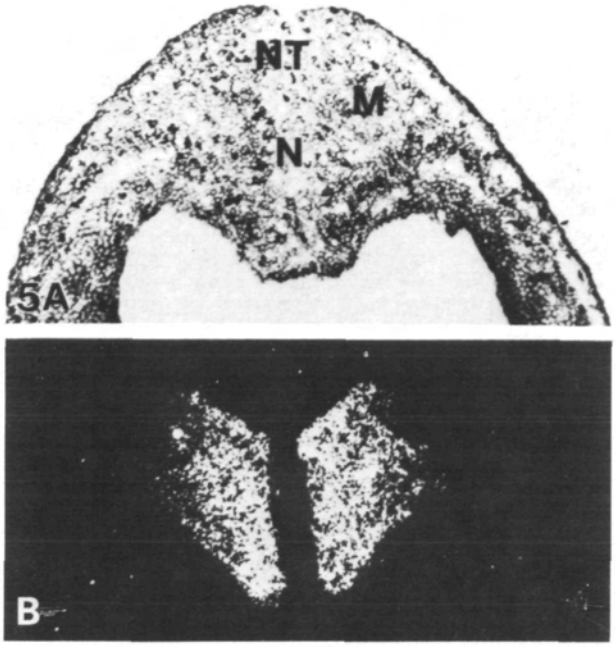


Fig. 5. A probe for a neural cell adhesion molecule serves as an early marker of neural differentiation. The figures show transverse sections through the neural folds region of a *Xenopus* embryo. (A) Phase contrast; *NT*, neural tube; *N*, notochord; *M*, muscle. (B) *In situ* hybridization with an ^{35}S-RNA probe synthesized from an N-CAM cDNA; the neural tube shows strong hybridization, the autoradiographic grains appearing white under dark-field illumination. (From Kintner & Melton, 1987.)

by the responding tissue. In contrast, an instructive induction is one in which the responding tissue is to some extent uncommitted and requires a specific signal from the inducing tissue telling it in which of two or more directions to differentiate.

Examples will make the distinction more clear. One is provided by the later stages of mouse pancreas differentiation. From the 15-somite stage, the pancreatic mesenchyme can be replaced by mesenchyme from many other organ primordia such as the salivary gland, or even by embryo extract, and yet pancreas differentiation will take place (Rutter *et al.* 1964). At the 15-somite stage the endoderm epithelium is therefore largely committed to pancreas differentiation, and needs only a relatively unspecific inductive stimulus to complete the process (Wessells & Cohen, 1967). Other examples of permissive inductions occur in various adult tissues when enzymes are used to physically separate inducing and responding tissues. In such cases, the readdition of collagen, glycosaminoglycans and other extracellular materials will enhance the amount of terminal cell differentiation as effectively as inducer tissue, and may act by replacing materials that were removed during preparation and which are normally required to provide permissive conditions for terminal differentiation (Meier & Hay, 1975).

Instructive inductions can be recognized in different ways. A tissue is generally considered to induce instructively if it can force a responding tissue to change its path of differentiation and conform to the fate normally determined by that inducing tissue. This clearly happens in chick and mouse skin development. For example, a piece of ectoderm from a future feathered area of a chick embryo combined with mesoderm from the lower leg area will form scales characteristic of the leg and not feathers which would otherwise have been formed in normal development (Rawles, 1963; Fig. 7). Even more remarkable is the combination of chick corneal epithelium (destined to form the translucent cornea in normal development) with mouse mesoderm (which would normally induce mouse ectoderm to form hair). The result is that the chick epithelium forms feathers though these are morphologically fairly abnormal (Sengel, 1976). In these cases, the mesoderm is wholly responsible for redirecting the way the ectoderm differentiates. It is restricted, obviously, by the genetic repertoire of the responding cells, such that chick epithelium forms feathers and not hair.

A more stringent criterion for recognizing an instructive induction requires that a tissue can be induced to differentiate in two different ways apart from the way it would if receiving no induction at all. Thus *Xenopus* early gastrula ectoderm will form epidermis if cultured in isolation, but can be induced to form nerve if induced by mesoderm, or muscle if induced by blastula vegetal cells (section 2 above). At least one of the two latter must give specific information during induction and must therefore be instructive.

The answer to the question whether tissue inductions are permissive or instructive is that there are clear examples of each kind. Indeed both types seem to occur within the same system. In the mouse, for example, endoderm isolated at the 9-somite stage will only form pancreas if associated with its own mesenchyme. At the 15-somite stage, the endoderm will form pancreas if induced by any kind of mesenchyme, a permissive effect described above. Similarly, liver induction in the chick is at first instructive and subsequently permissive (Le Douarin, 1964). It seems likely that it may be a general principle that an instructive induction is needed at first, with progressively more permissive inductions at later stages, as developmental options are narrowed, to produce a morphologically normal tissue. However, it is important to appreciate that, even when inductions are

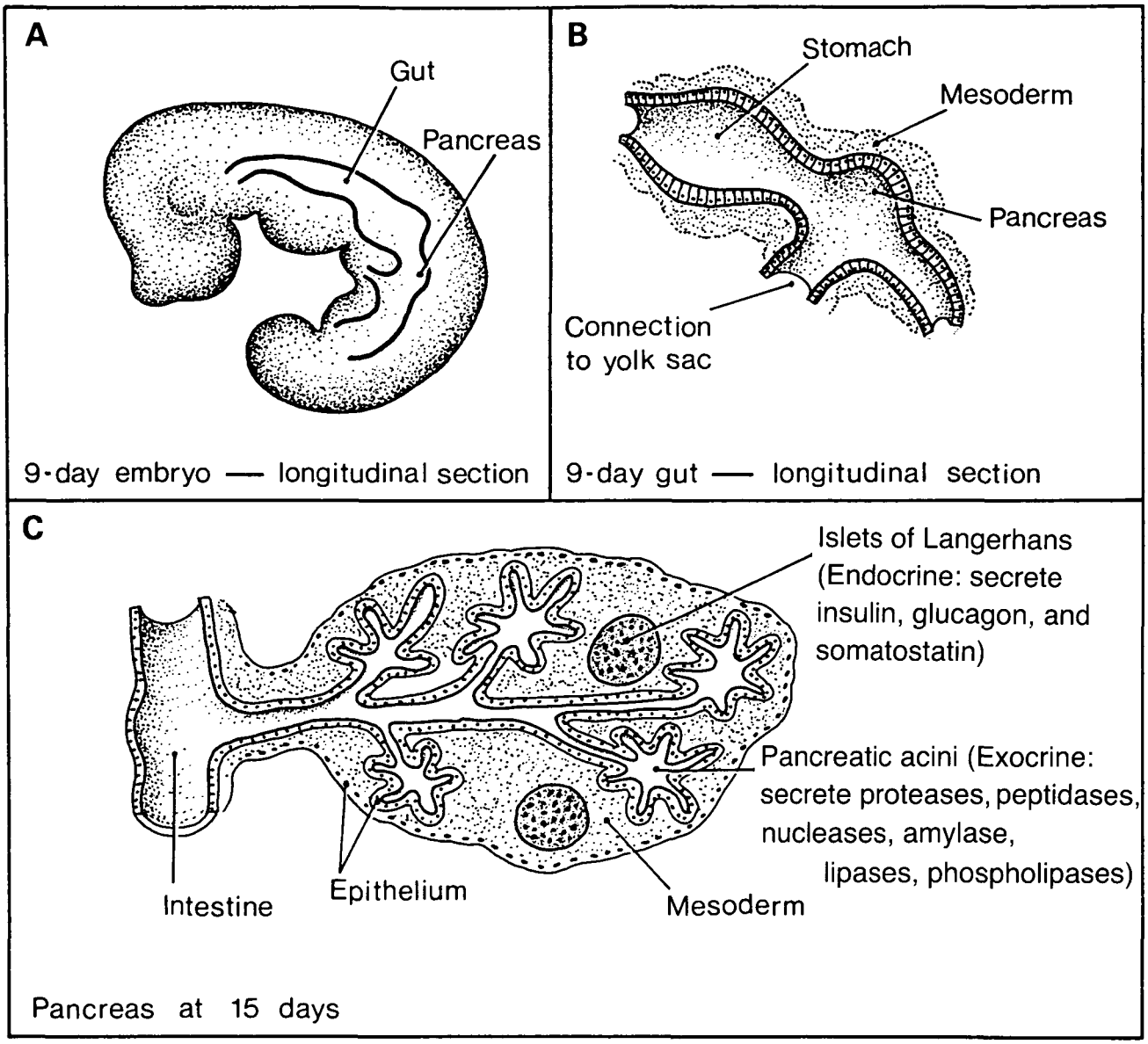
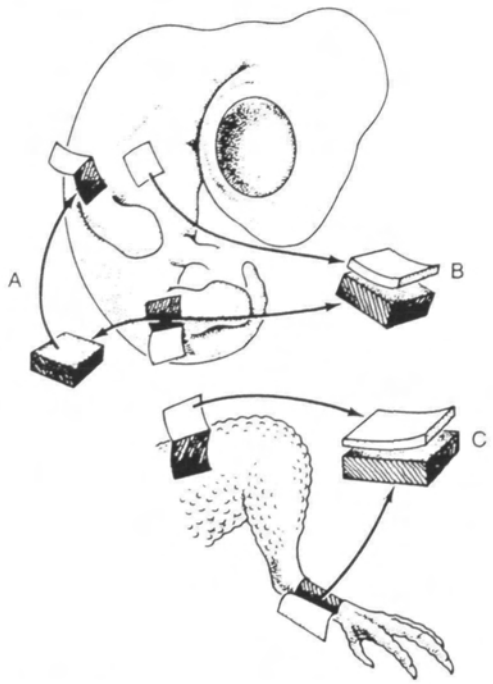


Fig. 6. Development of the pancreas in the mouse. (A,B) Longitudinal sections through the pancreas forming as an evagination of the gut. (C) Diagram of the pancreas at 15 days; acini and islets are derivatives of the epithelium induced by the adjacent mesoderm. (From Wessells & Rutter, 1969.)

described as instructive, the ways in which the responding tissue can differentiate are already limited; one of the most important aspects of an induction is therefore the predisposition of cells to respond in a particular way.

Does the classification of an induction as permissive or instructive give any useful information about its molecular basis? A permissive inducer could be something as unspecific as amino acids, glucose or other nutrients, which would be unlikely to tell us anything useful about the inductive response. On the other hand, an instructive response resembles in several ways those signalling mechanisms in which well-characterized receptors have been identified, hormones and growth factors being examples. It is therefore a reasonable assumption at present that instructive inductions involve specific receptor molecules. The fact that LiCl has strong inductive effects (see p. 297) does not exclude the involvement of a receptor, since Li^+ might affect the pathway of induction beyond the stage of receptor activation.

We will return later to the important question whether the instructive or permissive nature of induction, so far discussed at the tissue level, can be extended to individual cells. But first it is useful to review temporal aspects of induction, which emphasize the importance of the responding tissue.



A. Leg mesoderm in wing → leg feathers
B. Feather-area mesoderm plus nonfeather-ectoderm → feathers
C. Scale-area mesoderm plus feather-area ectoderm → scales

Fig. 7. The mesodermal induction of epidermal differentiation in chick development is instructive. (From Wessells, 1977.)

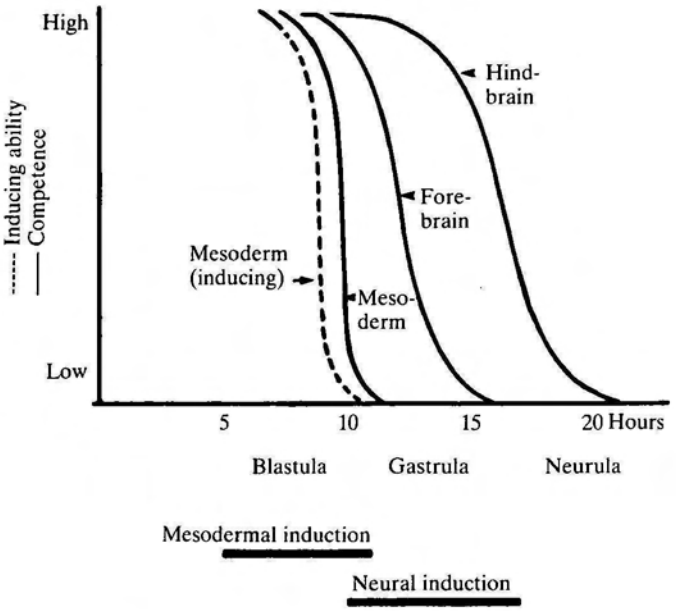


Fig. 8. Inductive capacity and competence to respond are rapidly lost after the time in normal development when these activities normally take place. The two curves on the left of the figure apply to mesodermal induction and those on the right to neural induction. (From results of Gurdon *et al.* (1985) and Dale *et al.* (1985).)

(4) The timing of the response

Much useful information has come from a careful description of timing in inductions. The first important generalization is that the time for which tissues can respond to induction (their state of competence), as well as the time for which other tissues can induce, are both strictly limited in duration. This has emerged most clearly from early embryonic inductions such as those that form mesodermal and neural structures in Amphibia. The stage at which the inductive and responsive capacities are acquired is unclear, but both terminate abruptly soon after the time when these inductions are complete in normal embryos (Fig. 8). Inductions that take place much later in life, and on a much longer time scale, appear to have similar timing characteristics. For example, the dermis and epidermis in chick development acquire and lose inductive capacity and responsiveness over just the few days when this induction normally takes place (Rawles, 1963).

We can now ask an important question, whether the time of response to an induction is determined by the inducing or responding tissues. This requires the combination of inducing and responding tissues of different ages, an experimental situation that can be achieved once the duration of the inductive and responsive phases is known. Using a sufficiently well-defined criterion by which to recognize when induction has taken place, such as muscle actin transcription, it is clear that the timing of induction is determined almost entirely by the developmental state of the responding tissue, and not by the time since it was exposed to inducing tissue (Fig. 9). One way in which this could operate is as follows. An endogenous series of events, perhaps including the activation of one gene by another, could permit the synthesis of muscle-specific actin, but only at the normal time in development and only if the cells have received an inductive signal at some previous but not precisely defined time.

Another useful piece of temporal information is for how long the inducing signal must be applied to be effective. Using the amphibian muscle induction system, inducer contact must be maintained for 1–2 h to give a response, but the amount of induction (actin gene transcripts, in this case) increases with contact times of up to about 5 h (Gurdon *et al.* 1985). With heterogenous inducers, much shorter exposure times of 1 h or less are sufficient to elicit a response (e.g. Smith, 1987), though it seems hard to eliminate the possibility that some part of the inducing pellet or solution used in such experiments has been left behind at the time of its removal. The later in

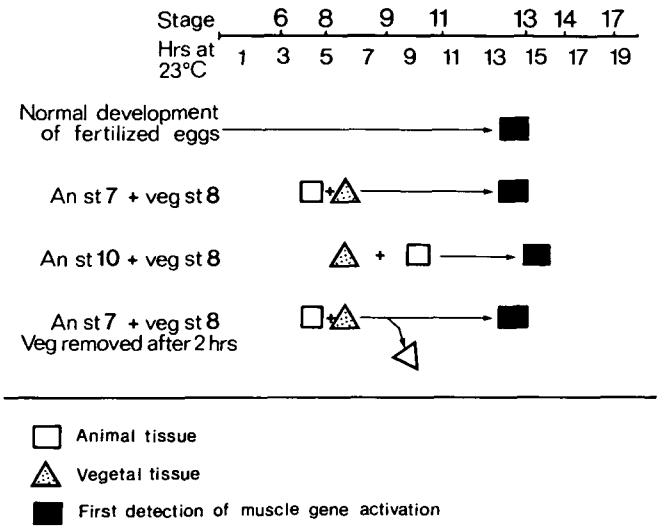


Fig. 9. The time of an inductive response is determined by the responding, not inducing, tissue. Animal and vegetal tissues, removed from a blastula (stages 7 or 8) or from an early gastrula (stage 10), were placed in contact according to the time scale at the top of the figure. They were then frozen at different times ranging from 11 to 17 h after fertilization, and analysed using a muscle actin gene probe as indicated in Fig. 2. (From Gurdon *et al.* 1985.)

development that induction takes place, the longer is the minimum exposure time; in the case of the mesenchymal induction of pancreas differentiation, 24 h are required (Wessells, 1977).

The most significant temporal aspects of embryonic inductions can be summarized as follows. First, the minimum time from the start of induction to the final response is long, being measured in hours or days as is also the case for the action of most hormones and growth factors. Second, the abilities to respond and induce are of strictly limited duration. Third, the time of response is governed by the state of the responding tissue and not by the time that has elapsed since the inducer is placed in contact. In these last two respects, inductions appear to differ fundamentally from all other known cell signalling systems. In the case of hormones, growth factors and neurotransmitters, the competent state (i.e. possession of a receptor) is a permanent property of responding cells, and the response is timed by the arrival of signalling molecules.

(5) The localization of the response

From the point of view of developmental significance, much the most important aspect of an induction is its localization, i.e. the selection of cells that are to respond or not to respond to the induction. We have emphasized above how the responding tissue plays a predominant part in the timing of the response. On the other hand the inducing tissue seems to be a major contributor to the *position* of the response.

The first important principle is that the number of cells that are capable of responding to an inductive stimulus greatly exceeds the number of cells that actually do so. Good examples of this come from early amphibian inductions. At a gross level, there is clearly some predisposition of a responding tissue since not all cells in an embryo are equally competent; for example only cells in the animal half of a blastula can respond to inducing vegetal cells by forming mesoderm. However, only cells in close proximity to the inducing tissue actually respond (Fig. 4C). It is also clear, from experiments with various configurations of inducing and responding tissues, that the proximity of inducing and responding cells to each other is important, rather than their distance from the animal and vegetal poles of an embryo (Nieuwkoop *et al.* 1952; Gurdon, unpublished data). Therefore within a tissue, it is the proximity of inducing cells as well as geographically limited competence that determines the localization of the response.

At the level of single cells, the situation is more complicated. All blastula cells in the animal half of an embryo are capable of responding to vegetal induction to become mesodermal, but not more than 40 % in fact do so (Dale *et al.* 1985). Similarly, both inner and outer layers of *Xenopus* gastrula ectoderm can respond to a mesodermal inducer by becoming brain, but only the inner cells actually do so (Asashima & Grunz, 1983). Is it just a question of proximity to inducing cells, or do other factors come in?

We have so far discussed induction from the point of view of tissues, which consist of hundreds or even thousands of cells. It would greatly simplify further analysis if we could analyse the response at the level of individual cells. The possible complications of describing inductive effects in terms of whole tissues are exemplified by referring back to our discussion on instructive *versus* permissive effects. We quoted instructive inductions in which a particular tissue can be made to differentiate in divergent ways according to the kind of inducing tissue; this is the case when *Xenopus* ectoderm is induced by mesoderm to form nerve or by endoderm to form muscle. The simplest interpretation of this result is that individual cells are caused to divert their uninduced direction of differentiation from epidermis into nerve or muscle. However, there are several other interpretations. The responding tissue might consist of a mixture of cells which are individually predisposed to respond to different inducers. The interpretation is further complicated by the fact that nearly all tissues that respond to induction undergo some proliferation and cell

rearrangement and in some cases there is selective cell death. For all of these reasons it is very hard to know how individual cells in an induced tissue were positioned with respect to the inducing tissue *at the time of the induction*. There are two further reasons why it is very desirable to be able to eliminate cell division in induction experiments. One is that any biochemical description of postinductive events is hard to interpret if these events could be related to cell proliferation and not to induction. The other is that the elimination of cell division would test whether 'quantal mitosis' is involved in this example of cell differentiation (Dienstman & Holtzer, 1975).

In the case of amphibian ectoderm, it has been possible to suppress all cell division and cell movement with colchicine or cytochalasin, and to show that muscle gene transcription is nevertheless induced (Gurdon & Fairman, 1986). This does not mean that the later morphogenetic aspects of induction proceed under these conditions, but at least the mechanisms leading to gene activation in roughly the normal number of cells is independent of cell division and rearrangement. The same conclusion does not appear to have been established for other inductive systems, probably because the much slower time scale involved would require a longer exposure to inhibitors that might be harmful. In no system has it so far been possible to suppress DNA synthesis and yet obtain an inductive effect.

To eliminate cell division and cell movement simplifies the analysis of inductive systems, but a major breakthrough would be achieved if the process could be made to take place in *single* responding cells. This appears never to have been achieved, and we must ask if this is significant. Is an inductive response simply the sum of individual cell responses, or is some cooperation between responding cells required? So-called 'mass effects' have been described, mostly as a result of experiments in which the size of the responding tissue is progressively reduced (Lopashov, 1935; Muchmore, 1957; Grunz, 1979). It is usually found that there is a size of tissue or number of cells below which no response takes place. However, this could merely reflect the common observation that cells differentiate best when surrounded by other cells, rather than by culture medium (e.g. Jones & Elsdale, 1963).

A particularly valuable series of experiments has been carried out by Heasman, Snape, Smith & Wylie (1986) in which it is found that single vegetal cells of a blastula, which will differentiate only poorly in isolation or in small groups, will nevertheless do so normally if surrounded by other cells, but it is unimportant whether these surrounding cells are the same as, or different from, the differentiating cell. This therefore fits the idea that the mass effect is unspecific and may merely represent a permissive environment. The most informative experiment would be one in which a single responsive cell would be exposed to normal inducing tissue, since this single cell should receive as much inductive influence and have as favourable a cellular environment as would many such cells. Some important experiments leading in this direction have been described by Heasman *et al.* (1984, 1985), who injected single marked vegetal cells into blastulae. They found that single early blastula cells differentiate in conformity with their surrounding cells, whatever type these are (Fig. 10A). Elegant single-cell transfer experiments have also been performed by Gimlich & Gerhart (1984) & Gimlich (1986); however, the one or two blastomeres transferred from the 64-cell stage subsequently divide (Fig. 10B), and the transplanted cells are inducing rather than responding. Our own work in progress, using a muscle-specific antibody, suggests that single animal cells are much less easily induced than a group of these cells, when underlaid or surrounded by vegetal cells. These unpublished results and those of Heasman *et al.* (1984) suggest a process by which gene activation or differentiation of a cell is facilitated by other cells of *like kind*. This may be described as a 'community effect' to distinguish it from the unspecifically beneficial effect of any other surrounding cells.

A community effect is not the same as homoiogenetic induction (Mangold & Spemann, 1927; Spemann, 1938), by which cells induced to differentiate in one way can in turn induce their neighbours to do the same. Homoiogenetic induction is usually thought to include an amplification step, by which a cell having received an inductive signal itself makes more inducer, thereby influencing its neighbours in the same way. The inductive effect can therefore be passed along *within* the responding tissue. Results consistent with this idea were obtained by Kurihara & Sasaki (1981) who found that an inducing signal cannot be transmitted through aged, and therefore nonresponsive, tissue. An amplification process is known to take place during amphibian oocyte maturation, in which the maturation-promoting factor (MPF), when phosphorylated, induces oocyte maturation as well as the phosphorylation of other MPF molecules (review by Masui & Clarke, 1979). While homoiogenetic induction may well involve an amplifying or relay process, it can also be explained by a much simpler process, in which inducer molecules are passed on, without amplification, from one inducing cell to the next; the response would cease when the amount passed on is below a threshold concentration. It is important to appreciate that homoiogenetic induction is not the same as a community effect. The

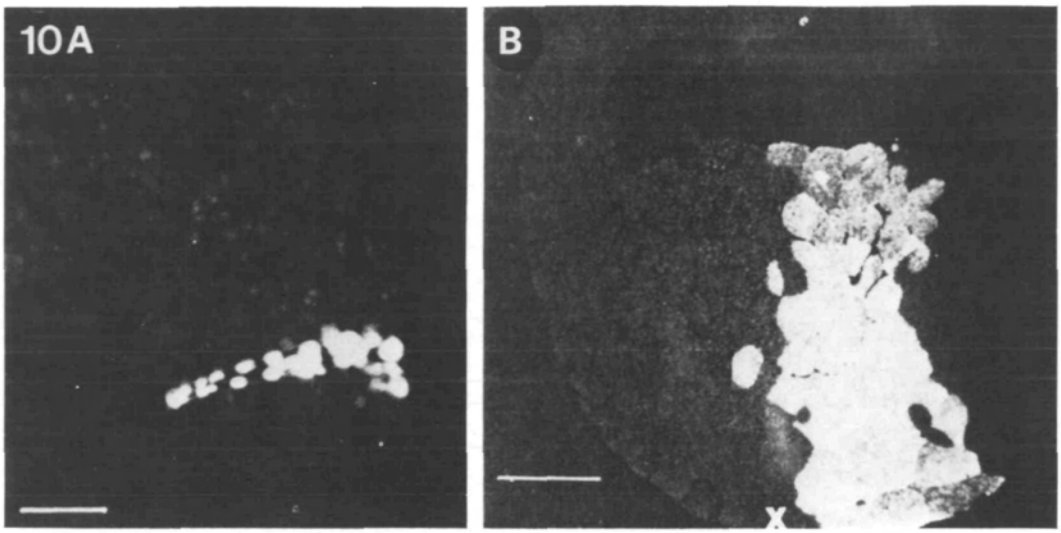


Fig. 10. Single-cell transfers in amphibian embryos. (A) A single rhodamine-labelled animal cell of a midblastula was injected into the blastocoel of another, unlabelled, host blastula. When the host embryo had reached the swimming tadpole stage, progeny of the injected cell, which would normally have formed epidermis or nerve, were found in sections of the gut (shown). The differentiation of the transferred cell conforms to that of the surrounding host tissue. (From Heasman *et al.* 1984.) (B) A single dorsal vegetal cell was labelled by injection of fluoresceinated–lysine–dextran at the 64-cell stage, transferred to the ventral vegetal region of another unlabelled 64-cell embryo. The labelled cell integrated into the host and divided (as shown); dorsal vegetal cells transplanted in this way induce a secondary axis in the host embryo. This shows that a single 64-cell blastomere (or its daughter cells) possesses axis-inducing activity. (From Gimlich & Gerhart, 1984.)

two would be distinguished by placing a single responsive cell in normal inducing tissue; failure to respond could be explained by a community effect, but not by homoiogenetic induction.

When single-cell assays are used, another important characteristic of induction responses becomes evident. This is the sharp demarcation between groups of cells which do or do not respond to induction. Many induced tissues in a vertebrate contain coherent blocks of cells of the same type, such as muscle, notochord and the neural tube. One might expect such groups of cells to be formed in part by the effects of localized cell proliferation and cell movement. However, when these processes are inhibited as mentioned above, induced cells nevertheless form a localized group with a sharp demarcation between cells that are positive or negative for the muscle marker antibody (Fig. 11). This seems most simply explained by a threshold effect such that any individual cell responds fully or not at all (Slack, 1983, for a theoretical discussion). A demarcation of response could also be enhanced by the community effect suggested above.

In conclusion, the localization of an inductive response in those cases where it has been appropriately investigated may be most simply explained as follows. First of all, responsiveness is restricted to competent cells. It is further limited by the rate of diffusion of inducing substances and by the proximity of competent cells to its source. Therefore the only cells to respond would be those near enough the inducing tissue to receive a concentration of inducer above their threshold level of response during their competent life. The precise localization of the response in tissues such as muscle and nerve would be achieved by homoiogenetic induction and by a community effect in which cells can respond to an induction in a given way only if their neighbours are undergoing the same response.

(6) The nature of an inducer

Much of what we know about molecules involved in induction concerns the nature of the inductive signal. We may first ask whether it is necessary for inducing and responding cells to make direct physical contact with each other, or whether the inductive signal can be conveyed by diffusible molecules. Since the original experiments of Grobstein (1956), numerous tests have been carried out with inducing and responding tissues separated by a filter. Millipore filters have tortuous pores of somewhat variable size, and this makes it hard to know whether the cytoplasmic processes often seen in the pores are continuous from one side of the filter to the other. In contrast, nucleopore filters have straight pores of rather constant size (Wartiovaara, Lehtonen, Nordling & Saxen, 1972). In early work (e.g. Grobstein, 1956), it

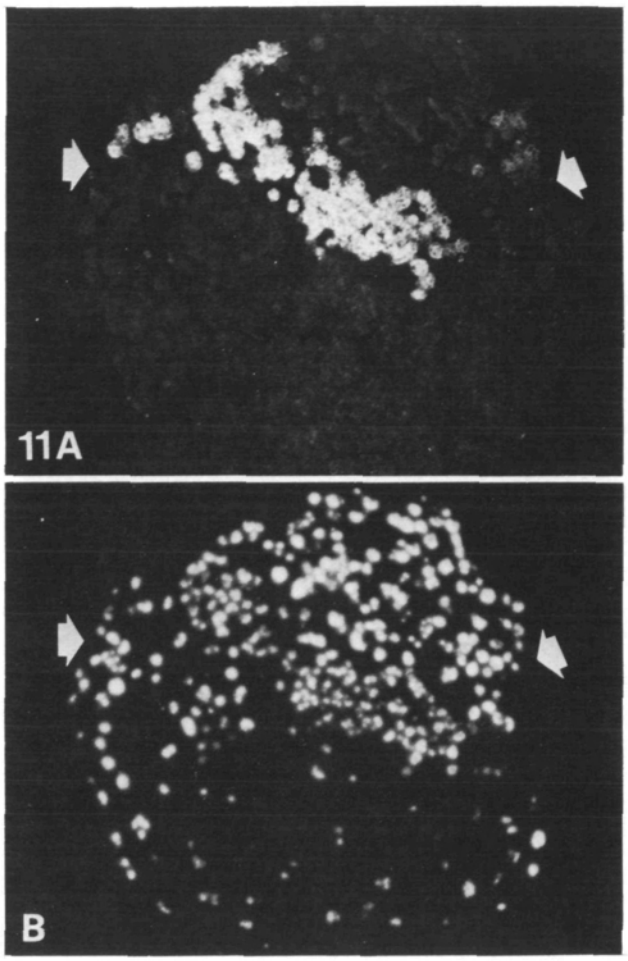


Fig. 11. Induction in the absence of cell division. Animal and vegetal tissues from a *Xenopus* blastula were placed in contact, and the conjugate immediately transferred to solid medium containing 10 $\mu g\,ml^{-1}$ cytochalasin B (CB). This drug inhibits cytoplasmic cell division, while permitting nuclear division. When control embryos had reached the heart-beat stage, the conjugate was fixed, sectioned, and processed for binding of muscle-specific antibody (see Fig. 4). No cell division has taken place since the start of induction, although control conjugates (without CB) would have increased their cell number about five times. Animal cells close to the inducing vegetal cells have undergone muscle gene activation and there is a sharp demarcation between animal cells that have responded to induction and those that have not. (A) Stained with 12/101 antibody; (B) stained with Hoechst. White arrows indicate the junction between animal and vegetal cells.

was assumed, perhaps correctly, that cytoplasmic processes did not reach right through a Millipore filter, but nearly all recent work has used nucleopore filters in which it can be seen when cytoplasmic penetration has been achieved. It is found that the thicker the filter and the smaller the holes, the longer it takes for cytoplasmic penetration to occur. In several very careful quantitative analyses, a clear correlation has been established between the passage of cell processes right through the filter and an inductive response: the removal of the inducer before filter penetration eliminates induction and the inductive effect is stronger the greater the amount of penetration (e.g. Wartiovaara, Nordling, Lehtonen & Saxen, 1974; Meier & Hay, 1975; Saxen & Lehtonen, 1978). This conclusion suggests that cellular contact between inducing and responding tissues may be required for successful induction. However, it is also compatible with the view that the signal is conveyed by a network of extracellular materials (Grobstein, 1967) or even by labile diffusible molecules. Most inductions where contact seems necessary are of the permissive type; these include mouse kidney tubule induction by mesenchyme and chick corneal induction by lens (Wartiovaara *et al.* 1974; Meier & Hay, 1975; Saxen & Lehtonen, 1978), and may involve extracellular materials (section 2). It is somewhat easier to interpret those inductions where cell contact is *not* required. These include instructive inductions such as chick lens by the optic vesicle (Karkinen, 1978) and *Xenopus* mesoderm by vegetal cells (Grunz & Tacke, 1986), as well as permissive ones such as the neuralization of newt ectoderm (Toivonen, Tarin & Saxen, 1976). In these cases, it is clear from the use of filters, as well as from the existence of soluble inducing extracts, that an inductive signal can be conveyed by diffusible factors.

The fact that some inductions can be transmitted without physical contact does not mean that this is how it happens in normal development. During mesoderm induction in normal *Xenopus* blastulae, gap junctions are known to link *Xenopus* blastula cells (Palmer & Slack, 1970; Regen & Steinhardt, 1986). However, mesodermal induction causes muscle gene activation to a *quantitatively normal extent* in embryonic cells which are in Ca^{2+}-/Mg^{2+}-free medium (i.e. in close proximity but not stably attached to each other) (Gurdon, Brennan, Fairman & Mohun, 1984; Sargent, Jamrich & Dawid, 1986), as well as in embryos in which gap junction communication has been suppressed by anti-gap-junction antibodies (Warner & Gurdon, 1987). The conclusion that gap junctions or other stable cell contacts are not required in induction is at present applicable to the only case where this has been precisely tested. Previous experiments with the same anti-gap-junction antibodies gave larval defects that were consistent with the idea that they resulted from impaired neural induction (Warner, Guthrie & Gilula, 1984); however, as the authors point out, the injected antibodies begin to lose their effect by the neural stage and the importance of gap junctions for neural induction is a suggested rather than proven conclusion.

Attempts to identify inducing substances have had a long history of slow progress. The difficulty of working with the more permissive inductions, such as the neuralization of newt ectoderm, soon became evident when it was found that almost any disturbing condition will cause induction (see Introduction). Much the most useful information has come from the use of heterogenous inducers. (Heterogenous means of different origin, i.e. not derived from the natural inducing tissue. Heterogeneous, sometimes used in error, means of diverse composition.) The great merit of heterogenous inducers is that they are available in fairly large quantities, that they have strong effects and therefore that they are suitable for biochemical purification. Very largely through the work of Tiedemann's laboratory over several decades, it seems clear that some inducers are macromolecular and contain proteins (Table 1). Recently Smith (1987) has discovered a very convenient source of heterogenous mesodermal inducer, from the culture medium of a *Xenopus* cell line, derived originally from a metamorphosing tadpole. This seems to be a heat-stable protein of about $16\times10^3 M_r$ (16K), and since it has the great merit of being soluble, there appear to be very good prospects for the eventual purification of this material. However, as yet no macromolecular inducer molecule has been fully purified or identified. There will always be concern that a heterogenous inducer may be substantially different from natural inducers, and some effort has been made to purify natural inducers from amphibian blastulae (Faulhaber, 1972). But the source of material is limiting, the effect of it weak during assay, and direct purification at present seems a forbidding task. It must be borne in mind that a few pure substances have strong inductive effects. For example, LiCl has long been known to have vegetalizing effects on sea urchin and amphibian embryos (Masui, 1961; Slack, 1983; Davidson, 1986). However, recent studies show that Li^+ can also have dorsalizing and neuralizing influences (Kao, Masui & Elinson, 1986; Breckenridge, Warren & Warner, 1987). It seems that the effects of Li^+ on development may well include secondary consequences of a generally disruptive effect on cell interactions, and may not give useful insight into any normal inductive process.

While discussing inducers, we should comment on an apparent characteristic that different concentrations or exposure times of the same inducer can have dramatically different developmental effects. For example, a 10-fold increase in the concentration

Table 1. *Many different substances act as embryonic inducers*

Inducer	Source	Assay	Composition	Reference
Vegetalizing	Guinea pig bone marrow (ethanol extract)	*Triturus* gastrula implant	—	Toivonen (1953)
Vegetalizing	9–13 day chick embryo	*Triturus* gastrula or ectoderm sandwich implants	30 K protein	Born *et al.* (1972) Schwartz *et al.* (1981)
Vegetalizing	Carp swimbladder (ethanol pellet)	*Triturus* gastrula ectoderm sandwich implant	—	Kawakami *et al.* (1976, 1977)
Vegetalizing	*Xenopus* cultured cell medium (soluble)	*Xenopus* blastula ectoderm	16 K heat stable protein	Smith (1987)
Neuralizing	Guinea pig liver (ethanol pellet)	*Triturus* gastrula implant	—	Toivonen & Saxen (1955)
Neuralizing	HeLa cells (ethanol pellet)	*Triturus* gastrula implant	—	Saxen & Toivonen (1958)
Neuralizing	*Xenopus* eggs and embryos	*Triturus* gastrula implant	Protein (from microsomes and yolk)	Faulhaber (1972) Janeczek *et al.* (1984)
Cartilage enhancing	10 day chick cartilage	Chick somite chondroitin sulphate synthesis	Chondromucoprotein	Kosher *et al.* (1973)
Pure substances				
Lithium ions (vegetalizing and other effects – see text)		Amphibian embryos (many species)	LiCl	Masui (1961) Kao *et al.* (1986)
Concanavalin A (neuralizing)		Newt (*Cynops*) gastrula ectoderm	Purified ConA	Takata *et al.* (1981)

Inducers effective in early embryos are classified only as vegetalizing or neuralizing. Vegetalizing includes 'mesoderm-inducing', on the grounds that the formation of mesodermal structures such as muscle and notochord probably results from the induction of vegetal cells which themselves induce animal cells into mesodermal cells. 'Neuralizing' factors include those described as inducing archencephalon (forebrain), deuterencephalon (hind-brain), and spinal cord, since these different morphological structures can be induced by different concentrations and exposure times of the same extract. It is assumed, as explained in the text, that neuralization of test embryos is caused by a direct neuralizing factor, and not by induced mesoderm cells which in turn induce ectoderm into neural structures. The chick-cartilage-enhancing factor acts permissively, increasing a substantial background (uninduced) level of chondroitin synthesis.

of vegetalizing inducer or in the time of exposure to it, can cause *Xenopus* ectoderm to form substantial amounts of muscle and nerve; by comparison, blood is formed at low doses or epidermis with no inducer at all (Grunz, 1983). This might suggest that different concentrations of the same factor can activate different genes in equivalent cells. However, this effect could well be explained if higher concentrations of inducer 'vegetalize' (make into vegetal cells) increasing numbers of animal or ectoderm cells, and if these vegetalized cells cause secondary inductions, according to the scheme shown in Fig. 1A, as suggested by Minuth & Grunz (1980). Another simpler interpretation, and one to be preferred until disproved, is that the heterogenous inducer used in this experiment contains several factors which independently induce different cell types, a conclusion supported by some inducer mixing experiments of Asahi, Born, Tiedemann & Tiedemann (1979).

This leads us to consider an extreme proposition, namely that any one cell at a particular stage in development can respond to only one kind of inducer; the concept is that a cell has only two options, to be induced or not, and that if induced it can respond in only one way. It would have to be supposed that amphibian early gastrula ectoderm cells though superficially similar are in fact heterogeneous, some being able to respond to vegetal cells or vegetalizing inducers by becoming mesoderm, and others to mesoderm cells or neuralizing inducers by forming nerve. It might also be supposed that a strong vegetal inducer would vegetalize a sufficient number of blastula animal cells sufficiently quickly for them in turn to induce some of the remaining animal cells to become mesoderm, which could subsequently induce nerve. It might therefore be that neuralizing inducers differ from vegetalizing inducers only in their faster or stronger action, enabling secondary inductions to proceed as far as neural differentiation. The strongest argument against such sequential effects of one induction is that a primary inducer is unlikely to be able to have its effect fast enough for secondary inductions to take place before competence, which is strictly limited in duration, has been lost. Another is that low doses or short exposure times of a neural inducer would be expected to give mesodermal inductions, but this is not observed (Saxen & Toivonen, 1962). However, these points do not argue against a heterogeneous population of responsive cells in the early gastrula. A decisive test of this important point will probably require not only early molecular markers (section 2), but also single cell inductions (section 5).

(7) Early steps in the inductive response

A molecular understanding of embryonic induction requires a knowledge of events that lead from the appearance of inducer at a responding cell's surface to the earliest differentiation event, such as gene activation.

Do inducers need to enter cells? In a few cases it seems clear that they can act at the surface of the recipient cell and do not need to enter, as do steroid hormones, to have their effect. This is true for a crude chick embryo fraction as well as for concanavalin A, both of which induce neural differentiation in newt embryos, when covalently bound to Sepharose beads which do not enter cells (Tiedemann & Born, 1978; Takata *et al.* 1981). In contrast, a vegetalizing inducer of the same chick origin and assayed in the same way loses activity if bound to Sepharose, though it can be subsequently recovered in active form (Born, Grunz, Tiedemann & Tiedemann, 1980).

Do initial responses include changes in free Ca^{2+} and cyclic AMP content, protein phosphorylation, oncogene activation, and other events often associated with major changes in cell activity? Pictet & Rutter (1977) found that neither cAMP nor cGMP derivatives can substitute in pancreatic epithelium for the mesenchyme-inducing factor, an effect which would have been expected if an increase in these substances was a direct effect of induction. Similar results have been obtained by Grunz & Tiedemann (1977). The strong effect of LiCl on development might suggest the involvement of inositol triphosphates, since Li^+ is a specific inhibitor of inositol metabolism (Berridge, 1986); but if Li^+ has a generally disruptive effect on development (p. 297), inositol metabolism might well be disturbed for reasons unconnected with induction. It will require an induction system in which cell proliferation either does not accompany induction, or one in which it can be suppressed, before a useful investigation of early postinduction events can be undertaken (as discussed above).

It is important to know whether protein synthesis is required during early inductive events. A short exposure to cycloheximide is sufficient to largely suppress protein synthesis in embryonic amphibian tissue, in a way that is nontoxic and reversible. Grunz (1970) established that cycloheximide treatment causes a prolongation of the period of competence, suggesting that the termination of competence may be actively induced by mRNA translation. Recently it has been found, by transcript and two-dimensional gel protein analyses, that protein synthesis, and therefore mRNA translation, is absolutely required during the first two thirds (6 h) of the vegetal induction of *Xenopus* blastula tissue into

muscle (Cascio & Gurdon, 1987), but not during the last 3 h. It is not known whether transcription is also required during this induction period, or whether the mRNA required is preformed (and therefore in this case maternal). However, these results clearly suggest the value of screening cDNA libraries for induction-related clones (p. 301).

Much recent work concerned with gene activation has been successfully directed towards the identification of cis-acting sequences in front of genes, presumed to interact with gene-specific proteins or RNAs. Success has recently been achieved in obtaining correct tissue-specific expression of muscle actin genes injected as DNA into *Xenopus* eggs, and the same transferred genes can be activated in animal cells by vegetal induction (Wilson, Cross & Woodland, 1986; Mohun, Garrett & Gurdon, 1986; Fig. 12). Mohun *et al.* (1986) have also found that the sequences needed for gene activation by induction lie within 400 bases upstream from the promoter of this gene. This seems an encouraging start on an analysis that aims to work backwards from an early inductive response (actin gene activation). The hope will be to find factors that bind to this upstream region of the induced actin gene; these might be the products of a gene whose translation occurs during the cycloheximide-sensitive phase at the start of induction. Since the whole induction process is, in this case, completed in 7–9 h, this is about the timing expected if induction were to cause the transcription and translation of a gene or genes, whose products directly activate the actin gene.

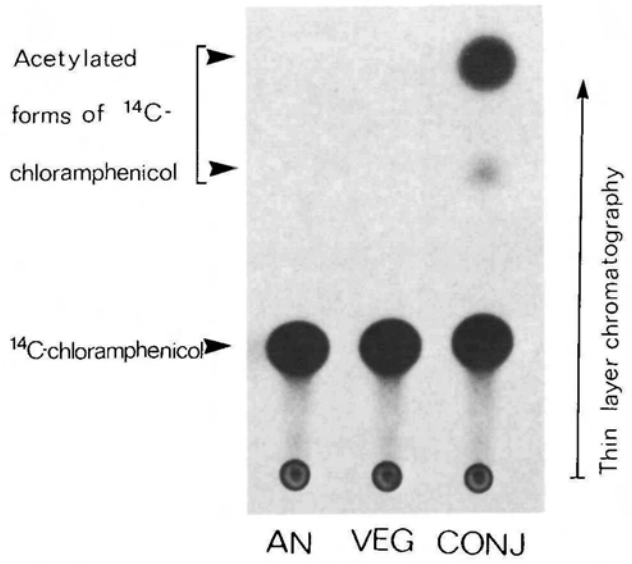


Fig. 12. A rapid gene transfer assay for DNA sequences involved in a response to embryonic induction. A *Xenopus* cardiac actin gene promoter (3 Kb) has been fused to a bacterial chloramphenicol acetyl transferase (CAT) gene. DNA of this kind is injected into fertilized eggs of *Xenopus* from which animal and vegetal regions are isolated at the blastula stage and cultured separately (AN, VEG) or after conjugation (CONJ). The embryo pieces are cultured overnight until controls have reached the neurula stage, when extracts are assayed for CAT activity. Acetylated [^{14}C]chloramphenicol shows that the CAT gene has been expressed by activation of the muscle-specific cardiac actin gene promoter. The positive signal in the conjugates results from the activation of the actin gene promoter in animal cells by induction from vegetal cells. (From Mohun *et al.* 1986.)

(8) Comparison with other cell signalling systems

Of all the cell signalling systems that operate between cells, embryonic induction is the least understood at the molecular level. In most other cases the ligand, and often the receptor, have been well characterized and even sequenced at the nucleic acid or protein levels. It is therefore useful to compare the biological properties of embryonic induction with other cell interactions. How similar is embryonic induction to these other systems?

Table 2 summarizes most of the conclusions we have reached in the preceding discussion. In several respects embryonic induction appears to differ fundamentally from all other known types of cell interaction. First, induction appears to be the only case in which the responsive or competent state disappears soon after the reaction is completed. In others, the possession of a functional receptor is an indefinite property of responding cells once they have acquired it. One exception to this generalization, and therefore resemblance to embryonic competence, exists in the action of a lepidopteran eclosion hormone, a neurosecretory peptide; the central nervous system, on which this hormone acts, is competent to respond for only a few hours, which coincide with the appearance and disappearance of two phosphorylatable proteins (Morton & Truman, 1986). A second difference between induction and other systems, is that the timing of an inductive response is determined by properties of the responding tissue (section 4), and not as in other systems by the time when the ligand, such as hormone, growth factor, neurotransmitter, etc. reaches responsive cells. A third difference is that several totally unrelated chemical substances, such as Li^+ and a guinea-pig bone marrow protein, can have the same specific effect (such as mesodermal differentiation) emphasizing the large extent to which inductions seem to promote a response to which cells are already committed. In all these respects, induction differs from other cell interactions in the overriding importance of the responsive or competent state. It seems as if responding cells undergo a series of endogenous processes which require, at any time within quite wide limits, the contribution of inducer

molecules, which can cause a switch from the uninduced to one of a small number of induced directions of differentiation. When we consider the purpose of embryonic induction in development, differences from other cell-signalling systems make sense. Embryonic induction brings about an irreversible differentiation, or more precisely determination, of cells in one part of an embryo. The exact position and time of the induction needs to be precisely coordinated with many other events in development. In contrast, most hormones, growth factors and neurotransmitters are required to give *reversible* effects, often timed in relation to an effect of the external environment on an organism.

In a few respects embryonic induction shares characteristics with certain kinds of cell interaction, but not others. Hormones are effective over very long distances, being conveyed in the blood stream between their origin in an endocrine organ and the responding tissue; on the other hand, normal inducers are effective only over short distances of a few cell diameters, and often affect only adjacent cells

Table 2. *Embryonic induction compared with other cell interactions*

Interacting system	Main biological effect	Duration of responsive state	Response timed by	Time from induction to main response	Distance from inducer source to responding cells
Embryonic induction					
e.g. *Xenopus* embryo	Mesoderm and nerve differentiation	Terminates rapidly	Responding cells	Hours or days	Few cell diameters
Hormones[1,2]					
e.g. Oestradiol (steroid)	Increased gene activity in oviduct	Long term	Hormone release	Hours	Blood stream (from ovary)
Erythropoietin (46 K glycoprotein)	Proliferation of early erythrocytes in bone marrow	Long term (in early erythrocytes)	Hormone release	Days	Blood stream (from kidney)
Lymphokines[3]					
e.g. Interleukin-2 (15 K protein)	Cell division and differentiation of T cells	Long term	Lymphokine release	Hours	Few cell diameters (from T cells)
Growth factors[4]					
e.g. Nerve GF (2×118 aa)	Survival and growth of neurones	Long term	NGF release and distance	Hours or days	Few cell diameters
Epidermal GF (53 aa)	Stimulates cell division	Long term	EGF release and distance	Hours	Few cell diameters
Prostaglandins[5]					
Thromboxane, PGE_2, PGI_2 (small fatty acid derivatives)	Blood platelet aggregation	Long term	Prostaglandin release	Seconds or minutes	Few cells (unstable)
Neurotransmitters[6]					
Acetylcholine, enkephalins and endorphins (short peptides)	Modulation of nerve conduction	Long term	Transmitter release	Microseconds to milliseconds	Nanometers to microns (rapid degradation)
Morphogens[7]					
Hydra head activator (11 aa)	Control of head regeneration	Long term	Head activator and inhibitor release	Hours or days	Activator (few cells)
Hydra head inhibitor ($<500\,M_r$)	Control of head regeneration	Long term	Head activator and inhibitor release	Hours or days	Inhibitor (many cells)
Slime mould DIF and cAMP ($<300\,M_r$)	Spore or stalk differentiation	Long term	Morphogen release	Seconds or minutes	Few cells (rapidly degraded)

aa, amino acids; K, $\times 10^3\,M_r$.

References

[1] Standard textbooks.
[2] Goldwasser (1975); Metcalf (1981).
[3] Farrar *et al.* (1982).
[4] Levi-Montalcini & Calissano (1979); Yankner & Shooter (1982); Carpenter (1985).
[5] Samuelson *et al.* (1975).
[6] Iversen (1984).
[7] Schaller *et al.* (1986); Gross *et al.* (1981); Loomis (1982).

less than 1 μm away. Neurotransmitters are effective over the same short distances as inducers, but differ in having extremely rapid effects, of the order of milliseconds, compared to inducers whose main effects are seen hours or days later. The single most important characteristic of embryonic inducers is that they cause major changes in the direction of cell differentiation. In this respect, they differ from growth factors, whose effects are usually to enhance the proliferation of an already determined cell type. The nerve growth factor does not initiate cell division, nor does it cause a change in the direction of cell differentiation; it stimulates the outgrowth of axons from cells already committed to nerve differentiation (Levi-Montalcini & Calissano, 1979). There are several other 'local mediators' of cell interactions, such as prostaglandins, histamine, enkephalins, etc. which share many properties with inducers, but which do not cause substantial changes in cell differentiation.

(9) Prospects for further analysis at the molecular level

It has commonly turned out, in molecular biology, that progress on a difficult problem has been achieved by selecting a particularly favourable biological system for analysis. A good example is the identification of the nerve growth factor. This substance is produced in very small amounts by many tissues but happens to be present in the submaxillary gland at a concentration 10^3 times higher than elsewhere in the mouse, thereby permitting its initial purification (Cohen, 1960) and the subsequent characterization of its receptor. It may therefore be helpful to consider which kinds of inductive system are likely to be most useful, and what attributes such a system should have, for future molecular analysis.

At the cellular level, a great simplification would be achieved if an experimental induction system could be made to work at the level of single cells cultured in isolation, as has been done for terminal differentiation of astrocyte and oligodendrocyte nerve cells (Temple & Raff, 1985). The fact that inductive systems so far analysed appear to depend on an interaction between multiple responding cells complicates analysis (p. 294). Single cell assays are, of course, impossible for inductions recognized by the morphogenetic arrangement of multiple cells, but these kinds of induction processes are likely to be hardest to analyse in molecular terms. A second substantial simplification could be achieved at the cellular level, if cell division, cell rearrangement and eventually DNA synthesis can be eliminated, as discussed in section 5.

A particularly successful route towards the molecular analysis of any complex biological process is to obtain mutants that inactivate, one at a time, the various gene products involved. Once a mutation of interest has been secured, it is a matter of time, in *Drosophila*, before the gene sequence and coding capacity can be determined, though this does not necessarily reveal its function. The organisms most favourable for genetic analysis, namely *Drosophila* and the nematode *Caenorhabditis*, are also those whose development is least obviously affected by inductions, and there is at present no experimental inductive system with isolated tissues available in these species, like those discussed for vertebrates. Nevertheless it seems certain that interesting mutants affecting cell interactions will be found and analysed. A good example is the nematode mutant lin 12 (Greenwald, 1985), in which the DNA sequence encodes multiple copies of a cysteine-rich 40–50 amino acid peptide; this is present many times in the sequence of the precursor to the mammalian epidermal growth factor, and once in the mature growth factor itself. A *Drosophila* mutation that has turned out to be of special interest is *Notch*, the developmental effects of which are complex but suggest the involvement in cell interactions (Akam, 1986). The DNA sequence of *Notch* also reveals many repeats of a sequence capable of coding the same cysteine-rich peptide found in epidermal growth factor genes (Wharton, Johansen & Artavanis-Tsakonas, 1985). We should bear in mind the common observation, especially from cell hybrid experiments, that cell components involved in gene activation seem to be conserved between distant species, but are quite different from one tissue to another (Ringertz & Savage, 1976; Blau, Chiu & Webster, 1983). It is commonly found that a sequence from one organism, such as the *Drosophila* homeobox, can be used to find similar sequences (though not necessarily functionally equivalent) in other species, such as mice and frogs. Putting these observations together, it may well be rewarding to screen cDNA libraries prepared from developmental stages where inductive processes take place, using low stringency hybridization with probes from other organisms for receptors of known growth factors, hormones, etc. Using subtracted cDNA libraries (Sargent & Dawid, 1983), or subtractive hybridization, it should be feasible to isolate cDNAs for mRNAs present at a frequency of 10^{-4} in the total poly(A)$^+$ RNA of an embryo. This would correspond, in a *Xenopus* blastula, to about 100 molecules of one type of mRNA per cell, a minimum concentration expected for a message whose product needs to be synthesized in substantial amounts within only a few hours.

(10) Conclusion

The overall impression conveyed by this survey of embryonic inductions is that these processes are very complex, probably consisting of several reciprocal interactions between inducing and responding tissues, each step enhancing, little by little, progression towards the eventual differentiation. Most inductions seem to take place as follows. Both competence and inducing ability are acquired by restricted populations of cells for a limited time. The proximity of the first, apparently instructive, inducer further restricts the number of cells within the competent population that will respond. Especially with inductions that take place late in development, several further, usually permissive, inductions from other inducing tissues enhance the initial response. Tissue structure may be finalized by interactions among responding cells with each other.

In retrospect it is not surprising that so many steps should be required for the reliable formation of something as complicated as an embryo. Any manufacturing process in which a complicated machine is assembled requires controls to ensure the coordinate operation of different steps. If the formation of an embryo were to take place without frequent interactions between different regions, errors in the final product would often arise.

I believe that the greatest obstacle to the molecular analysis of induction over many decades may have been the imprecision and late appearance of the assays used, which often depend on morphological assessment many days after the inductive response has started. It may also have been necessary, faute de mieux, to concentrate on identifying inducer molecules; but compared to ligands for other cell interactions, embryonic inducers seem less specific, since they can be substituted for by other substances, and therefore harder to purify. For embryonic induction to be accessible to the powerful methods of molecular analysis now available, it seems essential to use, as an assay, a single early response, such as the expression of one gene. Nucleic acid technology has probably now reached a sufficient level of precision and efficiency of operation to be usefully applied to the analysis of inductive responses, working from the response backwards, rather than from the inducer forwards.

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