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Inductive interactions in early amphibian development and their general nature.

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SUMMARY

After a short discussion on cell interactions in general and inductive interactions in particular, the almost completely epigenetic nature of amphibian development is emphasized.

In the symmetrized egg undergoing cleavage a large-scale inductive interaction occurs which leads to the formation of the meso–endoderm. Meso–endoderm formation gives rise to the morphogenic process of gastrulation. In the ensuing triple-layered embryo inductive interactions are strongly enhanced. The following large-scale inductive interaction leads to the formation of the neural anlage. This is again followed by the morphogenetic process of neurulation or neural tube formation. Subsequent interactions between the germ layers of the triple-layered embryo give rise to the formation of the regional pattern of organ anlagen. Finally, the most promising approaches to the nature of inductive interactions for mesoderm and endoderm formation are discussed.

INTRODUCTION

Before describing the individual inductive interactions occurring successively in the developing amphibian egg and embryo, a few words ought to be said about the possible nature of cellular interactions.

Interactions between the constituent parts of the egg and embryo constitute the basic requirement for the development of a complex, multicellular organism. Without such interactions no further development occurs. Interactions can and will take place between different compartments inside a cell, between different cells, and between different cell groups provided they differ sufficiently from each other, so that 'messages' released by one component can be recognized by the other as distinct 'signals' to which the latter can react.

In any interaction a distinction should be made between an action system and a reaction system. Interactions are in principle *reciprocal* in nature, but the reciprocal actions usually alternate in time. The predicate 'inductive' is by definition restricted to those interactions which cause the reaction system to switch into a *new* pathway of differentiation. These should be distinguished from interactions which only support the pathway of differentiation already in progress. Inductive interactions therefore refer to *pluripotential* systems which are open to a *choice* of potential

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pathways. It must be realised that an inductive interaction implies the suppression of the pathway of differentiation already in progress, as e.g. the suppression of epidermal differentiation during meso–endodermal or neural induction (Grunz *et al.* 1975). This may be the first step in the induction process.

During the course of development both interacting systems may pass through successive phases of inductive activity and responsiveness or competence. The appearance of a given competence is often the consequence of previous inductive action(s), as e.g. the appearance of lens competence in the placodal ectoderm under the influence of the pharyngeal endoderm (Jacobson, 1966), or of liver competence in that region of the endodermal epithelium which has come into direct but transient contact with the presumptive heart mesoderm (Le Douarin, 1974). Competence may also develop independently, however, as e.g. the successive appearance of meso–endodermal, neural and placodal competence in the ageing ectoderm during blastulation, gastrulation and neurulation (Nieuwkoop, 1958, 1963, 1973). However, the great majority of developing competences are the consequence of the transient or permanent apposition of parts of the embryo which are characterized by different pathways of differentiation and which have been brought into direct contact by morphogenetic movements (see below).

The amphibian egg

The unfertilized amphibian egg on the one hand represents a very large and highly differentiated cell, but on the other hand is characterized by a minimum of spatial heterogeneity; it consists of only two different moieties, viz. a totipotent animal moiety and an endodermal vegetal moiety. Each shows a different cytoplasmic composition with different amounts of yolk platelets and other cell organelles, as well as a different composition of the outer membrane with different membrane fluidities. The two membrane domains meet at the equator of the egg, forming a sharp boundary (Dictus *et al.* 1984).

Fertilization leads to dorsoventral polarization of the egg. Sperm entry in the animal hemisphere leads, among other things, to the formation of a dorsal vitelline wall, an extension of the vegetal yolk mass into the animal hemisphere opposite the sperm entry point. This configuration is thought to be the site of most intense interaction between the two primary moieties of the egg, thus determining the dorsal side (Nieuwkoop, 1977; Gerhart, 1980). The accompanying formation of the grey crescent seems to be only an epiphenomenon. Rotation of the egg under the influence of gravity may overrule the polarizing effect of the sperm; the side along which the heavy yolk mass descends becomes the future dorsal side. The accompanying shift of the yolk mass inside the egg leads to a drawing-out of its upper edge (Born's crescent). The latter seems to be functionally equivalent to the dorsal vitelline wall.

Although fertilization is usually considered to represent the starting point for embryonic development, oogenesis and spermatogenesis, egg and sperm maturation, and the fusion of egg and sperm actually form integral parts of the entire

process of development. Development in fact starts with the formation of the primordial germ cells as the forerunners of the next generation. The development of the amphibian egg and embryo is characterized by a stepwise increase in heterogeneity and spatial complexity due to the interaction of its constituent parts and is therefore completely *epigenetic*, with the sole exception of the spatial structure of the egg or of the primordial germ cell, which represents the only *preformistic* aspect of development.

Development of the fertilized, symmetrized egg starts with the process of cleavage, during which the very large amphibian egg becomes divided up into ever smaller blastomeres, without intervening growth phases as found in the normal cell cycle; thus a more normal nucleocytoplasmic ratio is restored. This ratio had been profoundly disturbed during oogenesis, when enormous growth of the oocyte occurred without mitosis. In the amphibians cleavage is synchronous during the first 10 to 12 cleavage cycles, when only DNA replication occurs. Around the mid-blastula stage cleavage becomes asynchronous and mRNA and tRNA synthesis starts. This seems to be due to the dilution and final exhaustion of an inhibitory factor during the formation of an exponentially increasing number of nuclei (Newport & Kirschner, 1982*a, b*).

It must be emphasized that cleavage, though leading to interblastomeric cell wall formation, does not appreciably affect the spatial distribution of the egg cytoplasmic domains and cellular inclusions except for blastocoel formation. Neither is it likely that cleavage is essential for the interaction between different moieties of the embryo, since the process of pseudogastrulation, which may occur in overripe unfertilized eggs in the absence of cleavage, shows great similarity in external appearance and timing to normal development up to the end of gastrulation; a development up to a stage equivalent to the slit-blastopore stage would be impossible without inductive interactions.

Induction of meso–endoderm

During cleavage blastocoel formation takes place by intercellular fluid accumulation. The blastocoel spatially and functionally separates the original animal and vegetal moieties of the egg except for the peripheral region, where they remain in direct contact with each other (Nieuwkoop, 1973). In this peripheral region the first large-scale inductive interaction takes place, which leads to the formation of the 'meso–endoderm'. This is formed from the totipotent animal moiety under an inductive influence emanating from the vegetal yolk mass, an interaction which is strongest on the dorsal side (Nieuwkoop, 1969*a, b*; Boterenbrood & Nieuwkoop, 1973) (see Fig. 1). In the region just above the future blastopore, adjoining the endodermal yolk mass, the future pharyngeal endoderm as well as the most dorsal gut endoderm is formed (Nieuwkoop & Ubbels, 1972; Koebke, 1976) (see Fig. 2). Beyond this induced endoderm, the mesoderm develops: the axial mesoderm with differentiation tendencies for notochord and somites dorsally and dorsolaterally, the nephrogenic mesoderm laterally, and the lateral plate mesoderm ventrally. All

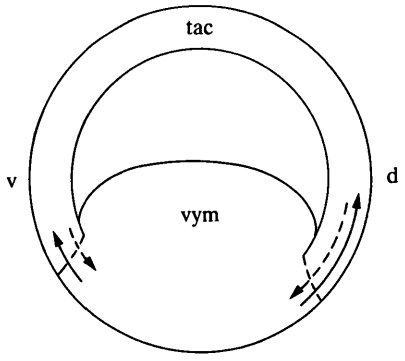

Fig. 1. Diagram of reciprocal actions between the endodermal vegetal yolk mass (*vym*) and the totipotent animal cap (*tac*) of the amphibian blastula. ⟶ meso–endoderm induction in animal cap, - - → flask cell induction in peripheral region of the vegetal yolk mass.

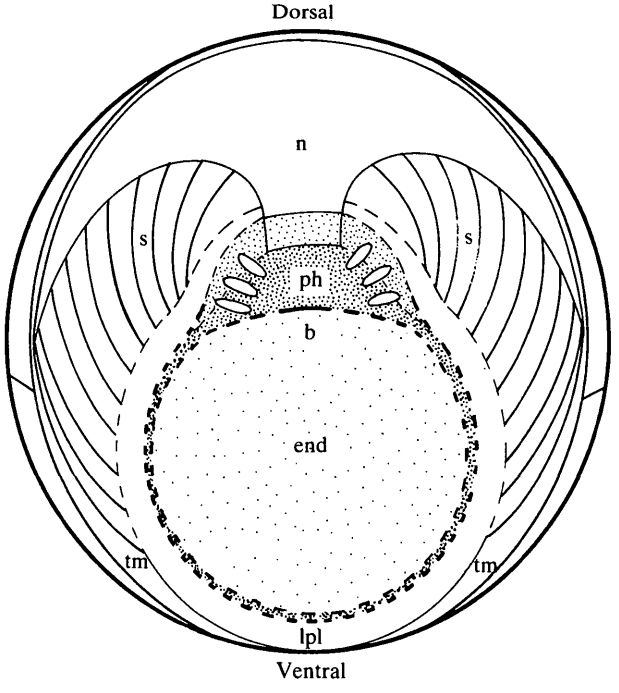

Fig. 2. Fate map of endo- and mesodermal organ anlagen in the very early urodele gastrula, seen from the vegetal side. *b*, Initial blastoporal groove; *end*, endodermal yolk mass; *lpl*, lateral plate mesoderm; *n*, notochord; *ph*, pharyngeal endoderm; *s*, somitic mesoderm; *tm*, tail mesoderm. Stippled area represents induced endodermal structures. (after P. D. Nieuwkoop & G. A. Ubbels, 1972 and J. Koebke, 1977).

these induced structures, together called the 'meso–endoderm' and representing the so-called marginal zone, are formed as a result of different intensities of one and the same inductive action (Grunz, 1983).

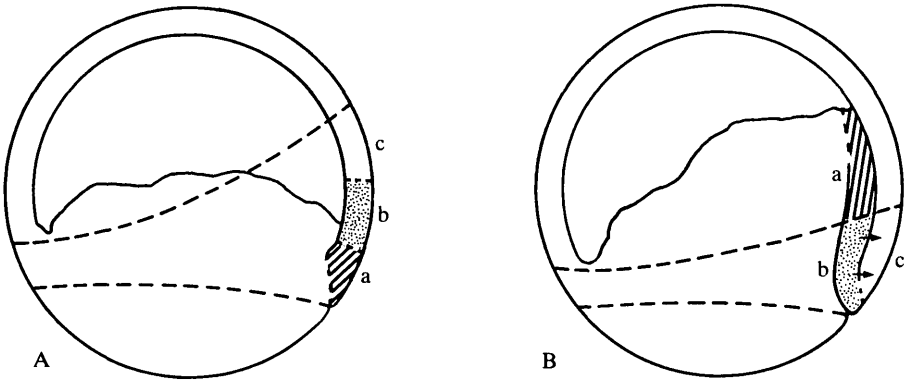

Fig. 3. Diagram of the development of head and trunk inductors in a urodele amphibian embryo during the gastrulation process. (A) Location of presumptive pharyngeal endoderm and anterior and posterior archenteron roof at an early gastrula stage, the presumptive anterior archenteron roof with chordomesodermal differentiation tendencies and the presumptive posterior archenteron roof still without mesodermal differentiation tendencies. (B) Interactions (→) between the just-invaginated presumptive anterior and the still uninvaginated presumptive posterior archenteron roof at a slightly older gastrula stage with transformation of the presumptive anterior archenteron roof in prechordal endo- and mesoderm (head inductor) and development of the presumptive posterior archenteron roof into trunk inductor with chordomesodermal differentiation tendencies.

Meso–endoderm induction is a slowly propagating process, so that only the lower half of the marginal zone, the future anterior half of the archenteron roof, is formed at the early gastrula stage (Kaneda & Hama, 1979). A subsequent interaction between the just invaginated 'anterior' mesoderm and the still uninvaginated 'posterior' mesoderm seems to be responsible for the transformation of the former into prechordal endomesoderm, the head inductor, and of the latter into posterior axial mesoderm, the trunk inductor (Kaneda, 1980, 1981) (see Fig. 3). The tail inductor develops still later, transforming the most caudal portion of the neural plate into tail somites as part of the neural induction process (see below). Loss of mesodermal competence may be the cause of the spatial extension of the meso–endoderm, with the exception of the formation of the tail somites, for which a separate period of mesodermal competence seems to be required, which arises during the regional segregation of the neural anlage (Bijtel, 1936; Spofford, 1953; Niazi, 1969) (see below under neural induction).

Gastrulation and neural induction

Mesoderm induction is followed by the complex process of gastrulation, by which the single-layered blastula is transformed into a triple-layered embryo. In the urodele amphibians, which have an 'external' marginal zone, occupying part of the outer surface of the embryo, this transformation is accomplished by the invagination of the endo- and mesoderm together through the blastopore. Invagination begins with flask cell formation in the peripheral region of the endodermal yolk

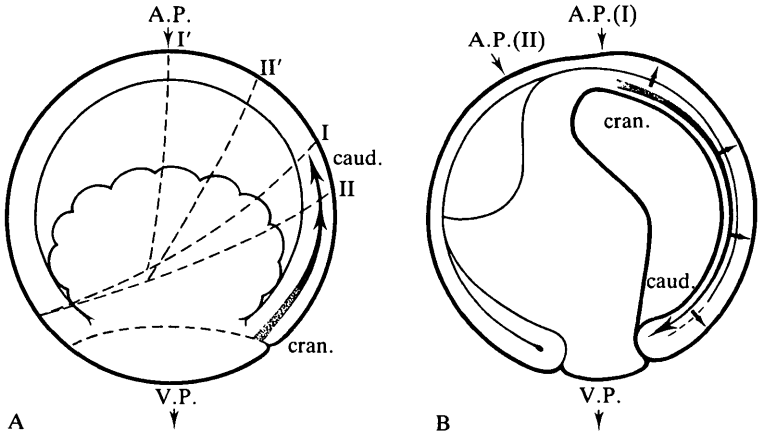

Fig. 4. Diagram of amphibian gastrulation, showing reversal of craniocaudal axis (large arrows). (A) Early gastrula: with I and II, extension of marginal zone in urodeles and anurans, respectively; I' and II' extension of cranial border of presumptive neural area in both groups. (B) Advanced gastrula with position of aminal pole (AP) in both groups. VP, vegetal pole; small arrows, inductive interactions.

mass, leading to blastoporal groove formation. Flask cell formation must be due to an interaction between the two primary moieties of the embryo, representing the reciprocal aspect of meso–endoderm induction (Nieuwkoop, 1969*a*; Doucet-de Bruïne, 1973) (see Fig. 1 on p. 336). After invagination the flask cells flatten out and finally form part of the squamous epithelium of the archenteron. Invagination around the blastoporal lip is followed by an active migration of the frontal edge of the endo- and mesoderm along the inner surface of the animal, ectodormal cap of the spherical embryo. The ectoderm forms a fibrillar meshwork used for contact guidance by the frontal edge cells (Nakatsuji & Johnson, 1982). In the anuran amphibians, which have an 'internal' marginal zone which is fully covered by presumptive endo- and ectoneuroderm, gastrulation consists of a separate rolling-in of the mesoderm around an internal lip and the subsequent invagination of the endoderm through the blastopore. It seems likely that these two separate processes are again followed by an active migration of meso- and endoderm along the inner surface of the ectoderm, as in the urodele amphibians.

Gastrulation implies a reversal of the anteroposterior axis of the meso- and endoderm (see Fig. 4) and leads to an interaction of the three layers of the triple-layered embryo over their entire inner surfaces, confronting parts of the embryo which were initially far apart, and thus strongly enhancing the possibilities for inductive interaction (Nieuwkoop, 1977). The first consequence of gastrulation is the large-scale interaction between the mesodermal archenteron roof and the overlying ectoderm, which leads to the formation of the neural plate as the anlage of the central nervous system (CNS).

The formation of the CNS is due to two successive inductive actions exerted by the endomesodermal archenteron roof: an initial 'activating' or neuralizing action,

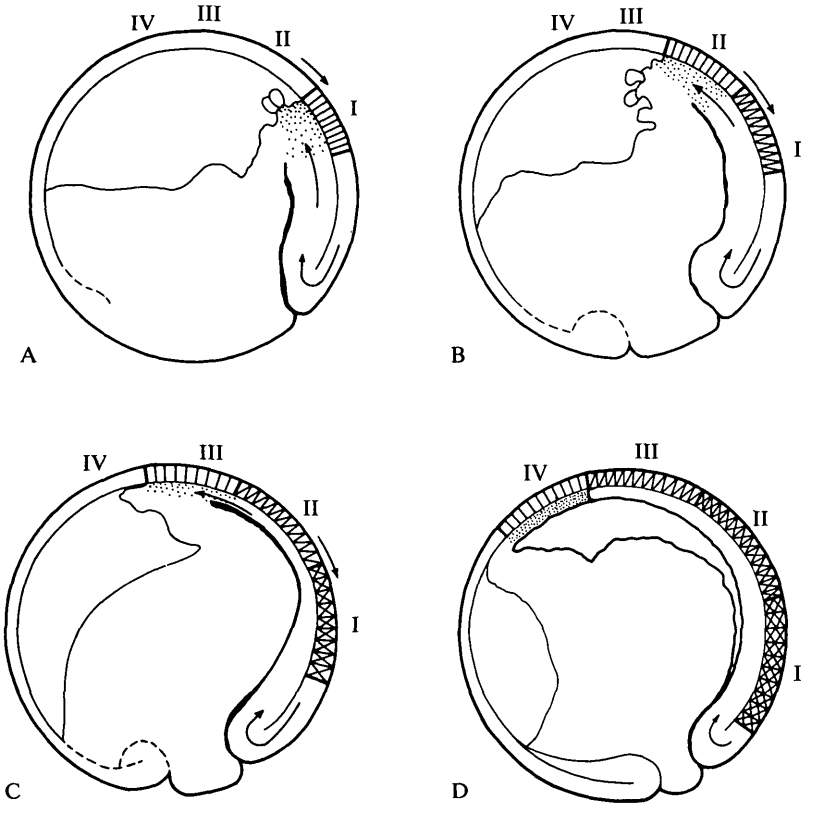

Fig. 5. Diagrammatic presentation of four successive stages (A to D) of posteroanterior progression of neural induction during gastrulation. (A) Interaction of prechordal endomesoderm (stippled) with most posterior region of presumptive neurectoderm; (B, C and D) Interaction of prechordal endomesoderm with successively more anterior regions of presumptive neurectoderm, and simultaneous confrontation of the more posterior regions of the neuroectoderm with more and more posterior regions of the archenteron roof, leading to progressively stronger transformation (increasingly dense hatching).

which is mainly exerted by the prechordal endomesoderm, liberating neural differentiation tendencies in the overlying, highly competent ectoderm, and a subsequent 'transforming' or caudalizing action exerted by the chordomesoderm upon the already neuralized ectoderm. In the amphibians both actions take place during the gastrulation process, when the invaginated endomesoderm moves upwards along the inner surface of the ectoderm with the prechordal endo- and mesoderm moving in front of the chordomesoderm (see Fig. 5). Both inductive actions are mainly restricted to the median region of the archenteron roof, i.e. the median prechordal endomesoderm and the notochordal anlage. Here the interacting layers are in intimate contact during the upward movement of the endo- and mesoderm; they become firmly attached to each other as soon as the upward movement ceases (see Nieuwkoop, 1973).

Mechanism of induction

In the amphibians both the meso–endodermal and the neural induction process are four-dimensional processes spreading from cell to cell in the three-dimensional, spherical embryo during a rather long period of embryonic development. During this period the intensity of the inductive action and the character and intensity of the competence of the reacting ectoderm change. The propagation of the two inductive actions cannot be based on simple diffusion, since, after replacement of part of the neurectoderm by competent gastrula ectoderm at an increasing distance from the dorsal midline from where the induction spreads, the propagation of the neuralizing action seems to cover the same distance (Albers, unpublished observation). Depending on the thickness of the implanted gastrula ectoderm a substantially larger neural structure is formed on the operated than on the non-operated side. Implantation of ectoderm taken from increasingly older gastrulae demonstrates that the spatial extension of the neural anlage is essentially determined by the loss of neural competence during the lateral (and anterior) propagation of the inductive action in the overlying ectoderm (Albers, unpublished observations).

Although amphibian embryos are in principle very suitable for the analysis of inductive interactions, since isolated reactive material can easily be confronted with different inductive stimuli exerted either by different embryonic tissues or by different purified compounds, it must be admitted that not much insight into the processes involved has been gained up till now. The only thing that is clear is that very different tissues and chemical compounds can elicit a specific response from the competent reaction system, the latter apparently being thoroughly prepared to be switched into a new developmental pathway. For instance, meso–endoderm induction can be achieved by, among other things, a protein isolated from 9-day chick embryos (Tiedemann, 1966) as well as by treating the competent ectoderm with Li ions (Masui, 1961). Recent experiments of Hoperskaya *et al.* (1984) show that extracellular matrix material of Bruch's membrane can also evoke extensive mesodermal inductions in competent gastrula ectoderm, which points to a possible role of the extracellular matrix in meso–endoderm induction. Likewise, neural induction can be achieved by, among other things, a high-M.W. nucleoprotein fraction isolated from 9-day chick embryos (Tiedmann, 1966) as well as by treating the competent ectoderm with a medium of either low or high pH (Holtfreter, 1947*a, b*, 1948). An inductive action of extracellular matrix material has not yet been demonstrated for neural induction.

Holtfreter (1968) called attention to the fact that inductive interactions and morphogenetic movements alternate during embryonic development. Davenport (1979) pointed out that morphogenetic movements seem to be the consequence of instabilities that have arisen in the spatial configuration of the embryo, e.g. due to the appearance of new (positive or negative) affinities between adjacent cells caused by previous inductive interactions; such instabilities call for a readjustment of the entire system into a new, more stable configuration. For instance,

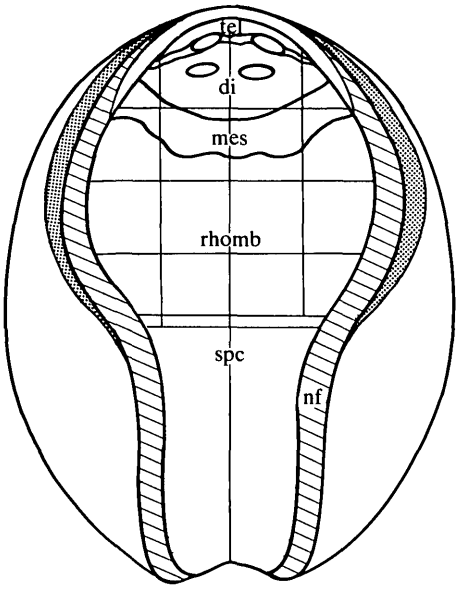

Fig. 6. Localization of the cephalic placodal ectoderm (stippled) outside the anterior neural plate, and localization of cephalic and trunk neural crest (hatched) in the neural folds, formed respectively under the influence of the spreading activating and transforming inductive actions in the ageing reaction system (ectoderm and neurectoderm respectively). *di*, presumptive diencephalon; *mes*, presumptive mesencephalon; *nf*, neural fold; *rhomb*, presumptive rhombencephalon; *sp.c*, presumptive spinal cord; *tel*, presumptive telencephalon. (Redrawn from C. O. Jacobson, 1959 and adapted to the views of P. D. Nieuwkoop, 1963 and B. Albers (unpublished).).

meso–endoderm induction causes changes in cell behaviour which lead to gastrulation, and neural induction evokes cell properties which are responsible for the process of neurulation or neural tube formation as well as for neural crest migration.

Later inductive interactions

The cephalic placodes form just outside the neural anlage from ectoderm that has lost its neural competence but still retains competence for placodal ectoderm formation, at the time the laterally and anteriorly propagating neural activation process reaches the boundary of the neural anlage. In analogous fashion the neural crest may be formed in the peripheral region of the neural anlage when the neurectoderm begins to lose its competence for the laterally propagating transforming action (Nieuwkoop, Johnen & Albers, 1985) (see Fig. 6). Apart from the appearance of mesectodermal differentiation tendencies in the cephalic neural crest prior to the initiation of cell migration, neural crest cells are apparently still pluripotent. Their stepwise determination partly occurs under the influence of surrounding tissues during their migration, but mainly after they have reached their final destination sites (le Douarin *et al.* 1979; Cochard & le Douarin, 1982).

The further development of the CNS occurs partly by self-organization and partly under the influence of new inductive actions emanating from the underlying archenteron roof. Examples of autonomous processes are the essentially concentric segregation of the prosencephalon into telencephalon, diencephalon and eye anlagen (Boterenbrood, 1970), the segregation of the rhombencephalon into basal and alar plates and the similar segregation of the spinal cord into a ventral motor column, lateral shunt systems and dorsal sensory elements (Rohon-Beard cells). Examples of processes dependent on inductive actions are those responsible for the bilateral development of the telencephalon and eye anlagen by an inhibitory influence from the median prechordal plate, and for the similar duplication of the basal plate of the rhombencephalon and the ventral motor column of the spinal cord by inhibitory influences from corresponding regions of the underlying notochord (See Källen, 1965). The craniocaudal segregation of the spinal cord seems to be causally connected with the preceding segmentation of the somitic mesoderm (Strudel, 1970).

The development of the complex vertebrate eye also occurs stepwise; it starts with the evagination of the primary eye vesicle from the ventral diencephalon. The primary eye vesicle then becomes firmly attached to the overlying ectoderm, inducing the lens anlage. The subsequent invagination of the lens anlage and formation of the lens vesicle causes the reinvagination of the primary eye vesicle, leading to the formation of the double-walled secondary eye vesicle. The segregation of the latter into the internal retinal layer and the external pigmented epithelium respectively takes place under the influence of the attached lens vesicle, which promotes cell division and inhibits the spatial extension of the retinal anlage, and of the cephalic mesenchyme (chiefly of neural crest origin), which inhibits cell division and promotes expansion of the outer layer (Lopashov, 1961; Hoperskaya, 1972). The subsequent differentiation of the lens vesicle occurs under a humoral influence emanating from the neural retina and stimulating lens fibre formation in the basal portion of the anlage (Reyer, 1966; Gunia & Tumanishvili, 1972). The appearance of lens antigens is apparently not restricted to the lens fibres, which synthesize α-, β- and γ-crystallins, but also occurs, though at a low level, in adjacent eye structures such as the lens epithelium, the corneal epithelium, the iris and the pigmented epithelium, all of them tissues from which lenses can regenerate under experimental conditions in the adult amphibian eye (Clayton, 1982). Clayton therefore suggests that lens competence may reflect a low level of specific mRNA transcription, while lens induction involves a marked enhancement of an already existing process rather than the initiation of new synthesis.

Subdivision of the mesoderm

The regional segregation of the mesodermal mantle occurs primarily under an inductive influence spreading with decrement from the mediodorsal chordomesoderm and leading to the differentiation of the segmental somites, the segmental nephric anlagen and the unsegmented lateral plate mesoderm at increasing

distances from the notochordal anlage (Yamada, 1937, 1939*a*, *b*, 1940). The notochordal cells form large vacuoles, while the notochordal anlage as a whole forms an outer, extracellular sheath. Notochordal differentiation is stimulated by the overlying neural plate induced by the archenteron roof, demonstrating again the reciprocal nature of inductive interactions (Nieuwkoop & Weijer, 1978). Somite formation is largely an autonomous process showing an anteroposterior time sequence. The subsequent segregation of each somite into a dorsolateral dermatome and underlying myotome and a medioventral sclerotome occurs under the influence of the overlying epidermis and of the adjacent notochord and ventral spinal chord, respectively. The extracellular matrices of the latter two structures seem to be responsible respectively for the segregation of the sclerotome into the two components of the vertebral cartilages, i.e. the vertebral body and the vertebral arches. The cartilages of the visceral skeleton are formed from the cephalic meso–ectoderm under local inductive influences of the pharyngeal endoderm. An anteroposterior progression in adhesiveness of the ventral surface of the somites guides the Wolffian duct anlage, which develops from the caudal portion of the pronephric anlage, during its outgrowth ('migration') towards the cloaca (Steinberg & Poole, 1982). The outgrowing Wolffian duct stimulates the differentiation of the segmental mesonephric anlagen, which by themselves are only capable of the initial steps of differentiation (Gipouloux & Delbos, 1977). The gonadal anlagen develop from the genital ridges, which are formed in the coelomic epithelium on either side of the dorsal mesentery under the influence of the notochord and the Wolffian ducts (Nieuwkoop, 1946). The primordial germ cells of the anurans seem to be formed in the endodermal yolk mass near the vegetal pole of the egg around preformed cytoplasmic inclusions (the so-called germ plasm)(Bounoure, 1939; Blacker, 1970), but in the urodeles they are apparently induced in the ventrocaudal mesoderm by the adjacent endoderm (Nieuwkoop, 1946; Maufroid & Capuron, 1977). In both groups they migrate actively towards the genital ridges. The bisexual gonadal anlagen are composed of a cortical component derived from the genital ridge and a medullary component originating from the interrenal mesenchyme (Witschi, 1957). In the male the medullary component proliferates and forms the testicular tubules, which later connect to the Wolffian duct, while in the female the cortical component proliferates and forms the main anlage of the ovary. The Müllerian ducts are split off from the Wolffian duct and develop into the female gonadal ducts.

The mesodermal 'germ layer', with its pronounced dorsoventral and craniocaudal polarities, undoubtedly constitutes the leading element in the establishment of the regional organization of the amphibian embryo. However, additional roles in the establishment of the ultimate three-dimensional pattern of the embryo are played by a weak animal–vegetal polarity of the ectoderm, which finds its expression in a vegetal–animal decrease in mesodermal competence (Sutasurya & Nieuwkoop, 1974) and a corresponding increase in epidermal differentiation tendencies (Grunz *et al.* 1975; Grunz, 1976), and by a much more pronounced anteroposterior and dorsoventral polarity of the endodermal archenteron.

Epithelial–mesenchymal interactions

Epitheliomesenchymal interactions govern subsequent organogenesis. They can be divided into ectomesenchymal and endomesenchymal interactions. The ectomesenchymal interactions lead to the development of the skin and its derivatives, such as the cement glands in the anurans and the balancers in the urodeles, and to the formation of hatching glands in both. They are also responsible for tooth formation and for the development of body appendages (fins and limbs). The endomesenchymal interactions govern the development of the various regions of the digestive tract with their specific glands, as well as the formation of the thyroid glands and the anlagen of the lungs. The various organ anlagen show different types of inductive interaction between the epithelium and the adjacent mesenchyme. In some epitheliomesenchymal interactions the mesenchyme plays the leading role, as e.g. in feather formation and in the development of the liver, while in others the epithelium shows a strong self-organizing and self-differentiating capacity and the mesenchyme is more passive in its behaviour, as e.g. in the development of the cement gland and in that of the pancreas anlagen. In other cases again the two components are of equal, often alternating importance for the development of the ultimate pattern, as e.g. in tooth formation and in limb development. In nearly all epitheliomesenchymal inductive interactions the extracellular matrices of both epithelium and mesenchyme play an important role (Grobstein, 1967; Wessels, 1970).

Specificity of signals and responses

Finally we come to the important question of specificity in inductive interactions. Although a certain specificity cannot be denied to the inductive action, whatever it may be – the mesodermal inductive action is most probably not identical to the neural inductive action, although both act upon the same ectoderm, though with slightly different competence maxima – the main specificity is localized in the reaction system, which is apparently thoroughly prepared to be switched into a specific new developmental pathway. The reaction system evidently needs a stimulus, since in its absence it does not enter the new pathway, but the stimulus may be of an unspecific nature, particularly when the competence of the reaction system is high.

It seems likely, therefore, that inducing factors represent nothing but *transient products of the cellular differentiation* of the action system. They may vary from ionic changes and simple metabolites all the way up to high-M.W. extracellular matrix components. On the basis of these considerations it does not seem promising to continue a direct search for specific chemical inductive factors. Instead, the changing physiology of both the action and the reaction system during the critical period of their inductive interaction ought to be studied, and special attention ought to be paid to differences in structure and function of the two systems, since their interaction is essentially based upon these differences.

For further details of germ cell formation and inductive interactions in the chordates the reader is referred to the monographs by Nieuwkoop & Sutasurya (1979) and Nieuwkoop, Johnen & Albers (1985).

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