Fate maps and cell differentiation in the amphibian embryo – an experimental study

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SUMMARY

The aim of this paper is to test two different fate maps for the amphibian blastula with respect to their predictions concerning the process of cell differentiation. The first of these fate maps is the one proposed by Vogt, according to which all three germ layers can be projected on to the surface of the embryo. The second is a revision which claims that only endoderm and ectoderm are located in the surface, while the mesoderm is represented by free cells in the interior of the embryo.

The testing has been performed by observing the differentiation of small explants of cells taken from various regions of the embryo.

It was found that the spontaneous cell differentiation comprises three patterns: undifferentiated cells (free interior cells and circumpolar endodermal cells), fibroblast-like cells (the remaining endodermal cells) and epidermis (ectodermal cells).

Further differentiation occurs only through induction, exerted either by the fibroblast-like endodermal cells or by heparan sulphate. When induced, the equatorial ectodermal cells give rise to swollen, hyaline cells (chordocytes), while the remaining ectodermal cells form a sequence of cell differentiation patterns, mesenchyme cells, nerve cells, melanophores and xanthophores.

The free interior cells differentiate into striated muscle cells and elongated collagen-producing fibroblasts.

Our results thus confirm the revised fate map, and they also give an insight into the mechanisms of the initial cell differentiations in the amphibian embryo.

INTRODUCTION

The present paper centres on three concepts, ‘cell differentiation’, ‘induction’ and ‘fate map’. In order to understand the argument it is necessary to begin with a specification of the implication of these words, when used in the present text. We shall do so on the basis of definitions found in Needham (1942). ‘[Cell] differentiation. Increase in number of kinds of cells; invisibly, as by determination and losses of competence; visibly, as by histogenesis.’

Up to the beginning of gastrulation all cells in the embryo, when isolated, have the spherical shape typical of embryonic cells, a miniature of the egg. During the ensuing development a large number of different cell types arise in the embryo, each representing a separate differentiation pattern.

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The cells belonging to a particular differentiation pattern are characterized by the fact that they synthesize a selected repertoire of the proteins coded for in the genome. The onset of differentiation must therefore be associated with the synthesis of new types of messenger RNA, and the process should be suppressed by inhibitors of RNA synthesis. This point has been confirmed repeatedly, including our experimental set-up (Lovtrup, Landström & Lovtrup-Rein, 1978).

We shall here distinguish between 'spontaneous' and 'induced' cell differentiation.

Thus, a spontaneous differentiation is one that is determined by factors intrinsic to the differentiating cell. From this definition it follows that it should be possible to expose the spontaneous differentiation patterns by culturing explants from the early embryo in a neutral medium.

An induced cell differentiation is one whose realization requires the participation of factors extrinsic to the cell. In the embryo such a factor must either reside in or on another cell, or be secreted by one, for instance into the blastocoel. If interaction between separate cells is going to affect cell differentiation, it is natural to presume that the cells in question belong to disparate differentiation patterns. Hence it may be expected that cells representing the spontaneous differentiation patterns are involved in the earliest instances of induced differentiations.

So far we have not mentioned the influence of the external medium, although it is known that the latter may influence certain differentiation processes (Landström, 1977). We shall not discuss this phenomenon in the present context, but rather argue on the presumption that the medium employed by us is neutral, i.e. that it does not suppress or otherwise modify any of the differentiation processes occurring in the normal embryo.

We have here used the word 'induced' in connexion with the process of cell differentiation. This usage does not involve an inconsistency as far as its meaning is concerned, but 'induction' is used also in other embryological contexts, and we must therefore try to establish whether the meaning implied by us coincides with the current one.

'Induction. The morphogenetic effect brought about by an organizer, inductor, evocator, etc. acting on competent tissue.' Here Needham mentions only the morphogenetic effect, thereby emphasizing that traditionally studies of embryonic induction generally have involved evaluation on the basis of morphological criteria. However, it must be stressed that embryonic induction is always associated with the appearance of new cell differentiation patterns. Thus, the induction of a neural plate is unthinkable without the simultaneous formation of the typical elongated neural plate cells. The presence of these cells is the criterion which shows that a neural plate has been formed. There are even good reasons to claim that the various new cell types are the true morphogenetic agents, and therefore the principal event in embryonic induction is the creation of new cell differentiation patterns, the ensuing morphogenesis being only a consequence of
this phenomenon. This question has been discussed at length elsewhere (Lovtrup, 1975). We present here results consistent with our previous interpretation of the induction phenomenon.

‘Fate-map. A map of an embryo in an early stage of development... indicating the various regions whose prospective significance has been established by marking methods.’

Fate maps have two sorts of implication. First, they account for the topological changes occurring during normal unperturbed embryogenesis. However, they also concern the fate of the embryonic cells as regards their prospective differentiation.

For instance, various morphogenetic movements ensure that the cells located in the mesodermal area of the fate map end up at the location proper to the mesoderm, i.e. at the dorsal side, surrounded by epidermis, neural tube, notochord and endoderm.

But the fate map also implies that, once the mesodermal cells have reached their station, they will differentiate into muscle cells and connective tissue cells (sclerocytes).

This means that the explant experiments mentioned above may be used for the testing of fate maps. For evidently wherever the fate map concerns a spontaneous differentiation pattern, explanted cells must differentiate in accordance with the prediction of the fate map. Here potency and predicted fate thus coincide.

The situation is less clear-cut as far as the induced differentiation patterns are concerned, because a process of induction is involved. However, it seems possible to conclude that if it is possible to subject the explanted cells to the same inductive stimulus as they encounter in vivo, then they should differentiate as predicted by the fate map. Under this particular assumption the fate map may be tested even in this case.

To avoid misunderstandings we shall stress that the proposed testing does not concern the geometrical details of the fate maps, only their prediction with respect to the location of the particular cell differentiation patterns.

The notion of correlation between fate maps and cell differentiation is well established; it was used by Oppenheimer (1940) and de Beer (1947) to challenge certain aspects of the currently accepted fate maps.

As far as the latter are concerned, it may be noticed that in the first quarter of the present century all embryologists seemed to agree that in the amphibian embryo the mesoderm resides in the interior, below the embryonic surface, a situation which, incidentally, befits this germ layer. The population of cells representing the mesoderm would then be the small spherical cells located in the ring-shaped groove between the ectoderm and the arching endoderm.

This situation was radically changed when Vogt (1925, 1929) published his fate maps, established by means of vital staining experiments. As shown in Fig. 1 A, Vogt interpreted his results to show that the mesoderm is located in the embryonic surface, surrounding completely the notochordal primordium. Vogt's
fate maps were readily accepted, and have formed the basis of interpretation of much embryological work since then. This was unfortunate, for they are wrong. It was correct to place the notochordal primordium in the embryonic surface, but the mesoderm lies beneath, in the interior, as stated above. It is a remarkable and deplorable fact that those who accepted the fate maps did not notice that Vogt was hesitant about their validity as far as the mesoderm is concerned. In fact, he stated that at the ventral side the mesoderm was probably represented by cells in the interior ('innere Randzone'), and he admitted that this might be in part the case even at the dorsal side. And on this point Vogt was right, for a careful scrutiny of his experimental records shows that they do not conform with his fate maps; agreement obtains only if all the mesoderm is placed beneath the surface (Løvtrup, 1966, 1975).

Furthermore, the fate maps imply a number of ruptures, between notochord and mesoderm, between mesoderm and ectoderm and between mesoderm and endoderm, none of which has ever been observed. In the urodele embryo Vogt described a rupture, separating endoderm and notochord; this phenomenon is a direct contradiction of his own fate maps. In the anuran embryo there is an outer pigmented cell layer, called 'epiectoderm', in the animal hemisphere (Løvtrup, 1966). The fact that this cell layer does not undergo any ruptures at all (cf. Brachet, 1921; Nieuwkoop & Florschütz, 1950), led the latter authors to disclaim the validity of Vogt's fate map for anurans, recently corroborated by the vital staining experiments of Keller (1976).

If the mesoderm consists of free interior cells, it cannot be projected on the embryonic surface and so a particular fate map is required. The revised fate maps for urodele ectoderm – endoderm and mesoderm, respectively, are shown in Figs. 1B and 1C.

Another circumstance intimating that Vogt's fate maps are wrong is found in the fact that Holtfreter's attempt (Holtfreter, 1938a, b) to correlate the cell differentiation patterns of explanted cells with the fate maps was a complete failure. In the present paper we propose to demonstrate that perfect agreement obtains between the revised fate maps and the differentiation patterns observable in cultured cells.
MATERIAL AND METHODS

Material. The present investigation was carried out with embryos from the axolotl *Ambystoma mexicanum*. Fertilized eggs were collected and allowed to develop in 7.5% amphibian Ringer.

Isolation and culture of cells. At a late-blastula stage, the jelly and the vitelline membrane were removed with a pair of watchmaker’s forceps, after which the embryos were rinsed in several changes of sterile 7.5% amphibian Ringer. Explants comprising about 15 cells were dissected out from various regions of the blastula by means of a glass needle and a hair loop, rinsed and cultured in Petri dishes in 4 ml of the standard solution of Barth & Barth (1959), containing 25 iu/ml benzyl-penicillin and 25 µg/ml streptomycin, with or without addition of heparan sulphate (1 µg/ml). The explants were placed on small glass coverslips with diameter 10 mm, placed on the bottom of the dish (three explants per coverslip and three coverslips per dish).

To survive, cells isolated from the embryonic interior, i.e. regions 3 and 7 in Fig. 2, must be cultured in a medium with a salt concentration corresponding to 1.5 x the standard solution.

The aggregates were regularly observed and photographed in an inverted microscope with camera attachment. All experiments were carried out at room temperature (~23 °C).

Scanning electron microscopy. At appropriate stages the cell cultures were fixed in 3% glutaraldehyde in 0.1 M-Tris-buffer, pH 7.3, for 24 h. The coverslips, with the cell aggregates attached to the glass, were rinsed in several changes of buffer and distilled water, and dehydrated in a graded ethanol series (30%, 50%, 70%, 80%, 90%, 95%, and absolute ethanol). The ethanol was substituted with amylacetate in a graded series of 25%, 50%, 75%, 100%. The slips were then dried in an Anderson critical-point drying apparatus, coated with a layer of gold and examined by scanning electron microscopy at the beam voltage of 15 kV.

Aggregates of undifferentiated cells and of uninduced ectodermal cells do not adhere to glass surfaces. These samples were therefore fixed and dried in small cups before they were gold-coated and examined in the microscope.

RESULTS

Samples were taken from ten different regions of the embryos, numbered as shown in Fig. 2. According to the revised fate maps, regions 1 and 9 represent dorsal and ventral endoderm; 2 and 8, the surface layer of the marginal or equatorial zone, which should contain the notochordal primordium, at least at the dorsal side; 3 and 7 are mesoderm; 4 neural, 5 and 6 epidermal, ectoderm. Finally, we have also explanted cells located near the vegetal pole (region 10).
Except for the last case, the differentiation predicted by the revised fate maps is more or less unambiguous. The situation is more dubious as regards Vogt’s fate maps. However, it is evident that the separation between notochord and mesoderm is superficial, thus region 2 might expectedly give rise to mesodermal cell types and region 4 to notochordal cells. Likewise, one might anticipate the appearance of mesodermal cells in explants from region 9.

As stated in the introduction, the outcome of the explant experiments depends upon whether or not inductive interactions are required for the cell differentiations to occur. The results reported in the next section show that the spontaneous differentiation potential of cells isolated from the blastula is quite narrow. This result is not unexpected, for it is well known that in the intact embryo the formation of the normal repertoire of differentiation patterns is dependent upon the process of primary induction. In the second section below we report the results obtained when this inductive interaction is allowed to occur.

Spontaneous cell differentiation patterns

If the cells are cultured in the standard solution, three distinct differentiation patterns are observed after 3 days, viz., ‘undifferentiated’, ‘vegetal’ and ‘animal’ cells.

(1) The cells which we call ‘undifferentiated’ have the spherical shape typical of embryonic cells. In the cultures these cells form irregular, unattached aggregates (Fig. 3A). The shape proper may not suffice to characterize these cells as undifferentiated embryonic cells, but we shall presently discuss some further observations which tend to support this view. A fair number of undifferentiated aggregates occurs in the explants from all regions (around 20–25%), but in the
FIGURE 3.
Scanning electron micrographs. A, Aggregate of undifferentiated vegetal cells (70 ×); B, surface of (A) (700 ×); C, surface of an aggregate of undifferentiated mesodermal cells (700 ×); D, vegetal fibroblast-like cell (Ruffini's cell) (700 ×); E, cells in the surface of an epidermal aggregate (640 ×); F, epithelial collagen inside (E) (700 ×); G, animal fibroblast-like cell (700 ×); H, epithelial collagen on chordocyte aggregate. The boundaries between three cells may be discerned beneath the collagenous sheath (1430 ×).
Fig. 4. Frequency of 'undifferentiated' cells after ten days, in explants from the various region of the blastula. In this and some of the following histograms lines skewed upwards to the left means absence, lines skewed upwards to the right presence, of heparan sulphate (HS). It is seen that in the absence of HS the incidence of undifferentiated cells is high in explants from the interior (3, 7) and the vegetal pole (10). In the presence of HS this pattern is radically changed as far as the interior cells are concerned, but the vegetal pole cells are refractive to the influence of HS.

regions 3, 7 and 10 the incidence is close to 90% (Fig. 4). The vegetal pole cells from region 10 are invariably larger than the mesodermal cells from regions 3 and 7 (Figs. 3B and 3C).

(2) In the light microscope the 'vegetal' cells have the appearance shown in Fig. 5A. When the cultures are followed for some days, the area covered by cells increases; the cells thus display the phenomenon of outgrowth typically observed in fibroblast cultures. The cells closest to the original explant form a kind of 'epithelium', but some lose contact with their counterparts. One such cell may be seen in Fig. 3D.

We have in previous publications contended that these cells represent Ruffini's flask-cells, attached to the bottom of the blastoporal groove in the amphibian embryo (Landström & Lovtrup, 1977a, b; Landström, Lovtrup-Rein & Lovtrup, 1976). But we should like to use a name for these cells which accounts for their cell class affiliation (cf. Willmer (1960) and Lovtrup (1974) on the subject of cell classification). Our 'vegetal' cells, as seen by scanning electron microscopy (Fig. 3D), are similar to published pictures of 'fibroblasts' (cf. Revel, Hoch & Ho, 1974). Therefore we shall use here the term 'fibroblast-like' cells, while suggesting that a better name would be 'solo-filocytes' (cf. Lovtrup, 1974).

Fibroblast-like vegetal cells primarily arise from endodermal cells outside the vegetal pole (70–75%). From the neighbouring regions, including the ectodermal cells in the marginal zone, some explants may differentiate in this fashion, but the most distal animal cells (regions 4, 5 and 6) never assume this differentiation pattern spontaneously (Fig. 6).

In the early amphibian embryo, the enzyme alkaline phosphatase is absent, or present at a very low concentration, but it begins to rise during gastrulation. It has been suggested that this phenomenon may be associated with the appearance
Fig. 5. Micrographs (150 x). A, Vegetal fibroblast-like cells (Ruffini's cells, region 1); B, animal fibroblasts-like cells (region 5); C, equatorial ectodermal fibroblast-like cells (region 2); D, mesodermal fibroblast-like cells (region 3); E, chordocytes; F, muscle cells; G, elongated fibroblasts; H, mesenchyme cells; I, nerve cells; J, melanophores; K, xanthophores.
Fig. 6. Frequency of vegetal fibroblast-like cells (Ruffini’s cells) after three days, in explants from the various regions of the blastula and in the presence and absence of HS (see the caption to Fig. 4). The preponderance of the differentiation pattern in the endodermal explants (1, 9) is evident. The effect if HS is negligible, except possibly in region 9.

Fig. 7. Frequency of ciliated epidermal aggregates after three days, in explants from the various regions of the blastula and in the presence and absence of HS (see the caption to Fig. 4). This differentiation pattern is spontaneous in the ectoderm, i.e. the external cells in the animal hemisphere (2, 4, 5, 6, 8). In the presence of HS this differentiation is suppressed except in the regions nearest the animal pole (4, 5, 6).

of Ruffini’s cells (cf. Løvtrup, 1974). These cells, in contrast to the undifferentiated cells, should thus be characterized by the possession of alkaline phosphatase. We have been able to confirm this proposition histochemically and electrophoretically (U. Landström and H. Løvtrup-Rein, unpublished experiments).

(3) The ‘animal’ cells form spherical, usually very regular, aggregates, in which ciliated cells occur interspersed among non-ciliated ones (Fig. 3E). This differentiation, representing the epidermis, is found in explants from the five ectodermal regions (2, 4, 5, 6 and 8), where 50% or more of the explants assume this pattern; a few ciliated aggregates may be formed also by the cells isolated from the mesodermal regions (3 and 7), but never by the vegetal cells (Fig. 7). When the vesicular aggregates are opened a meshwork of collagen is seen to cover the interior cell surfaces (Fig. 3F).
Induced cell differentiation patterns

Since only three cell differentiation patterns—undifferentiated cells, fibroblast-like Ruffini’s cells and epidermal cells—occur spontaneously, the appearance of further cell differentiation patterns must be the outcome of inductive interactions between these cell types. Since from the classical experimental embryology we know that the dorsal blastoporal lip, the site of Ruffini’s cells, is a potent inductor, it might be envisaged that Ruffini’s cells in some way must represent, or embody, the inductive agent.

To test this inference we added a few vegetal cells to explants of ectodermal cells. Under these circumstances the majority of the cell aggregates exhibit an outgrowth of cells, similar in shape to the vegetal cells (Fig. 5B). When the cultures are continued, these cells undergo further differentiation into mesenchyme cells, nerve cells and pigment cells (cf. below). This outcome corroborates our surmise, the outcome of the experiment showing that it is a miniature of the process of primary induction (Lovtrup, et al. 1978).

It is, of course, rather cumbersome to work with mixed explants, preferably one would like to add the inductor directly to the culture medium. Knowing that the vegetal fibroblast-like cells with great probability contain heparan sulphate (HS), we tested the conjecture that this substance may be the inductor, and found that at least it is an inductor, when applied in the range 0.1–1 μg/ml (Landström & Lovtrup, 1977a). We have utilized this discovery in the present paper; the ‘induced cell differentiation patterns’ means ‘those appearing in a medium containing 1 μg HS/ml’.

Under these conditions the following results were obtained:

1. The general effect of HS is to suppress the incidence of undifferentiated cells. This does not hold, however, for the cells in region 10, around the vegetal pole, which are completely refractory to the action of HS (Fig. 4).

2. HS does not have any striking effect on the differentiation of the fibroblast-like vegetal cells, they occur in the same regions and in about the same frequencies (Fig. 6).

3. In contrast to those described so far all the ecto-mesodermal regions (2–8) are profoundly affected by the presence of HS. To be sure, in all cases a certain fraction of the aggregates (13–25%) remains undifferentiated (Fig. 4). Likewise, the typical ectodermal differentiation pattern, ciliated epidermal vesicles, still occurs in regions 4–6 (16–22%), but not in the equatorial ectoderm and mesoderm, regions 2, 3, 7 and 8 (Fig. 7).

In the presence of HS a copious outgrowth of fibroblast-like cells is observed on the fourth day in the cultures from the equatorial (Fig. 8) and the animal regions (Fig. 13). It is possible from their appearance to distinguish the fibroblast-like cells arising in the different locations. Thus, although their general outline is like that of the vegetal cells, those arising in explants from the animal regions 4–6 are much smaller, containing little yolk and much pigment.
Fig. 8. Frequency of equatorial fibroblast-like cells (Fig. 5c, d) after four days, in explants from the various regions of the blastula and in the presence of HS.

Fig. 9. Frequency of chordocytes after seven days, in explants from the various regions of the blastula and in the presence of HS. This differentiation pattern is confined primarily to the external equatorial cells (2, 8) and exhibits a dorso-central gradient.

(Figs. 3G and 5B). The cells from the equatorial ectodermal regions (2 and 8) are intermediate between the animal and the vegetal cells (Fig. 5C). The mesodermal cells (regions 3 and 7) are more elongate and look more like typical fibroblasts (Fig. 5D). Perhaps they are smooth muscle cells.

Later these cells differentiate further into a wide spectrum of differentiation patterns:

(4) On the seventh day some explants from the equatorial ectodermal regions (2 and 8) form aggregates consisting of swollen hyaline cells which clearly are chordocytes (Fig. 5E). The frequencies for the two regions are 37 and 23%, respectively, suggesting that the dorso-ventral polarity affects this differentiation pattern. Chordocytes are also formed in a few of the mesodermal aggregates and in aggregates from the dorsal endoderm (Fig. 9). In some instances these cells secrete collagen which forms a sheath around the aggregates (Fig. 3H), similar to that observed in the normal embryo (Löfberg & Ahlfors, 1978). In fact, the aggregates may elongate to form notochord-like structures (Fig. 10A).

(5) Cross-striated muscle cells (Figs. 5F and 10B) appear in 40% of the dorsal mesodermal aggregates (region 3); the corresponding number for the ventral
Fig. 10. Scanning electron micrographs. A, Chorda-like structure (140 x); B, muscle cells (700 x); C, elongated fibroblast (700 x); D, mesenchyme cell (700 x); E, axons extending from an aggregate (150 x); F, nerve cell junctions with other cells (700 x); G, melanophore (700 x).
Fig. 11. Frequency of striated muscle cells after eight days, in explants from the various regions of the blastula and in the presence of HS. This differentiation pattern is confined primarily to the internal equatorial cells (3, 7) and exhibits a dorso-ventral gradient.

Fig. 12. Frequency of elongated fibroblasts (Figs. 5G and 10C) after ten days, in explants from the various regions of the blastula and in the presence and absence of HS (see the caption to Fig. 4). This differentiation pattern is unknown in explants from the non-equatorial ectoderm (4, 5, 6) and from the circumpolar endoderm. Elsewhere it is the most frequent differentiation, at least when induction with HS has taken place.

Mesoderm (region 7) is 24% again a distinct dorso-ventral polarity. Small amounts of muscle cells appear in aggregates from the equatorial ectodermal regions (2 and 8), and from the dorso-animal region (4), as shown in Fig. 11. The muscle cells first occur on the eighth day of culture.

(6) In cultures from the equatorial regions (2, 3, 7 and 8) and from the vegetal regions (1 and 9) elongated fibroblasts are found in almost all aggregates (Figs. 5G and 10C). These cells seem to be collagenocytes, secreting their differentiation product on to the surface of the culture dishes, as suggested by staining experiments with chlorantline fast red. The distribution is shown in Fig. 12.

The differentiation patterns deriving from the remaining ectodermal regions (4–6) are quite distinct from those discussed so far.

(7) In about two-thirds of all cultures mesenchyme cells appear on the fifth
Fig. 13. Frequency of the various ectodermal differentiation patterns induced by HS. Sparse dots, animal fibroblast-like cells after four days; squares, mesenchyme cells after five days; circles, nerve cells after six days; dense dots pigment cells after ten days.

day (Figs. 5H and 10D). Their frequency is shown in Fig. 13. Although we have not yet evidence to support this view, we believe that these cells are producers of glycosaminoglycans, probably hyaluronate. They should thus be myxocytes according to the terminology of Willmer (1960).

(8) Nerve cells are formed on the following day, as evidenced by long axons extending from the aggregates, making contact in many cases with free cells (Figs. 5, 10E and 10F; distribution, Fig. 13). It is possible to liberate the nerve cells by treatment with Ca^{2+}-free medium.

(9) Melanophores are observed around the ninth day (Figs. 5J and 10G; distribution, Fig. 13).

(10) At about the same day one may observe yellow spherical cells (imbedded in the aggregates, Fig. 5K). Since carotenoids are absent, and pteridines are present in early amphibian pigment cells (Bagnara, 1966); we conclude that those observed by us are pteridin-producing xanthophores. We have corroborated the absence of carotenoids by means of histochemical tests.

It may be noticed that when the values for mesenchyme cells, nerve cells and melanocytes in Fig. 13 are added, they amount to more than 100%, showing that more than one cell type may arise in each aggregate.

**DISCUSSION**

According to Vogt's fate maps the notochordal primordium and the mesoderm should both occur in the embryonic surface, but at different locations. In contrast, the revised fate map predicts that they should both be found in the equatorial region, but at different levels. A comparison between Figs. 9 and 11 bears out the corroborations of this prediction; our results clearly are a refutation of Vogt's fate maps.

Our observations agree in part, but not completely with the classical notion that each germ layer corresponds to a particular array of cell differentiation
patterns. Lack of agreement is particularly evident with respect to the elongated fibroblast-like cells, which originate from the endoderm, as well as from the equatorial ectoderm and mesoderm.

It seems that the results may be accounted for in a way less ambiguous than the one based on the concept of ‘germ layers’. Thus they may demonstrate the existence of four zones along the animal–vegetal axis.

(1) An animal zone (regions 4–6) which uninduced gives epidermis, but upon induction gives rise to neural plate–neural crest cells (myxocytes, neurocytes, melanophores and xanthophores). These cells always occur in a specific sequence, a fact which may explain their distribution in the embryo (Løvtrup, 1974).

(2) An equatorial zone which, after induction, contributes chordocytes (myxocytes), myocytes and elongated fibroblast-like cells (collagenocytes). It is possible to distinguish between an outer and an inner zone. The former is ectodermal and gives rise preferentially to the chordocytes, whereas the latter is mesodermal, the source of myocytes and sclerocytes.

It may be noted that this observation may be accounted for formally by assuming the existence of a polarity perpendicular to the surface of the blastula, a ‘normal’ polarity (Løvtrup, 1974).

(3) A vegetal zone (endoderm). The only differentiation product we have observed from this region is elongated fibroblast-like cells. We do not know whether these cells represent several disparate differentiation patterns, but evidently the factors, perhaps inductors, which normally ensure the development of the endoderm, are missing under the conditions of culture.

(4) Finally we have the cells in the vegetal pole region, which remain undifferentiated and, in fact, soon stop dividing. From analogy with the situation in the anurans (Bounoure, 1934), one might envisage that these cells represent the germ line. However, many observations suggest that in urodelans the germ cells arise in the lateral mesoderm (Humphrey, 1925, 1919; see Sutasurja & Nieuwkoop, 1974, for a discussion of recent literature). Rather, it may be that these cells are those large yolk-laden cells in the inner wall of the midgut which are digested during later development (Vogt, 1909; Holtfreter, 1943).

Apart from the ‘undifferentiated’ cells, there are only two spontaneous cell differentiation patterns in the amphibian embryo, Ruffini’s cells in the endoderm and epidermis in the ectoderm.

The former, residing in the invaginating endoderm, have the power to induce the ectoderm to assume the cell differentiation patterns that normally appear as the result of primary induction. That the endoderm embodies the inductor involved is not too far from ideas sustained by Boterenbrood & Nieuwkoop (1973).

We have not made mixed cultures with endoderm and mesoderm, but we have found that heparan sulphate may elicit primary as well as mesodermal induction. From this observation we tentatively conclude that the first round of cell
differentiations in the amphibian embryo is triggered by HS. Hence it appears that the many attempts previously made to distinguish between ‘primary’, ‘neural’ and ‘mesodermal’ induction are the consequence of reasoning based on wrong premises. Probably this misunderstanding may be traced to a lack of distinctions between the true inductive event, leading to the appearance of new cell differentiation patterns, and the various kinds of morphogenetic processes which may follow from the activities of these various cell types.

REFERENCES


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