Polarities in the Amphibian Blastula

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ABSTRACT

Two polarities are outlined by the pigment distribution in the surface of the fertilized amphibian egg--an animal-vegetal and a dorso-ventral polarity. A number of observations bearing on the mechanisms by which these polarities exert their function are presented here. They show that the animal-vegetal polarity acts by controlling the differentiation of the embryonic cells, whereas the dorso-ventral polarity ensures that the vegetative cells differentiate along a dorso-ventral temporal gradient.

In order to impose bilateral symmetry on a sphere a median plane must be defined. This may be achieved by any pair of lines (vectors) passing from the surface to the center. The amphibian egg is a sphere, the gastrula possesses bilateral symmetry, and we may therefore conclude (Løtrup and von Sydow, 1974) that two polarities must reside in the early embryo. In fact, both of these polarities are well known. The first of them, imposing radial symmetry on the egg, is visualized in the unfertilized egg through the distribution of pigment in the cortex; the center of the pigmented area defines the animal pole, the center of the unpigmented area the vegetal pole. The animal-vegetal axis outlines the animal-vegetal- or apical-polarity. This polarity is determined by the orientation of the egg in the ovary, the side turned towards the ovarial wall is the unpigmented, vegetal side.

The other polarity becomes visible when, shortly after fertilization, the pigment border rises at one side of the embryo, thus forming the grey crescent. This event defines a dorso-ventral- or median-polarity passing through the middle of the grey crescent area. The direction of the median polarity is determined by the fertilizing sperm the elevation of the pigment occurs in the median plane determined by the entrance point, but on the opposite, the presumptive dorsal side of the embryo.

Together these two polarities determine that blastopore formation, and hence gastrulation, begins in the median plane in the dorso-vegetal quadrant of the blastula.

The sphere with its vectors may be regarded as a model of early embryogenesis. If so, I shall raise a question which, I believe, is asked far too seldom; what are the implications of, what can we learn from, this model?

In the sequel we shall discuss this issue for each of the polarities, but first we shall consider their material substrates.

The Material Substrates

It has been known for a long time that the formation of the blastopore, outlined by pigment accumulation in the embryonic surface, is accomplished by the so-called Ruffini's flask cells. The latter, anchored by long necks in the blastoporal groove, are engaged in pulling the embryonic surface into the interior of the embryo.

The process of gastrulation, the orientation of which is determined by the two polarities, is thus concerned with the process of cell differentiation giving rise to the formation of the Ruffini cells. The polarities are particularly concerned with the spatial location of these cells, the apical polarity determines that they shall be situated in the vegetal hemisphere, and the median polarity that they shall prevail at the dorsal side. That the differentiation subsequently spreads around the circumference of the embryo is evidently a result predictable from the contribution of the apical polarity.

It has been suggested by Dalcq and Pasteels (1937) that materially the apical polarity is bound to the cytoplasm, and the median polarity to the cortex. This claim should be testable by experiments designed to dislocate the polaraties: off-hand one should expect a cortical polarity to resist such endeavours, while a cytoplasmic polarity might well be quite labile. In the egg the cytoplasmic composition varies along the animal-vegetal axis such that the ratio hyaloplasm/yolk is gradually diminished. Under the influence of gravity this distribution will be upset when the eggs are turned upside down, consequently the direction of any prevailing cytoplasmic polarity should be inverted. Clearly, if the theory of Dalcq and Pasteels was correct, the formation of Ruffini cells, and hence of the blastopore, should occur in the animal hemisphere turned downwards.

Inversion experiments of this kind were first made by Schultze (1894),

who squeezed embryos between two glass plates to prevent them from assuming their normal orientation. We shall not discuss these old experiments, but rather concentrate on the results published by Penners and Schleip (1928), who continued and extended the work of Schultze.

Like Schultze these authors observed many cases of double embryos but this may be and probably is, the outcome of interference with the median polarity. Further they recorded that two thirds of all observed blastopores in inverted embryos arose in the region of the grey crescent, but the remaining ones might occur at any part of the surface and in any direction relative to the original plane of symmetry This does not mean, however, that a blastopore would be formed in the normally pigmented animal surface, for it was observed that in almost every case the invagination occurred at the margin of an unpigmented yolk-containing area of the surface. In other words, whenever a blastopore arose in the animal hemisphere, the pigment had partly been replaced by yolk.

These results show that it is very difficult to invert the apical polarity. The circumstance that invagination tends to remain located near the original grey crescent in spite of the redistribution of the cytoplasm rather suggests a cortical connection. On the other hand, the cases where dislocation of the blastopore took place seem to suggest an influence exerted by the yolk. The available evidence thus does not allow for a precise location of the material substrate of the polarity.

The determination of the median polarity is much more labile, and its direction may be changed by a variety of expedients. One of these implies a forced rotation of the egg (Pasteels, 1946; Ancel and Vintemberger, 1948; Ubbels, 1980). If an unfertilized egg is exposed to this treatment, then the plane of rotation will define a median plane, as demonstrated by the

subsequent formation of a grey crescent. In fertilized eggs repeated rotations are required before the directive influence of the fertilizing sperm is overcome. In accordance with the original observations by Schultze, rotations may also in some instances give rise to double embryos.

Attempts to invert the median polarity, by applying temperature gradients to amphibian embryos before gastrulation has begun, were first made by Huxley (1927). These experiments and similar ones performed by others gave rise to various developmental abnormalities, for instance duplication of the neural plate. It was later shown by Glade, Durrill and Falk (1967) that complete inversion of the median polarity may be achieved if the temperature gradient is applied from the outset of development.

Similar results have been obtained by a different, but clearly related method, unilateral constriction of the oxygen supply. It was first observed (Løvtrup and Pigon, 1958) that if an axolotl embryo is placed in a tight-fitting glass-tube (Figure 1), blastopore formation will always begin at the oxygenated side.

As these experiments were resumed recently with Xenopus embryos (Landström and Løvtrup, 1975), it was found that when the ventral side of an embryo is turned outwards development is retarded by the amount of time normally required for the blastoporal groove to reach the ventral side. Otherwise there are no significant differences between the experimental embryos, but as is shown by Figure 2, the development in the anterior part of the body is retarded compared to that of the controls. It is thus possible to invert the polarity by 180° through unilateral restriction of the oxygen supply.

Possibly the most conspicuous result obtained through this experimental approach is the finding that when a ventral half-embryo, which normally does

not develop, is confined in a closed glass tube, then the formation of an embryonic axis is imposed upon the embryo, even though the anterior part of the embryo does not develop normally (Figure 3). It thus appears possible not only to invert an already prevailing median polarity, but to create one de novo through unilateral restriction of the oxygen supply.

These several observations permit a number of important conclusions concerning the material substrate of the median polarity. The association of the latter with the grey crescent might suggest that the polarity is bound to the cortex. This inference is not ruled out by the rotation experiments, since these may result in the formation of a grey crescent.

The temperature gradient experiments show that the function of the median polarity is based on activity differential along the dorso-ventral axis, and the oxygen restriction experiments demonstrate that this activity is associated with the oxidative metabolism.

These results, as well as those obtained in the rotation experiments, rule out that the polarity can be determined by a chemical substance, an "organizer", residing in the dorsal cortex. The experiments devised by Curtis to demonstrate this tenet were therefore refuted in advance. It is gratifying that Ubbels (1980) has been able to forward an acceptable explanation of Curtis' results.

The Function of the Apical Polarity

The studies on the material basis and the reorientation of the polarities have given important information, but they have contributed little to the understanding of the way the polarities exert their function.

In fact, it seems that our knowledge has for a long time sufficed to solve this problem by pure reason without making a single experiment. We have seen

that blastopore formation is the work of the Ruffini cells, and we furthermore know that the blastoporal groove spreads in a radially symmetrical fashion around the circumference of the egg.

Hence the inference that the apical polarity is responsible for the formation of Ruffini cells in the vegetal, and their absence in the animal hemisphere. From the classical induction experiments we even know what to expect in the latter case, namely, epidermal cells.

This deduction has been tested experimentally by observing the differentiation of cells explanted from the axolotl blastula (Løvtrup, Landström and Løvtrup-Rein, 1978; Landström and Løvtrup, 1979). The results obtained show that the blastula can be subdivided in three regions along the animal-vegetal axis: (1) the animal hemisphere, the cells of which differentiate into ciliated epidermal vesicles (Plate 1a), (2) most of the vegetal hemisphere from which explants give rise to an outgrowth of large yolk-laden cells (Plate 1b) which, on closer inspection, are seen to possess numerous filopodia by means of which they are anchored to the surface of the culture dish (Plate 1c); this must be Ruffini cells. (3) The cells closest to the vegetal pole - the circumpolar cells - form irregular aggregates covered by a coat (Plate 1d and 1e). Upon dissociation of these it is found that they have retained the spherical shape typical of embryonic cells. It thus seems that these cells remain undifferentiated, a finding which in fact corroborates various observations in the classical embryological literature. There is good reason to presume that the cover of these aggregates is Holtfreter's surface coat (Holtfreter, 1943).

Our prediction is thus corroborated and we know the function of the apical polarity on the cellular level. Important questions remain on the subcellular level of organization. First among these is the problem: What

influences the animal and vegetal cells to differentiate in disparate direction?

This question can be phrased more concisely, for as I shall show briefly, the animal cells may be induced to assume the differentiation pattern represented by the Ruffini cells, but uninduced they are prevented from doing so. This means that in the animal cell, presumably in the cytoplasm, there is a factor which prevents them from differentiating like the Ruffini cells. What is the nature of this factor? This question seems to be a cardinal problem in present-day developmental biology.

Proceeding with our logical reasoning and recalling that invagination of the Ruffini cells is a precondition for the induction of a neural plate, we may make a very important deduction: At the cellular level the Ruffini cells must be the primary inductor.

In fact, in mixed explants, containing both animal and vegetal cells, we observe the formation of cells similar to the Ruffini cells, except that they are smaller and contain pigment rather than yolk (Plate 1f and 1g). Apparently, the Ruffini cells have acted as homotypic inductors, impossing their differentiation pattern upon the animal cells.

On subsequent days the latter differentiate further into mesenchyme cells, nerve cells, melanophores and xanthophores, a spectrum typical of the neural crest.

The same result may be obtained if we add LiCl (10 mM) or heparan sulphate (0.1 - 1 ppm) to the cultures. Since we know that heparan sulphate is synthesized in the early embryo (Kosher and Searls, 1973: Hoglund and Lϕvtrup, 1976), and since heparan sulphate is a typical constituent of fibroblast-like cells (Kraemer, 1971), we have good reason to suspect that we have found the primary inductor, the substance which has been hunted by

embryologists for half a century. Incidentally, when special precautions are taken, the cultured Ruffini cells are seen in the scanning electron microscope to be covered by a rather heavy surface coat (Perris and Løvtrup, unpublished observations). We are at the moment trying to confirm the conjecture that this coat consists of heparan sulphate.

Evidently, if our suggestion is correct, we may infer that no trace of the primary inductor should be detectable before Ruffini cells have appeared, that is, when gastrulation has begun. This point has been corroborated recently by Malacinski, Chung and Asashima (1980).

The Function of the Median Polarity

If we continue our logical reasoning we may also arrive at a solution as concerns the function of the median polarity: it has to impose a dorso-ventral temporal gradient on the cell differentiation process controlled by the apical polarity.

The importance of this gradient becomes evident once it is realized that the process of gastrulation topologically is equivalent to the transformation of a hollow sphere to a double-layered sphere. It is easily appreciated (think of a rubber balloon) that this transformation is only possible if the invagination begins at one point on the surface; if it begins simultaneously around the circumference of the sphere the outcome must be two connected spheres. Thus, if the temporal gradient is suspended in the amphibian embryos, one may anticipate that exogastrulation takes place, leading to the separation of the germ layers. This inference is confirmed by observations made by Holtfreter (1933).

And now we also can understand the result obtained with the ventral halves reported above. In the half embryo in ambient oxygen Ruffini cells are

formed in due course. As indicated by the pigment accumulation in the surface they also attempt to carry out their normal function of pulling the surface into the interior, but as this activity is scattered at random, no invagination occurs. (Figure 3D). When the half embryo is confined in a glass tube the cellular activity is confined to a narrow region of the surface, and invagination is possible.

How can a polarity, presumably bound to the dorsal cortex, impose a temporal gradient on a process of cell division? Following a line of argument developed elsewhere (Løvtrup, 1974; 1980) it may be concluded that the only epigenetic mechanism available to the polarity before gastrulation is cell division. And if this point is accepted it follows that the polarity should be responsible for unequal cell divisions along the dorso-ventral axis. In other words, in the four-cell stage the dorsal blastomers should differ from the ventral ones in one or more parameters, e.g., size, contents or pigmentation. In fact, it is a classical observation that in the amphibian embryos the dorsal blastomeres generally are smaller than the ventral ones.

I shall not here discuss in detail how a cortical polarity can affect the process of cell division. However, it should be mentioned that the observed effect may be accounted for on three assumptions, namely (1) that microtubules from the centres are attached to the cortex during mitosis, (2) that such cortical elements like pigment granules (and also cytoplasmic elements like yolk platelets) may impede their attachment, and (3) that opposing sets of microtubules are in equilibrium when the numbers of counteracting microtubule subunits are identical. The last assumption implies that many short microtubules may balance a smaller number of long ones. On these premises the lower density of pigment on the dorsal side increases the likelihood of microtubule attachment there, and hence the observed disparity in cell size

(cf. Løvtrup, 1974).

We are thus facing the next question, how can smaller cell size entail earlier differentiation?

In order to approach this problem we may first notice that in the amphibian embryo, and in many others as well, the first cleavage divisions occur with great regularity. The cell divisions are often said to be "synchronous" although the synchrony is seldom complete. The phase of synchronous cell divisions, which lasts to about stage 7 in amphibian embryos (Hara, 1977), is distinguished by the fact that the G_1 and G_2 phases are absent or indetectable, and the S and M phases very brief and of the same duration (Graham and Morgan, 1966). As a consequence all the cells are either in the mitotic phase or in the interphase.

This means that the mitotic index, defined as the percentage of cells in the state of division at any one time, is either 100 or 0, and since the two phases are of nearly equal length, the mean index is about 50. During gastrulation 'asynchrony' prevails, the mitotic index falling below 10, and between these two stages a phase of 'desynchronization' occurs, distinguished by intermediate values of the index (Chulitskai, 1970).

It has been observed (Neifach, 1961) that if an early blastula is irradiated with X-rays, development stops before the onset of gastrulation. If the irradiation is performed at the mid-blastula stage or later, gastrulation occurs as usual, but further development is arrested. This shows that in the mid-blastula some events - presumably nuclear since they are sensitive to X-rays - take place, which are necessary for the execution of the process of gastrulation, and thus for the formation of the Ruffini cells. Once this has occurred, irradiation cannot prevent gastrulation, but only later embryogenetic processes.

Detlaff (1964) has pointed out that the loss of sensitivity to X-rays occurs during the phase of desynchronization. This means that the events preparatory to the differentiation of the Ruffini cells occur not later than during desynchronization. And since it is unlikely that the synchronously dividing cells can undergo differentiation, one may submit as a hypothesis that some cytoplasmic factor is present in the early embryo which at the same time sustains synchronous cell divisions and prevents cell differentiation. With time this factor becomes exhausted, and then desynchromization and cell differentiation both occur.

In order to test this proposition it is necessary to know the agents which ensure the early exceptional cell divisions. In this context it may be of relevance that, in spite of the mitotic activity going on, the total amount of deoxyriboside-containing material remains constant during early development in various amphibian species. A new increase occurs only in the mid-blastula (Hoff-Jørgensen and Zeuthen, 1952; Brachet, 1954; Løvtrup, 1955; 1966), thus approximately at the time when desynchroniation begins. The quantity present during the period of constancy lies in the range 0.05 - 0.10 µg, corresponding to several thousand times the DNA contained in a diploid nucleus.

The composition of the deoxyriboside-containing material has remained unknown for a long time, but recently it was shown that the egg of Xenopus laevis contains substantial amounts of the four deoxyribonucleoside triphosphates in approximately equimolar amounts (Woodland and Pestell, 1972). One may therefore envisage that during the period when the deoxyriboside-containing material remains constant the synthesis of DNA involves the utilization of preformed deoxyribonucleotides, and that desynchronization is an indication of their exhaustion.

If this conjecture and Dettlaff's hypothesis are correct then it may be

anticipated that an increase in the amount of cytoplasmic deoxyribonucleosides should have the following consequences:

(1) The period of synchrony should be prolonged. (2) As a consequence of (1) the onset of gastrulation should be delayed or suppressed. This effect should be dependent upon the amounts injected and be greatest when all four nucleotides are included. (3) As we have seen, the occurrence of gastrulation is dependent upon a process of cell differentiation, leading to the formation of Ruffini cells. It may be presumed that this event is preceded by the synthesis of the appropriate messenger RNA. If as the deoxynucleotides suppress cell differentiation, it may be envisaged that they do so by suppressing the protein synthesis associated with cell differentiation, either directly or by inhibiting the synthesis of messenger RNA.

These predicitions which, if true, will be of great importance for our understanding of the mechanism of early development, were tested in various ways.

To test the predicted effect on cell division a solution of deoxynucleotides, containing 0.8 nmoles of each of the nucleotides, corresponding to a total amount of about sixty-four times that present in the normal embryo, was injected into fertilized eggs of Xenopus (Landström, Løvtrup-Rein and Løvtrup, 1975). Control and experimental embryos were fixed, embedded, sectioned and stained by the Feulgen procedure to determine the mitotic index.

The results are given is Table 1. The value of 25 for the mitotic index at stage 9 shows that the cells are in the phase of desynchronization, the embryos have hardly begun to gastrulate. In the stage 11 controls the index has fallen below 10, but in the experimental embryos of the same age the index

is close to that of the stage 9 controls, the difference observed being insignificant. Thus, although the number of cells is significantly higher in the experimental embryos, they still have a mitotic index (21.6), indicating that desynchronization has just begun, whereas the mitotic index in the controls (7.7) is representative of asynchrony (Figure 4). The first of the predictions submitted above has thus been corroborated.

In the experiments aimed at elucidating the effect of the deoxynucleotides on the morphological development, three different concentrations, corresponding to four, sixteen and sixty-four times the amount present in the normal embryo, were injected in fertilized eggs. The effect of omitting one of the four nucleotides was also tested.

The results are shown in Figure 5, where 'arrested development' refers to cytolysis and developmental block. The non-arrested embryos were followed to the tail-bud stage; minor abnormalities were found in some of these. From the figure it appears that the injection procedure itself has little influence on morphological development, whereas the deoxynucleotides clearly induce developmental arrest. The effect is concentration-dependent, and it is greatest when all four nucleotides are injected. The latter point is borne out by the fact the mean value of the developmental arrest with the four mixtures of three nucleotides was 14 ± 1% (when corrected for the blank), whereas the corresponding interpolated value for the same total nucleotide concentration is 35%. The effects of the four mixtures of three nucleotides were closely similar, none of the nucleotides is therefore of special importance for the developmental arrest.

It is remarkable that in no case did we obtain 100% developmental block. The reason for this is probably that the egg cytoplasm is not sufficiently homogenous to ensure an even distribution of the injected nucleotides. But in

any case the second prediction has been corroborated.

The effect on the nucleotides on RNA synthesis is shown in Figure 6, which presents the electrophoretic pattern and the radioactivity distribution of RNA extracted from controls at stage 11 and from embryos of the same age injected with deoxynucleotides. The bulk of the RNA, as measured by UV absorbance at 254 nm, is represented by 28 S and 18 S ribosomal RNA and by transfer RNA. The amounts are similar in controls and in experimental embryos.

The radioactivity profiles, however, exhibit great differences. Very little incorporation occurred into the RNA of the injected embryos, but in the controls are found fractions with high specific activities throughout the gel. The species located between the ribosomal RNA peaks, and those moving ahead of them, have molecular sizes of about 22 S and 10-14 S, respectively. Molecules with similar sedimentation coefficients have previously been found in the early amphibian embryo. Having a DNA-like base composition (Brown and Littna, 1966; Bachvarova, Davidson and Allfrey and Mirsky, 1966) and being readily hybridizable with DNA (Denis, 1968), these fractions have been assumed to represent messenger RNA. The synthesis of these compounds is completely suppressed in the experimental embryos, and this effect of the deoxynucleotides may thus account for the developmental arrest. Hence, even the third prediction could be corroborated.

The results presented here thus suggest that the deoxynucleotides in the fertilized egg serve to carry the embryo through the phase of synchronous cell divisions and that, so long as these substances present, the cells are prevented from engaging in the synthesis of messenger RNA, and hence in cell differentiation. If this supposition is correct we may infer that exhaustion of the deoxyriboside-containing material in the embryo is a prerequisite for

the initiation of cell differentiation. And since the mentioned exhaustion must be dependent upon cell size - the amount of DNA per unit volume is larger in a small than in a large cell - it may be suggested that, by imposing a differential with respect to cell size, the median polarity ensures that the synthesis of messenger RNA, and thus cell differentiation, may begin earlier at the dorsal than at the ventral side.

Conclusion

The use of illustrative models is a general practice in the natural sciences. In the field of developmental biology one of the most famous models is "the epigenetic landscape", devised by Waddington (1957). And as far as the earliest development is concerned, the notions of "gradients" or "polarities" have been in use for a long time.

What do we gain from the use of models? In physics and chemistry this expedient has been extremely useful, because it has permitted the deduction, frequently in mathematical terms, of testable consequences of the theory embodied by the model. In this way the models have been of great importance for the progress within these sciences.

The situation is less felicitous in biology in general, and particularly in developmental biology. I do not recall any attempts to test the empirical implications of the epigenetic landscape, nor do I believe that, not withstanding their manifest existence, the notion of embryonic "gradients" has been used to devise experiments which have contributed to a deepened understanding of the nature and the function of those biological entites to which our models apply.

In making this observation I believe having put my finger on a weak point in biological tradition, namely that of being satisfied once a particular phenomenon has been named. This, of course, is a practice of long standing in the world of systematics, but it does not suffice in developmental biology, where we want explanations rather than descriptions.

Let us return to the "gradients" and "polarities". These words have been used rather indiscriminately for the phenomena observable in early embryos, and of late another concept has been added: "positional information". I believe that positional information is an obvious implication of a gradient as

well as of a polarity, and conclude that the additional concept has enlarged the vocabulary of trade, but not our understanding.

For a long time I have consistently talked of "polarity" where many others would use "gradient". I think that the work reported above may serve to substantiate my opinion. As we saw, the apical-or animal-vegetal-polarity is controlling the alternative between two particular patterns of cell differentiation, an either-or choice; in this case the concept of "gradient" is clearly inappropriate, "polarity" being preferable.

If "polarity" is thus accepted as the more general term, it is by no means excluded that a polarity may function gradientwise. As we have seen, this is the case with the median-or dorso-ventral-polarity, which acts by imposing a temporal gradient on the formation of the Ruffini cells.

I believe that the particular message of the present paper is that when we endeavour to obtain experimentally information regarding the nature of our models, in cas the polarities, it is extremely important to opt for working at the proper level of organization. If, as observed here, the polarities are concerned with processes of cell differentiation, then we must work on the cellular level if we want to get unambiguous results. The relative failure of many of the earlier studies on these problems depends, in my opinion, on the fact that the work usually was carried out with rather large explants, thus tending to obscure the proper interpretation of the results.

I mentioned above that models may facilitate the logical deduction of the implications of our theoretical notions about Nature. This brings me to another point where the present state of biology is defective. The logical deductions employed in the work described here were in most cases rather simple, but as development proceeds, everything becomes more complex and the chains of deduction long and impervious.

Yet, if we choose to follow the path staked out by Popper (1963), we must do this tedious brain work before we set out to make experiments. For the value of an experiment depends upon the importance of the theory which it is meant to test.

I think a common error committed by experimental biologists is that their work is designed to test theories of trifling substance, or none whatsoever. In this way biology is once more reduced to be a descriptive, rather than an explanatory, science.

References

Ancel, P. and Vintemberger, P. (1948). Recherches sur le determinisme de la symetrie bilaterale dans l'oeuf des amphibiens. Bull. Biol. France Belg, Suppl 31, 1-182.

Bachvarova, R., Davidson, E.H., Allfrey, V.G. and Mirsky, A.E. (1966). Activation of RNA synthesis associated with gastrulation. Proc. Nat. Acad. Sci. USA, 55, 358-365.

Brachet, J. (1954) Constitution anormale du noyau et metabolisme de l'embryon chez les Batraciens. Arch. Biol. 65, 1-72.

Brown, D.D. and Littna, E. (1966) Synthesis and accumulation of low molecular weight RNA during embryogenesis of Xenopus laevis. J. Mol. Biol. 20, 96-112.

Chulitskaia, E.V. (1970) Desynchronization of cell division in the course of egg cleavage and an attempt at experimental shift of its onset. J. Embryol. Exp. Morphol. 23, 359-374.

Curtis, A.S.G. (1962). Morphogenetic interactions before gastrulation in the amphibian, Xenopus laevis - Regulation in blastulae. J. Embryol. Exp. Morphol. 10, 451-463.

Dalcq, A. and Pasteels, J. (1937). Une conception nouvelle des bases physiologiques de la morphogenese. Arch. Biol. 48, 669-710.

Dettlaff, T.A. (1964). Cell divisions, duration of interkinetic states and differentiation in early stages of embryonic development. Adv. Morphogen. 3, 323-362.

Denis, H. (1968). Role of messenger ribonucleic acid in embryonic development. Adv. Morphogen. 7, 115-150.

Glade, R.W., Durrill, E.M. and Falk, R.J. (1967). The influence of a temperature gradient on bilateral symmetry in Rana pipiens. Growth. 31, 234-249.

Graham, C.F. and Morgan, R.W. (1966). Changes in the cell cycle during early amphibian development. Develop. Biol. 14, 439-460.

Hara, K. (1977). The cleavage pattern of the axolotl egg studied by cinematography and cell counting. Roux Arch. Develop. Biol. 181, 73-87.

Hoff-Jørgensen, E. and Zeuthen, E. (1952). Evidence of cytoplasmic deoxyribosides in the frog's egg. Nature. 169, 245-246.

Hoglund, L.R. and Løvtrup, S. (1976). Changes in acid mucopolysaccharides during the development of the frog Rana temporaria. Acta Embryol. Exp. 1976, 63-79.

Holtfreter, J. (1933). Die totale Exogastrulation, eine Selbstablosung des Ectoderms vom Entomesoderm. Entwicklung und funktionelles Verhalten nervenloser Orgone. Arch. Entw. mech. 129, 669-793.

Holtfreter, J. (1943). Properties and functions of the surface coat in amphibian embryos. J. Exp. Zool. 93, 251-323.

Huxley, J.S. (1927). The modification of development by means of temperature gradients. Arch. Entw. mech. 112, 480-516.

Kosher, R. and Searls, R. (1973). Sulfated mucopolysaccharide synthesis during the development of Rana pipiens. Develop. Biol. 32, 50-68.

Landström, U. and Løvtrup, S. (1975). On the determination of the dorso-ventral polarity in Xenopus laevis embryos. J. Embryol. Exp. Morphol. 33, 879-895.

Landström, U. and Løvtrup, S. (1979). Fate maps and cell differentiation in the amphibian embryo. - An experimental study. J. Embryol. Exp. Morphol. 54, 113-130.

Landström, U. Løvtrup-Rein, H. and Løvtrup, S. (1975). Control of cell division and cell differentiation by deoxynucleotides in the early embryo of Xenopus laevis. Cell. Diff. 4, 313-325.

Løvtrup, S. (1955) Chemical differentiation during amphibian embryogenesis. Compt. Rend. Lab. Carlsberg, Ser. Chim. 29, 266-314.

Løvtrup, S. (1966) DNA synthesis during the development of Rana temporaria. Exp. Cell Res. 43, 65-71.

Løvtrup, S. (1974). Epigenetics-A Treatise in Theoretical Biology. Wiley, London.

Løvtrup, S. (1980) The epigenetic utilization of the genomic message.

Løvtrup, S. Landström, U. and Løvtrup-Rein, H. (1978). Polarities, cell differentiation and primary induction in the amphibian embryo. Biol. Rev. 53, 1-42.

Løvtrup, S. and Pigon, A. (1958). Inversion of the dorso-ventral axis by unilateral restriction of oxygen supply. J. Embryol. Exp. Morphol. 6, 486-490.

Løvtrup, S. and Sydow, B. von (1974). D'Arcy Thompson's theorems and the shape of the molluscan shell. Bull. Math. Biol. 36, 567-575.

Malacinski, G.M., Chung, H.-M. and Asashima, K. (1980). The association of primary embryonic organizer activity with the future dorsal side of amphibian eggs and early embryos. Develop. Biol. 77, 449-462.

Neifach, A.A. (1961). Vergleichende strahlenbiologische Untersuchung der morphogenetischen Funktion der Kerne in der Entwicklung der Tiere. Sowjet-wissenschaft liche Beitrage. 8, 828-845.

Pasteels, J. (1946). Sur la structure de l'oeuf insegmente d'axolotl et l'origine des prodromes morphogenetiques. Acta Anat. 2, 1-16.

Penners, a. and Schleip, W. (1928). Die Entwicklung der Schultze'schen Doppelbildungen aus dem Ei von Rana fusca. Z. Wiss. Zool. 130 305-454.

Popper, K.R. (1963). Conjectures and Refutations. Routledge and Kegan Paul, London.

Schultze, O. (1894). Die kunstliche Erzeugung von Doppelbildungen bei Froschlarven mit Hilfe abnormer Gravitationswirkung. Arch. Entw. mech. 1, 269-305.

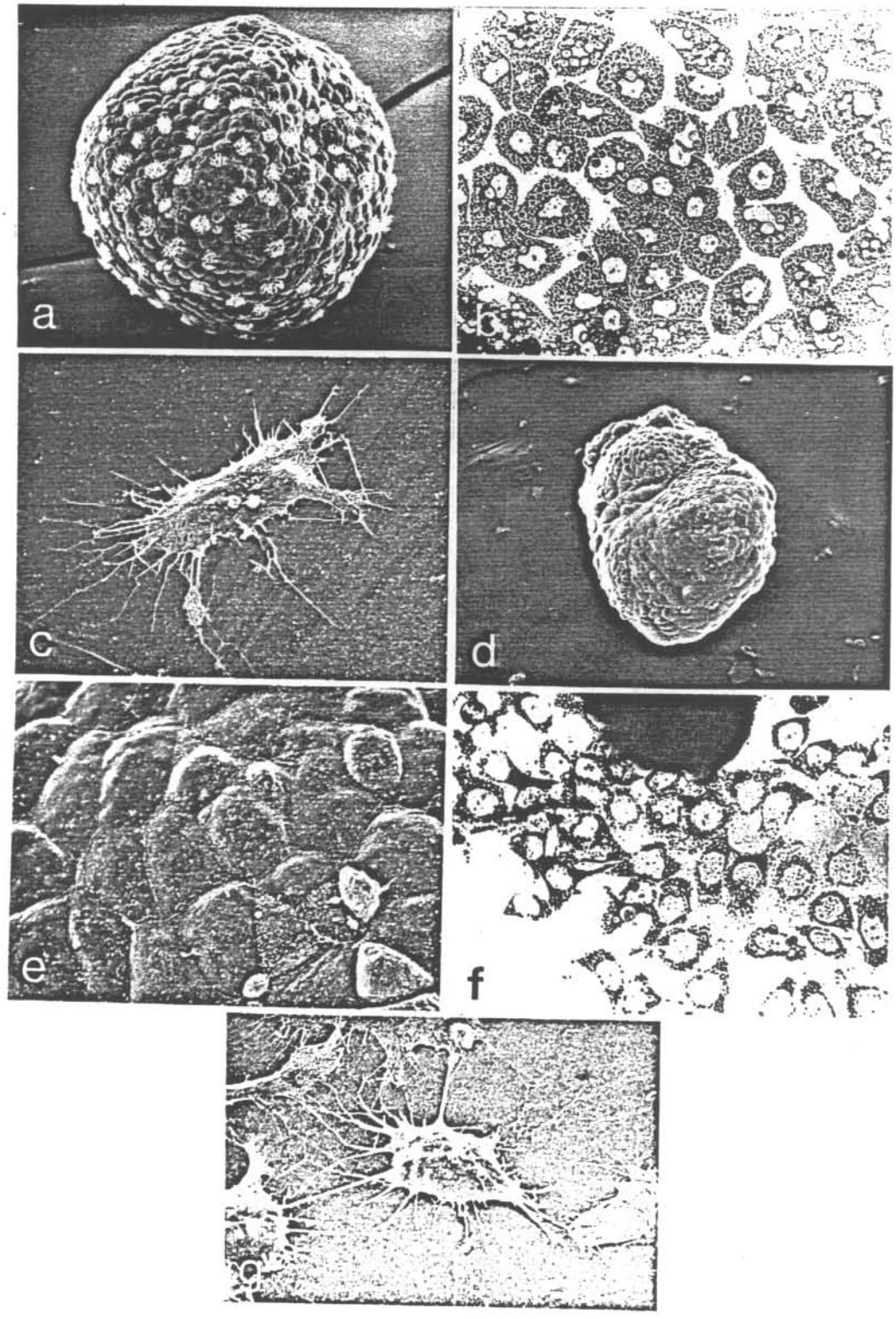
Ubbels, G.A. (1980). Egg rotation in Xenopus laevis, with special reference to the determination of dorso-ventral polarity Poster session (abstract), Conference in Padras, Greece, 1980.

Waddington, C.H. (1957). The Strategy of the Genes Macmillan, New York.

Woodland, H.R and Pestell, R.Q.W. (1972). Determination of the nucleoside triphosphate contents of eggs and oocytes of Xenopus laevis. Biochem. J. 127, 597-605.

Legend to Plate 1

All micrographs have been taken from cultures of Ambystoma mexicanum cells. (a) Vesicular aggregate composed of ciliated and non-ciliated epidermal cells. (150x). (b) Explanted endodermal cells, distinguished by the presence of yolk and absence of pigment (150x). (c) A cell of the kind shown in (b), showing the formation of numerous filopodia (750x). (d) Aggregate formed by cells near the vegetal pole (75x). (e) Closer view of (d), showing that the cells are covered by a coat blurring the cell contacts (750x). (f) Induced animal cells distinguished by the presence of pigment and absence of yolk. (150x). (g) A cell of the kind shown in (f) (750x). A comparison of (b) with (f) and (c) with (g) shows that we are here dealing with the same type of cell. From Løvtrup, Landström and Løvtrup-Rein (1978) and Landström and Løvtrup (1979.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Captions

Figure 1. Unilateral restriction of the oxygen supply to an embryo is accomplished by sucking it into a narrow glass tube. From Løvtrup & Pigon (1958).

Figure 2. Appearance of embryos of Xenopus laevis after 50 h confinement and 46 h in ambient oxygen. (A) Dorsal side initially turned outwards, stage of head region 32-34, stage of tail region 41-42. (B) Ventral side initially turned outwards, stages approximately as in (A). (C) Control, stage 41-42. From Landström & Løvtrup (1975).

Figure 3. Appearance of embryos of Xenopus laevis 65 h after fertilization. (A) Control, stage 33-34. (B) Dorsal half-embryo, stage 33-34. (C) Ventral half-embryo developed inside capillary tube. (D) Ventral half in ambient oxygen. The experimental embryos have had free access to oxygen for 15 h. From Landström & Løvtrup (1975).

Figure 4. The effect of injected deoxynucleoside triphosphates on cell division in Xenopus laevis embryos. Median sections of a) a control embryo (stage 11, 10 hr at room temperature) and b) an embryo of equal stage, injected with 64 times the normal amount of deoxyriboside triphosphates at the time of the first cleavage division. The fine dots represent interphase and prophase nuclei, the heavy dots metaphase, anaphase and telophase nuclei. Four subsections are indicated by stippled lines. From Landström, Løvtrup-Rein and Løvtrup (1975).

Figure 5. The effect of injected deoxynucleoside triphosphates on the morphological development of Xenopus laevis embryos. (a) Controls; (b) 7.5% amphibian Ringer's solution, a control of the

injection method; (c-i) Ringer's solution with added deoxynucleotides; (c) 4 x; (d) 16 x; (e) 64 x; (f) 16 x minus dATP; (g) 16 x minus dCTP; (h) 16 x minus dGTP; (i) 16 x minus TTP. The figure above each column represents the number of injected individuals, the figure inside the column the non-arrested ones. The hatched area represents the arrested embryos. From Landström, Løvtrup-Rein and Løvtrup (1975).

Figure 6. The effect of injected deoxynucleoside triphosphates on RNA synthesis in Xenopus laevis embryos. (a) Controls (stage 11, 10 h at room temperature; (b) embryos of equal age, injected with deoxynucleotides (64x). UV absorbance (-); radioactivity (...). The embryos were incubated in 7.5% Ringer's solution prepared with $NaH^{14}CO_3$ (5 µCi/ml). Subsequently they were rinsed with unlabelled medium and homogenized in 2-3 ml 0.01 M tris buffer, pH 7.4, containing 0.5% SDS, 0.5% naphthalene-1,5-disulphonate and 20 µg/ml polyvinylsulphate. RNA was extracted with 86% phenol containing 0.01 M tris buffer, pH 7.4, 0.5% SDS and 0.1% hydroxyquinoline, and DNA removed as described by Løvtrup-Rein (1970). The RNA was fractionated by electrophoresis on acrylamide-agarose gels and the radioactivity determined by scintillation counting. From Landström, Løvtrup-Rein and Løvtrup (1975).

Table 1. *Influence of injected deoxyribonucleotides on the mitotic index in embryos of* Xenopus laevis.
From Løvtrup, Landström and Løvtrup-Rein (1978).

	Hours after fertilization	Stage	Animal subsection	Dorsal subsection	Ventral subsection	Vegetal subsection	Whole section	Total no. of nuclei in all sections
Controls	7	9	27·2 (2·5)	27·0 (1·7)	27·7 (2·4)	19·6 (1·5)	25·3 (2·0)	634
	8·5	10	11·4 (0·5)	11·3 (0·2)	10·8 (1·0)	8·9 (1·0)	10·7 (0·6)	805
	10	11	7·9 (0·7)	8·1 (1·0)	8·6 (0·7)	6·6 (1·2)	7·7 (0·5)	948
Experiments	10	—	20·7 (2·5)	23·7 (1·0)	25·4 (1·9)	19·0 (4·6)	21·6 (2·3)	1115

Each value represents the mean of six median sections (three embryos per stage and two sections per embryo). The standard errors of the means are given in parentheses. The embryos were kept at room temperature during the experiment. Into each experimental embryo, newly fertilized, was injected 30 nl Ringer's solution containing 0·8 nmoles of each of the four natural deoxynucleoside triphosphates. At appropriate stages the embryos were decapsulated and fixed for 48 h in Smith's fluid (one change), and rinsed in dilute formalin for 3 days. They were then dehydrated, embedded in paraffin, sectioned at 12 μm, and stained by the Feulgen reaction after hydrolysis in 1 N HCl. Nuclear counts were made on drawings of the sections obtained by means of a drawing tube attached to the microscope. Only metaphase, anaphase and telophase nuclei were rated as mitotically active.

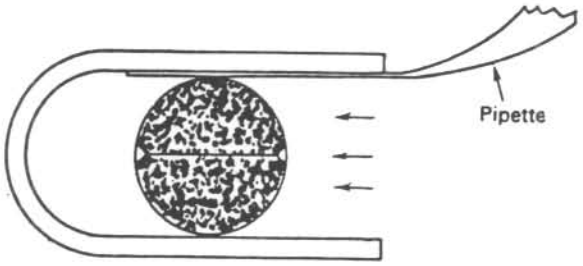

Figure 1

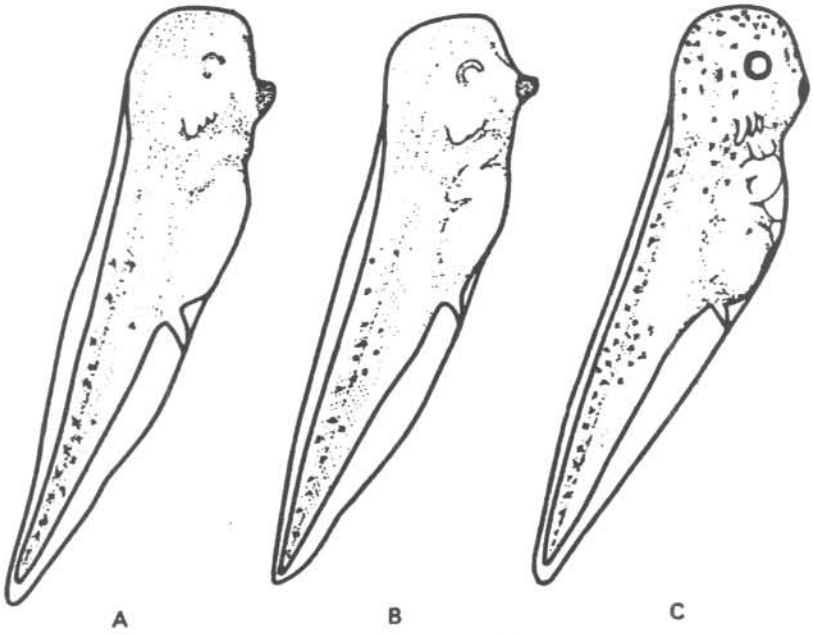

Figure 2

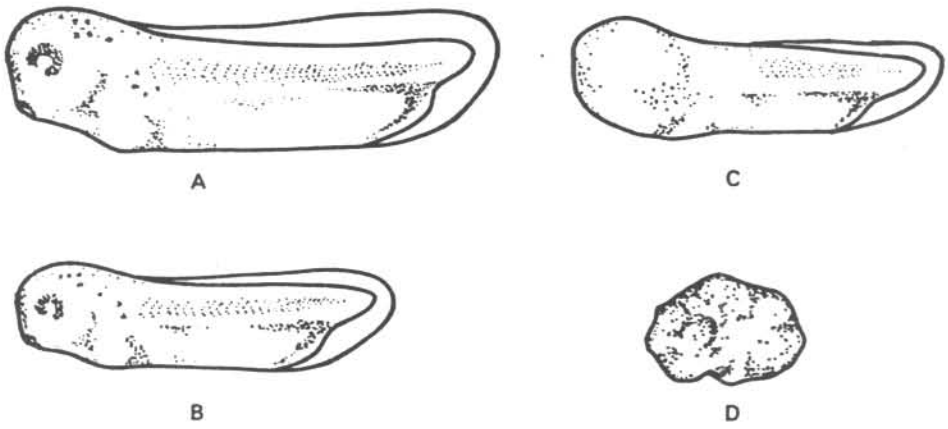

Figure 3

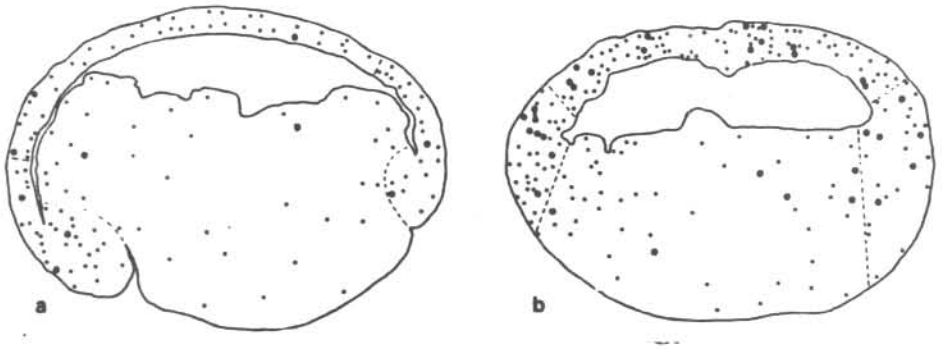

Figure 4

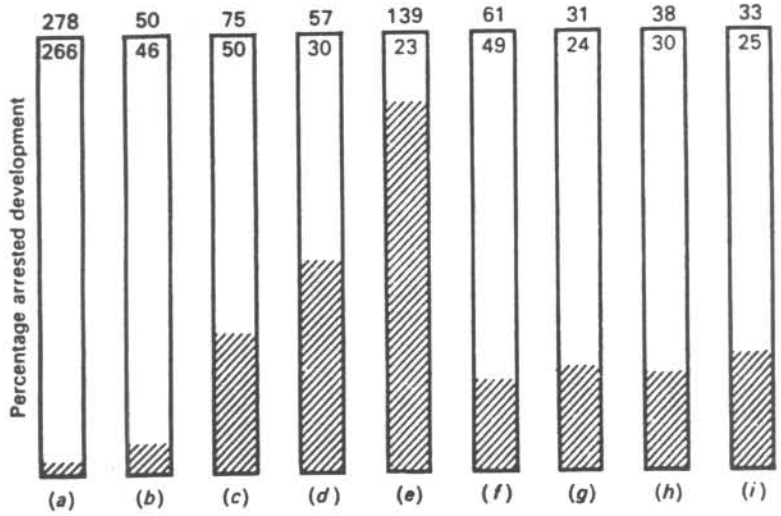

Figure 5

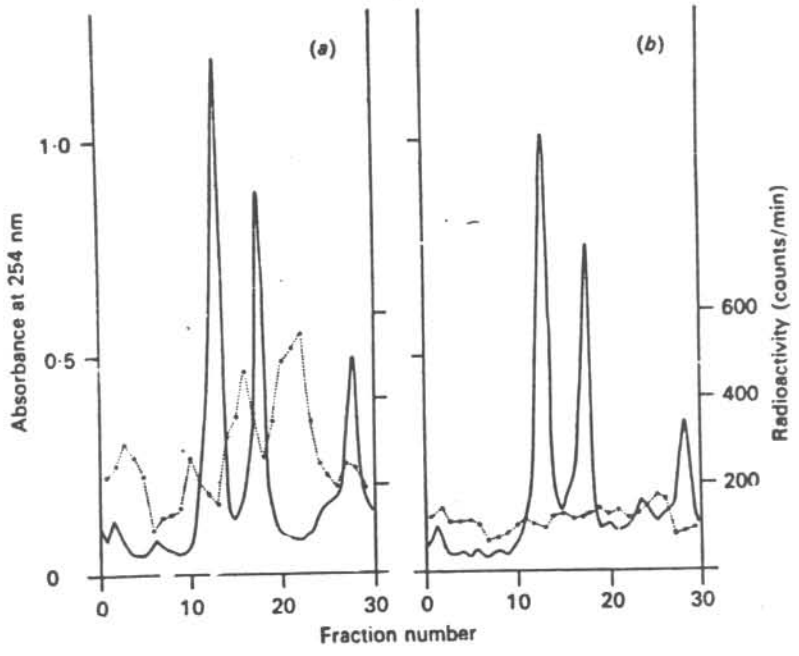

Figure 6