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Autor: Løvtrup, Søren

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possessing the f-property, and as we have seen above, it may even accelerate the rate of cell transformation. This acceleration is offset by a slight retardation of development, so no major change in the timing of the onset of synthesis may probably be anticipated, but a distinct enhancement of the rate of synthesis might be expected. Experiments show that the rate and extent of synthesis is unchanged. If one wishes to avoid any specific mechanism to be involved, I think that this result can be explained only by assuming interference with energy supply and utilization of reserve materials, as discussed above.

VII. Conclusion

1. Morphogenesis and phylogenesis

As has been discussed very briefly in the present paper morphogenesis is the outcome of the interplay, active or passive, between a limited number of different cell types. The egg cell, as well as the early blastomeres, belong to one and the same class, cells of the other types derive from the original ones through transformation processes. Of these there seems to be only two, consequently there can be only four different basic cell classes. All other kinds of cells may be regarded as further differentiations of each of these four types. It was shown above that the polarities of the sea urchin embryo determine the cell type distribution, and the same seems to hold for the amphibian embryo (LØVTRUP 1966). The primary morphogenesis can be considered the resultant of the interaction of these four cell types, and a few extracellular structures (cf. LØVTRUP 1965 a–c).

There are a number of interesting conclusions to be derived from the views presented here. BALDWIN (1937) stated: "Biologists have from time to time been impressed by the fact that the members of the animal kingdom fall into a relatively small number of types, in spite of a considerable degree of variation within each type ..." (l.c. p. 104). If the basic morphogenetic events, which of course must influence the pattern of all subsequent development, are determined mainly by the activity of these four cell types, then it seems obvious that the possible number of variations must be quite low. On the other hand, variations within each group of animals presumably is a result of differential protein synthesis, and here the possibilities are almost unlimited.

Another consequence is that phylogenetic evolution must largely be a result of changes in the cell distribution pattern during early embryogenesis. The first animal cell must have been a solitary cell, an amoeba or an amoebocyte. This archaeic cell is, to this very day, represented by each egg cell. The solitary amoeba represents, from an evolutionary point of view a blind alley; only when this cell had acquired the possibility to transform to other cell types were new roads open. The first new cell type which arose apparently was the epitheliocyte, probably in the flagellate form. The reversible transformation amoebocyte $\rightleftarrows$ flagellate can be observed in certain protozoa (cf.

WILLMER 1960). The flagellate is also a solitary cell, but the epitheliocyte exists in another form which is adhesive, often also ciliated or flagellated. With this cell type it is possible to build up multicellular structures, one of the simplest form being a spherical hollow body. If the cell transformation be reversible, cells may enter the cavity in the form of amoebocytes.

This form is also, unless new devices are invoked, a blind alley. This is maybe best illustrated by the stereoblastula, a structure which may arise if, in the sea urchin embryo, the formation of mechanocytes be suppressed by animalizing agents. Under these circumstances all amoebocytes may transform into ciliated epitheliocytes; no further development is consequently possible. It is not possible here to trace the various morphogenetic mechanisms which have allowed to avoid the formation of stereoblastulae in the various invertebrates, but we may dwell a moment at the sea urchin embryo. Here are two conspicuous traits of utmost morphogenetic importance. The first one is the hyaline membrane, a structure which is typical for amoebocytes (cf. WOHLFARTH-BOTTERMAN 1960); strong adhesion obtains between the cell surface and this extracellular membrane. It seems that this force may be overcome during cell division when the cells exceed a certain size, presumably spatial factors are mainly involved in this mechanism. The separation between the cells and this supracellular structure is of great morphogenetic significance, allowing the formation of a blastula, in its absence a number of solitary amoebocytes would arise from the subsequent cell divisions. The only possibility for formation of a multicellular structure under these conditions would be the transformation of the amoebocytes to epitheliocytes, but this would lead to a solid aggregate of cells, or at best to a stereoblastula.

The other trait is the apical formation of a new cell type, the mechanocytes, which through their pseudopodal activity can accomplish the invagination of the endoderm (GUSTAFSON 1961). At the time this happens most other cells seem to have become immobilized by the acquisition of epitheliocyte properties.

This kind of invagination is possible only in quite small eggs, for obvious spatial reasons. In larger eggs primary invagination results from the activity of mechanocytes, but the following event, the epibolic movements, results from the apposition of two layers of amoebocytes (cf. LØVTRUP 1965b). A prerequisite for this type of gastrulation is obviously that the transformation amoebocyte→epitheliocyte is delayed relative to the formation of mechanocytes. Another requirement is that the primary invagination, in contrast to that in the sea urchin egg, occurs outside the polar region, implying that it is bilaterally symmetrical. The factors determining the site of invagination in the amphibian embryo have been discussed in a recent paper (LØVTRUP 1965a).

The epibolic movements, involving intimate contact between cells of different types, since some of the invaginating cells are sf-cells, is the prerequisite for superficial cells transforming into the mechano-epitheliocyte

type (cf-cells), and the formation of this cell type in the embryonic surface is again a condition for the formation of a nervous system. The presence of mechanocytes able to produce elastic membranes is necessary for notochord formation, and thus for longitudinal stretching. The anchoring of the notochord to the neural plate by mechanocytes (the neural keel cells) allows for the enlargement of the brain, cf. Amphioxus, in which this attachment does not occur. I believe these few examples suffice to illustrate the kind of changes in cell transformation etc. which has made evolution possible (a more detailed discussion is to be found in LØVTRUP 1965b).

2. Biochemistry and phylogenesis

It seems possible to distinguish two phases in the history of the young branch of science, chemical embryology, specially with respect to the question about energy supplying mechanisms. At a certain stage it was observed that the processes obtaining in eggs and early embryos in various ways differed from those found in adult tissues, thus the difficulties associated with the demonstration of phosphorylation in embryos at a certain time led to the belief that lack of phosphorylation was typical for embryonic metabolism (cf. NEEDHAM 1942).

Later observations showed this view to be erroneous, and subsequently the contention was spreading that no significant differences exist between embryonic and adult energy metabolism. The various attempts to demonstrate glycolysis and cytochrome c in the sea urchin egg must be regarded as expressions of this opinion (cf. ROTHSCHILD 1956).

I have tried to show in the present paper that this standpoint may be wrong. Our present scanty knowledge of comparative biochemistry shows that on the chemical level there is a recapitulation of phylogenesis during ontogenetic development, and if we can extrapolate this principle back to the egg cell then we must expect this to represent a very early stage in animal evolution, viz., a solitary amoeboid cell. The closest relatives to the egg cell must thus be found among present day protozoa.

It is therefore interesting that the counterpart to a number of peculiar biochemical traits in the sea urchin egg concerning carbohydrate, and possibly also nucleic acid metabolism, as well as the content of phosphagen and various enzymes, has been observed in protozoa.

As during the development the original amoebocytes are transformed into other cell types these primitive features disappear and are replaced by others known from the tissues of metazoa. Thus the primitive, rather inefficient mitochondria disintegrate, and the extramitochondrial cytochrome oxidase as well as the enzymes associated with the special oxidative glucose localization disappear. Instead new types of mitochondria are produced, together with enzymes specific for the new cell types arising. There is no question that this comparative biochemical study could be extended to other features than those discussed here, to mention only one case I would like to point to the

observations of BÄCKSTRÖM (1956, 1957) that ascorbic acid increases during development and that it is higher in vegetalized and normal embryos than in animalized ones. This observation may bear some relation to the observation that this compound is a specific growth requirement for Trypanosomas, since these as suggested above may be protozoa which possess the f-property, being cf-cells. Ascorbic acid may also promote the growth of other protozoa (amoebae and flagellates, etc.) but in this case it may be replaced by other reducing agents (LWOFF 1951). I shall not discuss the implications for biochemical evolution inherent in the metabolic peculiarities of the sea urchin egg except by mentioning that glycolysis in animal development apparently does not represent the most primitive metabolic pathway for glucose utilization.

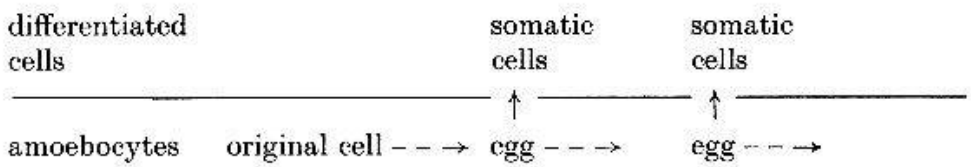
The thesis that an egg cell to all measures and extent is a very primitive cell may appear unlikely and incredible in view of the innumerable mutations which have occurred during animal evolution. Even if it can be stated with confidence that most of these mutations have been concerned with the synthetic capacities of differentiated cells at later stages of development, the possibility remains that certain mutations have been of direct influence upon the properties of the egg cell. About this there can be no doubt, the morphogenetic importance of such changes were discussed in the preceding chapter. Also on the chemical level changes might occur; I do not think it is entirely impossible to imagine that for instance cytochrome c might be present in other eggs than those af the sea urchin, claims to this have certainly been advanced (cf. ROTHSCHILD 1956).

However, there seems to be a limit to the extent of such changes, if this is transgressed ontogenetic development may no longer be possible. Thus with respect to the cell type it seems quite obvious that if the egg mutated into any of the other cell types, embryogenesis would be excluded, for no other cell type than the amoebocyte can form a blastula with further developmental possibilities.

Even concerning the chemical properties the range of permissible deviation may be narrow. If namely, as envisaged long ago by MONOD (1947) and SPIEGELMAN (1948), differentiation consists of the gradual establishment of unique enzyme patterns, resulting from interaction between nucleus and cytoplasm, then it seems to follow that a very specific sequence in the cytoplasmic changes be a prerequisite for normal chemical differentiation. Any mutation tending to interfere with this particular sequence would automatically lead to developmental arrest.

All these considerations suggest that there is terribly little margin for variations in the properties of the egg cell, and that all the changes in the genome which have been responsible for phylogenetic evolution have been expressed in cells at higher levels of differentiation.

The relation between the original mother cell of animal evolution, egg cells, and differentiated somatic cells may be illustrated as follows:



It follows that the egg cells in each generation must derive from the blastomeres before cell transformation or any other differentiation process has begun. Studies on the origin of germ cells support this contention (cf. BOUNOURE 1939; MINTZ 1961; BLACKER 1961). The present view on this question may probably best be summarized by quoting the last author: "the primordial germ-cells ..., or endodermal cells closely associated with them, are directly ancestral to the definitive gametes" (l.c. p. 28).

3. Biochemistry, preformism, and epigenesis

In closing the present discussion I would like to deal briefly with a question recurrent in papers dealing with chemical differentiation. It is suggested (recently by WRIGHT 1964) that enzyme determinations be of limited value because enzyme activity may depend as much on removal of inhibitors as upon synthesis of new enzyme protein. This point is of course correct, but it must be stressed that disappearance of an inhibitor is just as much a sign of differentiation as would be synthesis of any specific enzyme. However, the danger of the argument is the hidden preformistic point of view, i.e., that all the enzymes are there awaiting only to be activated, for instance by inhibitor elimination.

Obviously nobody would carry this argument to that extreme today, but the possibility is still discussed now and then in the literature: "The well-known question of 'preformation' or 'epigenesis' arises in trying to solve the problem of the origin of the enzymes. It is still to be proved whether the egg contains all the necessary enzymes or whether some of them are formed only at subsequent stages. Our knowledge of enzymatic properties is not sufficient to provide a precise answer, and is complicated by the confusion between the enzymatic molecule as considered as a chemical entity and enzymatic activity as displayed by the molecule itself in vivo [and in vitro]. An enzyme, in fact, may be present, but inactive ... a satisfactory solution to the problem will only be made possible by an objective examination of the data available and further research" (URBANI 1962, p. 98–99).

Although inclined to accept the epigenetic view URBANI discusses the preformistic one and decides that future research must decide the question. I am afraid that if we lean on this approach it will be as with the question of Creation, for each step our understanding advances the scope for participation of God diminishes, but there will always be plenty of possibilities for interference beyond the limits of our knowledge.

If the introduction of more and more refined techniques still led to negative results, it would be possible to reduce the maximum limits for the amounts of enzyme present, but it would be impossible to exclude that one

or two molecules of any enzyme was present, and thus the question remained unsettled. There is, in my opinion, a shortcut to the solution of the problem, i.e., the logical approach. I presume that, in contrast to opinions held in earlier times, nobody maintains today that the embryo is preformed in the egg, ready to develop by what may be called a growth process. In other words, none of the morphological entities, liver, brain ... etc. are present, only the genetical information required for the establishment of these structures, if and when the developmental processes proceed according to a certain, causally determined spatio-temporal pattern. However, if the various organs are absent it would seem an obvious inference that no organ-specific proteins can occur, since the synthesis of these compounds must depend upon the activity of the respective differentiated cells.

A particularly complex situation arises if it is contended that organ-specific proteins are present and that they, in order to exert their (determinative?) function must become distributed in the embryo in accordance with the organ and tissue differentiation. This mechanism would seem to imply that the substances be distributed according to a very intricate pattern already in the unfertilized egg. It is very difficult to see how such a requirement be reconcilable with various results obtained in experimental embryology, e.g. by the rotation experiments of ANCEL and VINTEMBERGER (1948). Anyhow, enzyme molecules are not self-reproducing units; what is required for synthesis is not an enzyme prototype, but the code which is present in the nucleus of any cell.

The question of epigenesis-preformism may also be approached from the phylogenetic point of view. According to this way of thinking the unfertilized egg, in spite of its higly complex organization in certain respects, must represent the archaic cell type, on the basis of whose properties all later development rests. It follows that this cell can contain only such substances as are typical for this stage of development, any enzyme or other protein which is characteristic for cell types derived from the original one by differentiation cannot be present, even though, of course, the template for their formation is present in the genome.

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ABRAMS R.: Synthesis of nucleic acid purines in the sea urchin embryo. Exp. Cell Res. *2*, 235–242 (1951).

AFZELIUS B. A.: The fine structure of the sea urchin spermatozoa as revealed by the electron microscope. Z. Zellforsch. *42*, 134–148 (1955).