The relation of the two blastomeres to the polar lobe in *Dentalium*

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Experiments on the influence of the polar lobe on the development of molluscs have shown that after removal of the polar lobe cleavage is radially symmetrical and indications of bilateral symmetry do not appear (Wilson, 1904: *Dentalium*; Clement, 1952: *Ilyanassa*). In normal development the polar lobe fuses with one of the two cells of the trefoil stage, and this cell becomes the posterior side of the embryo.

The question now arises whether the polar lobe fuses in an arbitrary way with one of the blastomeres at first cleavage, which then becomes the CD cell. Another possibility is that one of the two blastomeres at the trefoil stage is already predetermined to become the CD cell, with which the polar lobe always fuses. In the first case dorsoventrality is determined epigenetically; in the second case it is preformed. Morgan (1936) tried to solve this question by removing one of the blastomeres at the trefoil stage in *Ilyanassa*. Unfortunately, the other blastomere nearly always cytolyse. Only six eggs out of several hundreds survived. In four eggs the polar lobe fused with the remaining cell; in two it did not.

Eggs of *Dentalium* appeared to be suitable material for this kind of experiment, as they hardly ever cytolyse. An attempt was therefore made to solve this problem by using eggs of *Dentalium*.

MATERIAL AND METHODS

Eggs of *Dentalium antillarum* were obtained according to a method described in a previous paper (Verdonk, 1968). The diameter of the uncleaved egg is about 160 μ. Using a very thin, slightly bent tungsten needle, the blastomeres at the trefoil stage can be separated. In this way one blastomere with and one without a polar lobe is obtained.

Eggs and isolated blastomeres were cultured in filtered and boiled sea-water in salt dishes with a coverglass in an air-conditioned room at about 25 °C.

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RESULTS

In a first series of experiments the blastomeres were separated at the moment when the polar lobe was well rounded-off with respect to the blastomeres, so that no connexion between the lobe and the blastomeres could be seen. Altogether 103 eggs were operated upon. The polar lobe in 62 cases fused with the adjacent blastomere; in 41 it did not. Leaving out of consideration all cases in which the blastomere receiving the lobe did not cleave, the lobe fused in 37 cases and did not in 31.

Fig. 1. (1) Larvae from AB and CD halves isolated at the 2-cell stage; (2) and (3), larvae from blastomeres isolated at the trefoil stage. The polar lobe (l) has fused with one of the blastomeres in the left (2) picture. It remained unfused but attached to the embryo in the right (3) picture.

The best indication that the result is not influenced by damage to one of the blastomeres is provided by those cases in which both blastomeres continued cleaving after the operation. This happened in 54 cases. The lobe had fused in 28 of these cases; in 26 it had not. Consequently, the result approaches a one-to-one ratio very closely. This ratio can be expected if it is assumed that the polar lobe fuses with a predetermined CD blastomere in normal development, because it is combined arbitrarily with one of the two blastomeres in our experiment.
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On the other hand it is not easily explained by the opposite assumption that the polar lobe fuses at random with one or the other of the two blastomeres.

In a second series of experiments, the intervention was postponed until the moment when a connexion by a narrow stalk was observed between the polar lobe and one of the blastomeres. By this connexion the CD cell can be distinguished from the AB cell. This connection was cut and the lobe added either to the isolated AB or CD cell.

In 23 successful operations in which the lobe was added to the AB blastomere, it never fused except in one case, in which this blastomere did not cleave; apparently it had been damaged during the operation. When the lobe, after being separated from the CD cell, was added again to this cell, it fused in all of 26 cases. The fusion of the polar lobe was considerably retarded in some cases, so that the first lobe became directly the second lobe at next cleavage. The results of this second series of experiments corroborate those of the first series. They all indicate that at first cleavage the polar lobe fuses with a predetermined blastomere.

With respect to the further development of isolated blastomeres, it appeared that after separation at the two-cell stage, AB cells never produce an embryo with an apical tuft, whereas the embryos from the CD cells in 11 out of 25 cases show an apical tuft and prototroch (Fig. 1, (1)).

After separation of the blastomeres at the trefoil stage, one has to distinguish between cases in which the polar lobe fused, and those in which it did not. Forty-five per cent (9 out of 20) of the embryos originating from blastomeres with which the polar lobe had fused had an apical tuft (Fig. 1, (2)). When the polar lobe did not fuse, the embryos never showed an apical tuft. In these embryos the lobe can still be seen as a separate structure attached to the embryo (Fig. 1, (3)).

Whether or not the lobe fused with the blastomere to which it was added appeared to be of no importance to the fate of the other blastomere. From blastomeres isolated at the trefoil stage and missing the polar lobe embryos originated without an apical tuft and a prototroch in all cases (Text-fig. 1 (2, 3)).

DISCUSSION

The relation between the polar lobe and the blastomeres in dispermic eggs of Dentalium entalis has been studied by Schleip (1925). At first cleavage, such eggs divided spontaneously into three or four cells. In the first case the three blastomeres came together at the vegetative pole, where the polar lobe was formed. The lobe fused with three, two or one of the blastomeres or remained isolated. In the second case the four blastomeres were arranged either in one layer around the egg axis (the polar lobe fused with one, two or four of the blastomeres) or the four blastomeres showed a tetrahedral arrangement with one or three cells at the vegetative pole (in this case the polar lobe fused with one, two or three blastomeres). Schleip concluded that in normal development the polar lobe also
does not fuse with a predetermined blastomere, but at random with one of the blastomeres, with which it happens to come into contact. This blastomere then becomes the CD cell.

However, when first cleavage is normal and two blastomeres are formed, the polar lobe was never observed to fuse with both blastomeres but always with only one of them. We may therefore conclude with Raven (1958) that the observations of Schleip on dispermic eggs shed no light on the question of the determination of the dorsoventral axis in the molluscan embryo, as in these dispermic eggs the cleavage proceeds abnormally from the start.

Novikoff (1940) removed one of the blastomeres at the trefoil stage of the egg of the annelid Sabellaria. In 12 out of 13 eggs the lobe fused with the remaining blastomere, in one case there was no fusion. After puncturing one of the blastomeres at the trefoil stage in 30 eggs, the other cell with the lobe also cytolysed in 27 cases. In the three successful operations the lobe fused with the remaining blastomere. Taking the results of all experiments together the lobe fused in 15 out of 16 or in 94 % of the cases. Novikoff (1940, p. 144) concludes: 'This does not, of course, mean that in normal development it is a matter of chance into which one of the two cells the polar lobe will flow. But it does demonstrate that under the conditions of the experiment the lobe can in practically every case be made to fuse with either of the two cells.' As for these experiments, the eggs were denuded by a treatment with an isotonic solution of NaCl brought up to pH 9-6 by the addition of Na₂CO₃ (Novikoff, 1938); the results may have been influenced by this pre-treatment. If this is not the case, then the situation in Sabellaria and Dentalium is quite different.

The results of the experiments described in this paper show clearly that in Dentalium antillarum the polar lobe has a predetermined relation to one of the blastomeres at first cleavage, as in half the operated eggs the lobe fused with the remaining blastomere, while it remained free from it in the other half. As the cell that combines with the polar lobe becomes the posterior side of the embryo, this means that the dorsoventral axis, which together with the original egg axis determines the plane of bilateral symmetry, is already fixed at the beginning of first cleavage, and thus is predetermined in the uncleaved egg.

The further development of the isolated blastomeres shows that the lobe-dependent structures originate only after the lobe is incorporated into the blastomere. If it stays apart, it does not influence the differentiation of the cell to which it is attached. The same situation exists in Sabellaria (Novikoff, 1938). Consequently, the polar lobe as such cannot induce the formation of the specific lobe-dependent structures such as the apical tuft. The morphogenetic factor present in the polar lobe has to pass through the CD cell. The possibility, however, that during later development induction plays a role in the determination of certain structures in Dentalium cannot be excluded. Experiments of Clement (1962) indicate that in Ilyanassa a mechanism of induction is involved in the determination of some lobe-dependent structures.
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SUMMARY

1. The relation of the blastomeres to the polar lobe has been studied by separating the blastomeres at the trefoil stage in eggs of Dentalium antillarum.

2. The polar lobe fused in 50 % of the cases with the adjacent blastomere; in 50 % it remained separated, but attached to the embryo.

3. Lobe-dependent structures developed only after fusion of the lobe.

4. It is concluded that dorsoventrality and bilateral symmetry are predetermined in the uncleaved egg of Dentalium.

REFERENCES


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