Organogenesis in Cephalopoda: further evidence of blastodisc-bound developmental information

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SUMMARY

The basic mechanisms of organ differentiation in the Cephalopod embryo (telolecithal egg, discoidal cleavage) are studied. The results of ligation experiments, performed in early cleavage stages, confirm earlier conclusions of the author, drawn from transplantation/explantation and heat-shock experiments. The developmental information for cellular differentiation is shown to reside in the blastodisc; the yolk syncytium, in which a large part of the original egg cortex is incorporated, acts as nutritive substrate for the cellular material involved in organogenesis.

On the basis of these results, Arnold's induction model supposing an undisplaceable determining informational pattern laid down in the uncleaved egg cortex must be rejected.

INTRODUCTION

In an earlier paper dealing with experimental embryology of *Loligo vulgaris* (Marthy, 1973), we have shown that the yolk syncytium (= 'yolk epithelium') is not involved in organogenesis as a 'morphogenetic inductive map', a concept presented by Arnold (1965a). The same author's hypothesis (Arnold, 1968, pp. 182, 196) that the 'cortex' of the Cephalopod egg would represent 'a rather rigidly fixed system of specific prelocalized organ determining areas' thus became extremely doubtful (Marthy, 1972). In the present paper we give further, more direct evidence, obtained by ligation experiments on embryos at early cleavage stages, endorsing our earlier conclusions: in contrast to the hypotheses of Arnold (repostulated recently in this journal (Arnold & Williams-Arnold, 1974)), the concept is re-established that the cellular material actually forming the embryo is the primary carrier of developmental information (and not the transitory yolk syncytium which is largely derived from the original egg cortex).

MATERIAL AND METHODS

Ligation experiments in preorganogenetic stages have already successfully been performed on embryos of *Loligo pealei* (Arnold, 1968; Boletzky, 1970).

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In the experiments presented here, embryos of *Loligo vulgaris* (Fig. 1) and *Octopus vulgaris* (Fig. 3) were ligated, with human hair, transversally to the longitudinal egg axis. For ligation, the egg-case jelly was removed from *Loligo* eggs; eggs of *Loligo* and of *Octopus* remained in their chorion. In both cases, the ligation was made closer to the animal pole, but particular care was taken that the plasma cap, the area involved in cleavage, was not disturbed by the ligation procedure; about 60% of the total amount of the yolk was thus isolated. Whereas in *Octopus* embryos the hair loop can be contracted quickly without causing the yolk to break in the ligated region, *Loligo* embryos had to be ligated slowly and stepwise in order to avoid yolk breakage. After ligation, the embryos were kept in Petri dishes with filtered sea water, at room temperature (18–24 °C). The controls were single embryos in separate Petri dishes. Water was changed once a week.

We have retained Naef staging, which is well defined for many species. Stages 1–10 of Arnold merely define the cleaving stages between fertilization and stage I of Naef.

**RESULTS**

(1) *Loligo vulgaris*

Embryos were ligated at the 16-cell stage (stage 7 of Arnold, 1965b). Of seven completely ligated embryos, four could be observed beyond the appearance of the organ anlagen, stage VI of Naef (stages 16/17 of Arnold) up to stage X (about stage 21 of Arnold). The fragment containing the animal pole differentiated into a complete, correctly proportioned dwarf embryo with an outer yolk-sac of corresponding size (Fig. 2). It is particularly interesting that the development of the dwarf was chronologically parallel to that of the controls. From rough estimation of cell numbers, for given stages, it appears that the mitotic rate was unaltered in the dwarf embryos.

(2) *Octopus vulgaris*

(a) Five complete ligations were performed on eggs just starting cleavage (i.e. slightly prior to stage 4 of Arnold) (Fig. 4); two embryos died shortly afterwards. From the three remaining embryos that continued developing, one was fixed at stage VI, one reached stage XII, the last attained stage XX and hatched normally (Fig. 5). These results conform to the observations made with *Loligo*: the fragment containing the animal pole again developed into a well-proportioned dwarf embryo (some disproportions observed in the hatched animal may be due to the spatial conditions in the extremely narrow chorion).

(b) Five ligations were performed on embryos at the 8-cell stage (stage 6 of Arnold). Two embryos died prior to the appearance of the organ anlagen, two after stage VIII (stage 18/19 of Arnold); one survived until hatching. This dwarf embryo was similar to those shown in Fig. 5. As in the case of *Loligo* embryos,
Fig. 1. Four developmental stages in *Loligo vulgaris*. Note large cleavage spread territory in:

(A) Four-cell stage (stage 5 of Arnold). ——, Region where on embryos at the 16-cell stage ligation was made. Photograph shows artificially fertilized egg, not used in these experiments.

(B) Stage II–III (stage 12/13 of Arnold).

(C) Stage VI/VII (stage 16/17 of Arnold): appearance of the organ anlagen.

(D) Stage VIII (about stage 19 of Arnold), seen from the ventral side. *a*, Arm; *c*, chorion; *e*, in (A) territory which undergoes cleavage, in (B) cellular cap, in (C, D) embryo *sensu strictu*; *f*, free yolk; *m*, mantle; *o*, outer yolk-sac; *y*, eye.
Fig. 2. Dwarf embryo of *Loligo vulgaris* at stage X (stage 20/21 of Arnold). *a*, Arm; *e*, embryo *sensu strictu*; *o*, outer yolk-sac; *s*, egg case jelly.

Fig. 3. Four developmental stages in *Octopus vulgaris*. (A) Two-cell stage (stage 4 of Arnold). Arrow indicates first cleavage furrow. (B) Stage III. (C) Stage VIII. (D) Stage XVIII. *a*, Arm; *c*, chorion; *e*, in (B) cellular cap, in (C) embryo *sensu strictu*; *i*, inner yolk-sac; *m*, mantle; *o*, outer yolk-sac; *f*, free yolk; *y*, eye.
Fig. 4. Developmental stages of a dwarf embryo of *Octopus vulgaris*. (A) Uncleaved egg, (B) stage III, (C) stage XII, (D) empty chorion of hatched dwarf embryo, which was ligated at the 8-cell stage. e, In (B) cellular cap, in (C) embryo *sensu strictu*; f, free yolk; o, outer yolk-sac; x, degenerated yolk; y, eye.

the course of ontogenesis was chronologically parallel in the ligated embryos and the controls. Hatching of the controls took place 34–38 days after fertilization; the dwarf hatched after a total developmental time of 37 days (Fig. 4D).

**DISCUSSION**

Egg fragments containing the intact plasma cap undergoing cleavage are observed to develop into complete, well proportioned dwarf embryos. As to their small size, this result could be expected with the reduced amount of yolk available (Okada, 1927; Arnold, 1968, p. 191, table 3; Marthy, 1972); rather unexpected is that the developmental rate is independent of the amount of yolk. However, the crucial fact, in view of Arnold’s hypotheses (1965b, 1968), is the completeness of the embryo. Removal of cortical areas that were supposed to be determining in organ formation by Arnold (e.g. equatorial region of the egg for arm complex) has apparently no negative organogenetic effect. According to Arnold’s concept, our ligated embryos should lack arms and outer yolk-sac; this concept does not allow the supposition of any regulation mechanism. Thus our results definitely contradict Arnold who claims the existence of an organ-specific informational pattern in or on the cortex which cannot be displaced. The lack or the incompleteness of organs following local irradiation or local
Fig. 5. Newly hatched normal and dwarf embryo of Octopus vulgaris (shown in Fig. 4). The dwarf still has a small outer yolk sac (o). m, Mantle; v, visceral mass; y, eye.

Application of Cytochalasin B to the cortex of early stages, as observed by Arnold (1968, 1974) is, of course, undeniable, but there is, as our results emphasize, no evidence that the lack is caused by destruction of organspecific information. This does not mean that the egg cortex (comprising also the cleaving area at the animal pole!) might not have some role in determination, but there cannot possibly be any transfer, from the cortex, of organspecific information via yolk syncytium to cell material forming the embryo, as supposed by Arnold (1965a), for organogenetic stages.

Considering the results of Arnold and of our own work, we may put forward the following hypothesis: in Cephalopods (the generalization is intentional, see final paragraph) the cellular material actually forming the embryo is the primary carrier of developmental information. The yolk syncytium or its cortical precursor has no organspecific informational pattern other than 'yolk organ' information; by its nutritive function it is a crucial element that acts in realizing the potency of the overlying information carrier.
So far we have used the term ‘information carrier’ with a well-defined part of the egg (cleaving blastodisc); however, it must be emphasized that this part becomes increasingly complex with development, and for now, we can characterize its components only morphologically.

In early cleavage stages the blastomeres are arranged in a typical pattern (Watase, 1891; Naef, 1923; Lemaire, 1970; Arnold, 1971; de Leo, 1972) that becomes increasingly diffuse until a rather uniform monolayered blastoderm is present (stage I; stage 9/10 of Arnold). Germ layers are then formed, apparently by marginal delamination. At stage III (stage 13 of Arnold) the partially multilayered germ (in which the cellular material is differentially and symmetrically arranged, see final paragraph) lies on the yolk syncytium whose nuclei proliferate from the marginal ‘blastocones’ under the blastoderm (Fig. 1B). (It is this early germ, covering only a small part of the egg in Loligo vulgaris, that is able to differentiate into a dwarf embryo if some yolk is left with it (cf. Marthy, 1972).)

If we assume the presence of some sort of (e.g. region-specific) informational pattern in the egg surface, at early cleavage stages, it is only conceivable for the territory that undergoes cleavage. Any information ‘distributed’ during cleavage is then directly incorporated in the blastomeres as each of these retains a cortex fraction.

Global treatment (e.g. heat-shock, Marthy, 1972; cytochalasin B, Arnold & Williams-Arnold, 1974) as well as unfavourable environmental conditions (lack of oxygen, sublethal temperatures and salinities) affect all parts of the egg; the effect of any of these external factors depends on the developmental stage at which they arise. Alterations of the yolk syncytium cannot reasonably be considered as the cause of the alteration observed subsequently in the embryonic parts overlying it: the global treatment is most probably the common cause of both, alteration in the organogenetic cell material and in the yolk syncytium.

But why does local treatment outside the cleaving territory affect organ differentiation as observed by Arnold (1968, 1974)? The answer is apparently that the yolk syncytium has some morphogenetic significance; that this significance cannot be explained in terms of an induction mechanism, is demonstrated by the results reported in the present paper. The alternative answer can most probably be derived from the nutritive function of the yolk syncytium. It is very likely that local treatment disturbs the formation of an active, yolk-resorbing syncytium in the treated area so much that the cells overlying it subsequently are ‘starved’. This would then result in an irreparable time lag in local organ differentiation. In other words, only an intact, fully functional yolk syncytium enables the overlying cell material to differentiate in due time beyond the threshold of organ autonomy, around stage VII (Marthy, 1970; Lemaire, 1972; Marthy, 1973). Our current work is therefore focused on two aspects: (1) characterization of the cellular information carrier and (2) definition of the interrelationship between the information carrier and the nutritive substrate.
We have so far discussed Arnold’s hypotheses on the basis of experimental results. However, we must insist also on the fact that there are some incoherencies in the structural concepts of Arnold’s descriptions. From statements given by this author one might get the impression that the cellular cap at least from stage 13 (stage III, Naef) up to stage 16 (stage VI) is a rather homogenous structure (Arnold, 1965b, p. 27, fig. 3; Arnold, Summers & Gilbert, 1974). As to the ‘vertical’ division of cellular material, Arnold used for stage 16 the term ‘outer layer of cells (future body of the embryo)’ (1965a, p. 73), but later on characterized the same material as ‘two outer layers of cells’ (1974, p. 3). Furthermore, in *Loligo pealei* as well as in *Loligo vulgaris*, the germ is differentiated as early as stage III (stage 13 of Arnold) into an actual organogenic part and a peripheral area giving rise to the yolk-sac envelope. This phenomenon has also been observed in other Cephalopod species (Naef, 1923; Lemaire, 1970; Boletzky & Boletzky, 1973). The generalization of our hypothesis is justified on the basis of these common morphological characteristics which also contradict, by themselves, Arnold’s concept of an informational pattern which cannot be displaced residing in the uncleaved cortex.

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REFERENCES


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