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The Cleavage Initiation Site Establishes the Posterior Pole of the Hydrozoan Embryo

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Summary. When the first cleavage of the hydrozoan egg is reversibly suppressed, two cleavage furrows frequently form simultaneously at the time of the second cleavage. If these two cleavage initiation sites are far enough apart, each one specifies a site of gastrulation, and the embryo that forms develops into a two tailed planula larva. When two tailed planulae are induced to metamorphose, they form a polyp with two stalks and hydranths.

Key words: Embryogenesis – Cleavage – Polarity – Hydrozoan

Introduction

The hydrozoan planula larva has an anterior-posterior axis. The initial work on the role of polarity during hydrozoan embryogenesis asserted that this axis is set up in the egg during oogenesis (Teissier 1931). This argument is based on the observation that there is a positional congruence between the position of the germinal vesicle in the oocyte during late oogenesis, the site where the polar bodies are given off from the egg during oocyte maturation, the position where the first cleavage is initiated after fertilization, and the posterior end of the planula larva[1]. Recently the validity of the notion that the uncleaved hydrozoan egg has such a polarity has been tested by creating conditions where the first cleavage is initiated at a site some distance from the region where the polar bodies are given off (Freeman 1980). This work showed that the site where cleavage is initiated specifies the posterior pole of the planula larva and suggests that the polarity of the embryo is established at the time of the first cleavage at the cleavage initiation site. The present report examines the effect of two simultaneous first cleavages on the development of polarity in the hydrozoan *Phialidium gregarium*.

Materials and Methods

The accompanying paper (Freeman 1981) contains a description of the procedures used for obtaining *P. gregarium* gametes, for fertilizing these eggs, for marking them locally with vital dyes, and raising the embryos to larval stages. This paper also includes a procedure for inducing metamorphosis in three-day-old planulae by treating them with sea water containing Cesium, and describes the metamorphic response of these larvae following treatment.

The drug cytochalasin B was used to create eggs in which there were two simultaneous first cleavages. Cytochalasin was made up by dissolving 1 mg in 1 ml of dimethyl-sulfoxide. It was used at a concentration of 0.0005 mg/ml in sea water; this is just above the minimal concentration that blocks cytokinesis. After the cytochalasin treatment eggs were washed in several changes of sea water to dilute out the cytochalasin. Dimethyl-sulfoxide without cytochalasin B has no effect on cytokinesis or embryogenesis.

Eggs were centrifuged for 10 min at 3,000 g on a cushion of 1 part 1.1 molal sucrose and 2.5 parts sea water. After centrifugation the eggs were washed several times to remove the sucrose.

The normal development of *P. gregarium* is also described in the accompanying paper (Freeman 1981). After the egg is fertilized 50 min elapses until the first cleavage. Subsequent cleavages occur at roughly 50 min intervals. A hollow blastula is formed. At about 16 h of development gastrulation begins. This process takes place by unipolar ingression. By three days of development a mature planula larva has formed. The planula has an anterior-posterior axis which is defined by its shape and by its direction of locomotion.

Results and Discussion

The drug cytochalasin B reversibly inhibits cytokinesis in eggs but allows mitosis to take place (Schroeder 1972). When eggs which have been treated with cytochalasin B just before the first cleavage are removed from the drug, two cleavage initiation sites frequently form more or less simultaneously at the second cleavage cycle (Fig. 1). When two cleavage sites form simultaneously in hydrozoan eggs, they invariably form adjacent to each other. This is probably the case because the asters of each mitotic apparatus are quite small. The percentage of cytochalasin B treated eggs which form two cleavage furrows at the second division of the control eggs varies from 5–60%. If a cytochalasin treated egg does not form two cleavage furrows at the second division of the control eggs it will frequently form extra cleavage furrows at a subsequent division. Over 75% of the eggs which form these extra cleavage furrows at either the second cleavage or a subsequent cleavage develop into normal planulae. Those cases which do not develop normally arrest at early postgastrula stages.

By centrifuging cytochalasin B treated eggs prior to the second cleavage it is possible to move the cleavage initiation sites apart. Centrifugation temporarily stratifies the contents of the egg; the yolk takes up a centripetal position while the heavier cytoplasm takes up a centrifugal position, and the egg nuclei move to the interface between these two layers. Following centrifugation the cytoplasm begins to redistribute itself in a normal manner. Frequently the two nuclei created by the first mitosis take up different positions in the centrifuged egg prior to the second mitosis. Cleavage is initiated at the sites where the nuclei are. When cytokinesis is initiated each cleavage initiation site can be marked with a different colored vital dye in order to

1 Cleavage in coelenterates is unipolar. The furrow begins at one point on the egg surface and extends to the other side

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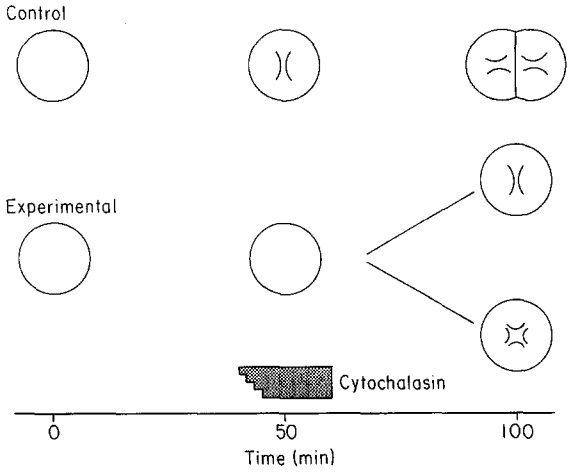

Fig. 1. The effect of reversibly inhibiting the first cleavage with cytochalasin B on the character of the second cleavage. The eggs are viewed from the pole where the polar bodies are given off. Eggs were placed in cytochalasin B 5–10 min prior to the first cleavage. They were removed from the drug 10 min after the initiation of the first cleavage in control eggs and washed in several changes of sea water. If an appropriate concentration of sperm is used to fertilize the eggs over 90% of the control eggs initiate the first cleavage within a five-minute period

Table 1. The effect of distance between cleavage sites on the development of two-tailed planulae[a] (3 days old)

Angular distance between cleavage sites (degrees)	Kind of planula	
	One tail	Two tails
0– 45	32	0
46– 90	19	5
91–135	3	14
136–180	0	9

[a] In these cases both furrows formed within a minute of each other. The angular distance between cleavage sites was measured with a goniometer eye piece

Table 2. The effect of synchrony of formation of cleavage sites on the formation of two-tailed planulae (3 days old)

Time between cleavage site formation (minutes)	Kind of planula	
	One tail	Two tails
1	3	23
2	7	1
3	3	0
4–10	7	0

establish the presumptive fate of that region during embryogenesis. When two cleavage furrows form simultaneously and these two sites are some distance apart in the *Phialidium* egg, the embryo gastrulates at two sites; each site corresponds to a region where cleavage was initiated (Fig. 2a, b). These eggs form two-tailed planulae (Fig. 2c); this form of planula is never seen under normal conditions.

One factor which influences the production of a two-tailed planula is the distance which separates the two cleavage sites (Table 1). When the two sites of furrow formation are within 45° of each other, either one enlarged site of gastrulation or two separate sites of gastrulation form. These larvae either form a normal planula or begin to form two tails; however regulation takes place in the two-tailed form and a normal planula results. The stained cleavage sites appear as two lateral stripes on opposite sides of the tail of the planula larva. When the two sites of furrow formation are more than 45° from each other, there are always two sites of gastrulation. As the distance between the two cleavage initiation sites increases, the probability of a planula with two tails increases. However, even when the distance between the sites of cleavage initiation is large (136°–180°), there is evidence for regulation because the stain is usually not at the tip of each tail but at the outer lateral edge of each tail, and the two tails form an angle which is less than the angle separating the two cleavage initiation sites. There is no evidence for regulation until after gastrulation.

The other factor which influences the production of a two-tailed planula is the synchrony with which the two cleavage sites form. In most cases both furrows are initiated within 30 s of each other; however, there are a number of cases in which the initiation of the second furrow is delayed for one or more minutes. Table 2 indicates the effect of cleavage delay on the

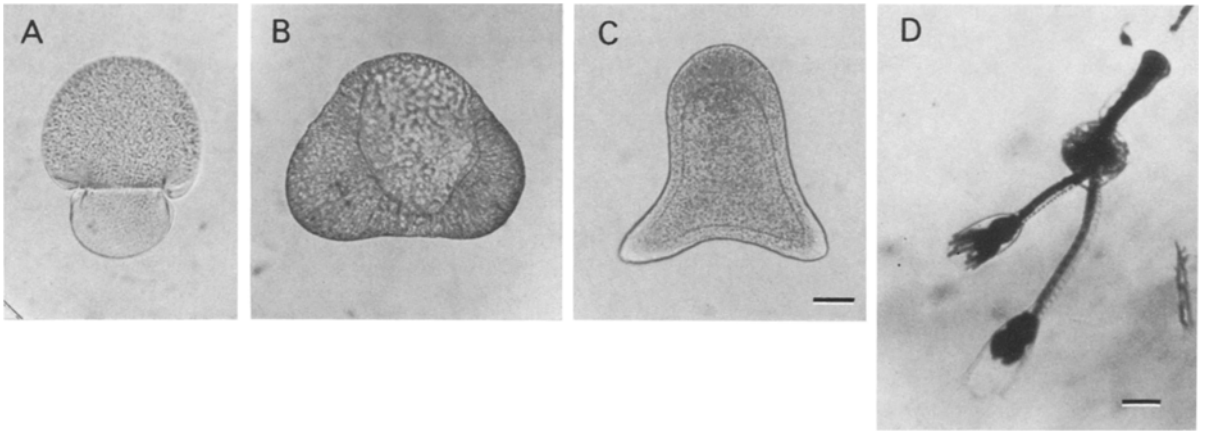

Fig. 2. A A cytochalasin B treated and centrifuged egg which is forming two cleavage furrows simultaneously. **B** A gastrulating embryo (19 h of development) which formed two cleavage furrows simultaneously. It is gastrulating at two sites. **C** A two-tailed planula larva (slightly flattened) ($2^1/_2$ days old). **A, B**, and **C** are at the same magnification. The bar indicates 50 μm. **D** A polyp with two stalks and hydranths derived from a two-tailed planula (five days after the initiation of metamorphosis). The bar indicates 100 μm

formation of two-tailed planulae for those cases in which the distance between cleavage sites is over 90°. If the second cleavage furrow is delayed one to two minutes, fewer cells move into this site at gastrulation; the initial tail that forms at this site is smaller than the other tail and is subsequently resorbed. If the second cleavage furrow is delayed for over three minutes there is only one site of gastrulation, that site is always specified by the first cleavage furrow and the planula that forms is normal. These observations suggest that within minutes after the formation of the first cleavage furrow events are initiated which prevent the formation of a second furrow from specifying a future posterior region.

The planula larva swims around for a few days and then settles on a substrate and metamorphoses to form a polyp. During metamorphosis the cells in the posterior part of the planula become the stalk and hydranth of the polyp (Teissier 1931). A metamorphosed *Phialidium* planula initially produces only one stalk and hydranth. When a two-tailed planula is induced to metamorphose, it invariably produces two stalks and hydranths simultaneously (Fig. 2d). If a planula initially begins to form two tails, but then regulates to give a one-tailed planula, this individual usually forms only one stalk and hydranth following metamorphosis. This observation indicates that events which take place at the first cleavage play a role in specifying stalk and hydranth formation after planula metamorphosis.

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