Polarization of Fucoid Eggs by a Calcium Ionophore Gradient

Abstract. *When the eggs of the brown alga* Pelvetia *were grown in a gradient of the calcium ionophore A23187, they tended to form their rhizoidal outgrowths on the sides that were exposed to the higher concentration of ionophore. This result supports the hypothesis that the formation of an intracellular calcium gradient is an essential step in the polarization of these eggs; the rhizoid forms at the pole that has the higher concentration of calcium.*

The eggs of the brown algae *Fucus* and *Pelvetia* have long been used for the study of the genesis of cellular polarity. When the eggs are released from the plant and fertilized in the surrounding seawater they, unlike most animal eggs, possess no developmental axes. In *Pelvetia* eggs, the first visible sign of polarity appears as a localized secretion some 5 to 6 hours after fertilization at 15°C (*1*). This "cortical clearing" leads, in about 2 hours, to the appearance of a rhizoidal precursor (germination) that represents the irreversible formation of the rhizoid-thallus axis of the plant. These events occur while the zygote is still single-celled; the first cell division does not occur until 18 hours after fertilization. The plane of the first division is perpendicular to the polar axis and results in the creation of a rhizoid cell and a thallus cell. Normally, the orientation of the axis is determined by unilateral light; the rhizoidal bulge forms on the shaded side of the densely pigmented cell. However, a variety of other vectors can polarize the egg (*2*).

Prior to the visible changes, there are a number of localized changes in the cell surface. Electrical current enters the region where the cortical clearing occurs (*1*). One ionic component of this current is calcium, since the influx of calcium becomes several times greater at the future rhizoid region than at the future thallus region, where the calcium efflux becomes greater (*3*); this finding led to the calcium-gradient hypothesis of localization. According to the hypothesis, the calcium gradient resulting from the asymmetrical distribution of ion leaks and pumps is an essential step in the polarization process, and acts to further polarize the cell by electrophoresis or other means. A prediction of this model is that one should be able to polarize a population of eggs grown in darkness by imposing an intracellular calcium gradient on them. The model was previously tested by putting the eggs in an extracellular calcium ion gradient (*4*). It was found that they tended to germinate on the side where calcium influx was greater. We have now devised a simpler method for imposing such a gradient. It involves the use of the divalent ion-hydrogen ion exchanger A23187 (*5*). Glass fibers (50 to 100 μm in diameter; comparable to the diameter of the eggs) were pulled from Pasteur pipettes, broken into 70-mm lengths, and cleaned with acetone after the ends were heat-sealed. The ionophore was dissolved in ethanol (2 mg/ml) just before it was used, and droplets were applied with a 33-gauge needle to the fibers as they lay on a microscope slide. The ionophore-bearing ethanol moved along the outsides of the fibers by capillary action and dried, leaving a coating of A23187. The uniformity of the coating was checked with an ultraviolet light (A23187 is fluorescent). The fibers were individually attached to slides with two small drops of silicone cement and were placed in petri dishes (10 cm in diameter). The cement was left to dry (about 30 minutes), and then the dishes were filled with natural seawater buffered with 10 m*M* tris buffer (*p*H 8.1). Recently fertilized *Pelvetia fastigiata* eggs [obtained as described in (*6*) from material collected in Monterey Bay, California, during the summer months] were added to the dishes and allowed to settle near the fibers. The eggs were allowed to grow undisturbed in darkness until 18 to 20 hours after fertilization. Because A23187 is only slightly soluble in water, the fibers acted as sources of the ionophore while the large volume of seawater acted as a sink. Eggs near the fibers were thus exposed to an ionophore gradient; it was expected that more calcium would enter the parts of the eggs nearer the fiber than the more distant portions, resulting in a cytoplasmic Ca^{2+} gradient.

When these eggs were examined 18 to 20 hours after fertilization, 60 to 80 percent of the germinated eggs had formed a rhizoid on the hemisphere nearer the fiber (Fig. 1). (Since the eggs glue themselves tightly to the substratum, the dishes could be moved without affecting the orientation of the rhizoids with respect to the fibers.) To determine the degree of polarization, we measured the angle of the rhizoid-thallus axis relative to the gradient; that is, relative to a line perpendicular to the glass fiber. If the rhizoid grew directly toward the fiber, the angle was 0°; if it grew directly away,

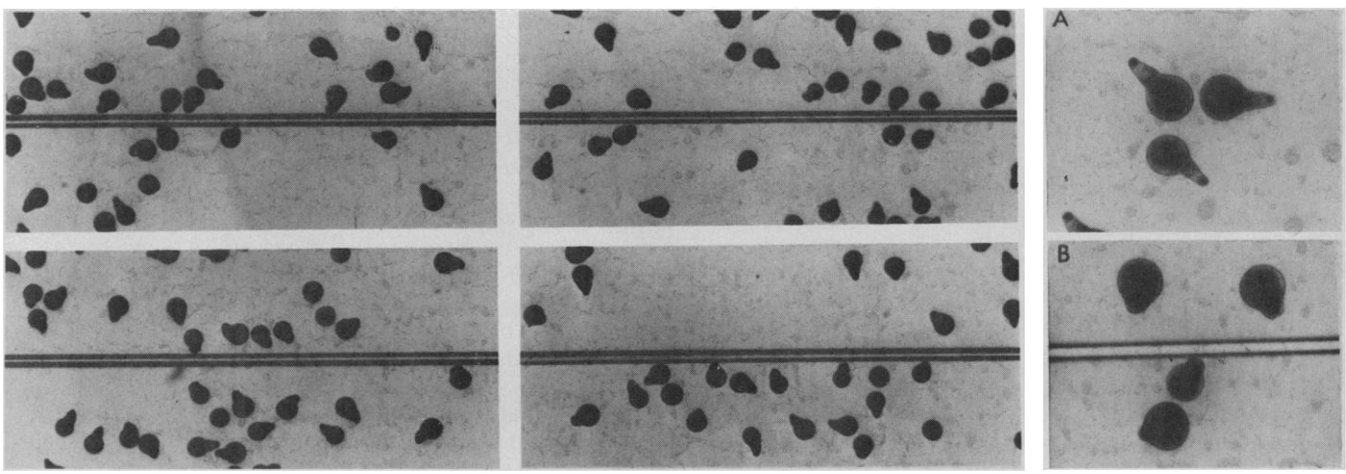

Fig. 1 (left). Eggs adjacent to sections of an A23187-coated glass fiber. The eggs were placed near the fiber 1½ hours after fertilization; these photographs were taken about 24 hours later. The average short diameter of the embryos is 90 μm. Some 78 percent of the embryos within one egg diameter of the fiber have formed their rhizoids on the sides near the fibers; 60 percent of the remaining embryos have done the same. Fig. 2 (right). Differences in rhizoid morphology between (A) eggs more than 1 mm from the glass fiber and (B) eggs adjacent to the fiber. The photographs were taken at the same time and show embryos from the same dish.

0036-8075/80/0104-0077$00.50/0

180°, and so on. These angles were measured to within 5° with an eyepiece protractor in an inverted microscope. From these angles, an average cosine for a particular population of eggs could be determined. An average cosine of +1 would mean that all rhizoids grew directly toward the fiber; −1 would mean that all rhizoids grew directly away from the fiber; and 0 would indicate no orientation.

Table 1 gives the average cosine as a function of the time between fertilization and placement of the cells near the fibers. If the eggs were placed near the fibers during the first 4 hours after fertilization, the average cosine was approximately +0.45. After that, the polarizing effect of the ionophore-coated fibers seemed to decline. Control eggs placed near uncoated fibers had an average cosine of almost zero. Similar experiments in which the potassium ionophore valinomycin was used produced little or no polarization. The results with A23187 were independent of *p*H from 8.1 to 6.5, and lowering the concentration of magnesium from 50 to 5 m*M* also had no effect.

The fraction of eggs that germinated was usually 10 to 20 percent smaller among eggs near the ionophore-coated fibers than among those farther away; however, in three cases, fewer than half of the eggs near the fibers germinated while all of the farther eggs germinated. In two of these cases, the average cosine was small but positive (+0.10, +0.16); in the third it was actually negative (−0.15). (These apparently aberrant cases are not included in the averages given in Table 1.) Another effect of proximity was that the morphology of rhizoids near the fibers was somewhat modified; they tended to be broader and shorter than those farther away (Fig. 2).

We examined the effect of A23187 on calcium influx in these eggs to verify that it was indeed acting as a calcium ionophore. Using methods described in (*7*), we found that the addition of A23187 to a nominal calcium concentration (5*M*) doubled the calcium influx from 0.1 to 0.2 pmole/cm²-sec.

To investigate whether the efflux of protons rather than the influx of calcium ions was responsible for the polarization of the eggs, we grew eggs on a Ronchi ruling (Edmund Scientific), a piece of glass that has alternate transparent and opaque strips, each 50 μm wide. The eggs were illuminated from beneath the Ronchi ruling by light that was passed through a red filter with a sharp absorption edge at 610 nm (Klinger Scientific, RG610). The intensity of the light reaching the cells was 1000 lm/m². Many eggs were approximately half illuminated by the red light. We reasoned that if a hydrogen ion gradient were maintained in the cytoplasm of the half-illuminated cells, the illuminated sides would be more alkaline than the dark sides, since photosynthesizing chloroplasts continuously take up CO_2. We observed that the zygotes were equally likely to germinate on either side. Of the 336 zygotes counted, 172 formed rhizoids on the shaded side and 164 on the illuminated side. These results are consistent with the finding that both unilateral red light and plane-polarized red light are ineffective in polarizing the zygotes (*8*, *9*). It seems unlikely, therefore, that the polarizing effects of the A23187 gradient are mediated by a transcytoplasmic hydrogen ion gradient.

Table 1. Polarization of *Pelvetia* zygotes by ionophore-coated fibers. At least 70 eggs were used in the determination of the average cosine in each experiment. Orientation measurements were made only of eggs whose bodies (the spherical portion excluding the rhizoidal outgrowth) were no farther than 100 μm from the fiber. All of these experiments were done with eggs in darkness in 10 m*M* tris-buffered natural seawater (*p*H 8.1, 15°C).

Fiber	Experiments (No.)	Time* (hours)	Cosine
A23187-coated	14	0–2	+0.45 ± 0.08
	10	2–4	+0.47 ± 0.19
	3	4–6	+0.31 ± 0.18
Uncoated	4	0–2	−0.01 ± 0.01
Valinomycin-coated	7	0–2	+0.07 ± 0.12

*Time between fertilization of eggs and placement of eggs near fibers.

In summary, we have shown that polarizing fucoid eggs grown near an A23187-coated glass fiber tend to form their rhizoids on the sides near the fiber. These results are consistent with the idea that the formation of an intracellular calcium gradient is a necessary step in the polarization process. We anticipate that the method used in this study will be applicable to the study of polarity and tropism in other cellular systems. Indeed, it has recently been shown (*10*) that the spores of the common moss *Funaria hygrometrica* respond to a gradient of A23187 by forming their rhizoids on the side of higher concentration.

KENNETH R. ROBINSON*
ROGER CONE†
Division of Molecular and Cellular Biology, National Jewish Hospital, and Research Center, Denver, Colorado

References and Notes

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11. We are grateful to R. Hosley for supplying the calcium ionophore A23187. Supported by NIH grant HD11925 to K.R.R.
* Present address: Department of Physiology, University of Connecticut Health Center, Farmington 06032.
† Present address: Department of Biochemistry, Princeton University, Princeton, N.J. 08540.

11 June 1979; revised 13 August 1979

Placental β-Endorphin–Like Peptides

Abstract. *Acid extracts of human placental tissue contain, by both radioimmunoassay and radioreceptor assay, β-endorphin–like material. Half of this material will not go through a 5000-dalton filter and on Sephadex G-200 has a molecular size between 25,000 and 50,000 daltons. Of the material going through a 5000-dalton ultrafilter, 80 percent is excluded on Sephadex G-25 and held back, very slightly, on Bio-Rad P6, indicating a molecular size of approximately 4500 to 4800 daltons. Thus, placenta appears to have macromolecular precursors from which a β-endorphin–like material is released, with a size approximately 12 amino acids longer than that of the pituitary hormone.*

The endogenous pituitary peptide β-endorphin competes with morphine for the same binding sites in the brain (*1*). This peptide has the same amino acid sequence as the last 31 amino acids in β-lipotropin (61–91) and is an endogenous, morphine-like analgesic (*2*). Amnionic fluid has been shown (*3*) to contain a significant amount of β-endorphin as judged by radioimmunoassay (RIA). Further, acid extracts of human placenta have been shown to contain (by RIA) both β-endorphin and adrenocorticotropic hormone (ACTH) as well as β-lipotropin (*4*). Recently, evidence has been presented that there is in the placenta a common precursor of lipotropin and β-endorphin with a number of mole-

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