

ELECTRICAL CURRENTS THROUGH THE DEVELOPING FUCUS EGG*

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The mechanism of localization or of pattern formation is a central problem of developmental biology. Much of patterning somehow occurs through interaction within single cells, or within groups of relatively similar cells; certain properties of the process are referred to in the concepts of fields and of gradients. However, there is available little clear speculation, let alone hard knowledge, as to the mechanisms in physicochemical terms. One appealingly simple thought, long ago advanced (and to a very limited degree supported) by Spek¹ as well as by Went,² is that patterns are established by self-generated electrophoresis (Spek's "self-cataphoresis").

Now, what might drive such transport? Localized changes in plasma membrane potential are one obvious source.

If the developing system were a single cell, then such inhomogeneities in membrane potential would drive current in loops going through the cytoplasm and back through the medium. Measurements of the extracellular currents, then, would reveal the internal ones without the substantial interpretative difficulties and high noise levels still associated with the use of intracellular microelectrodes. A knowledge of these intracellular currents supplemented by measurements of the internal resistance in turn would indicate the value of at least a portion of the internal electrical forces.

Accordingly, this is a study of the electric currents around developing eggs of the marine alga *Fucus furcatus*. These cells were used because they are exceptionally free of any built-in developmental pattern as well as of any need for the influence of other cells.

The unfertilized *Fucus* egg is a dense, nonmotile, radially symmetrical sphere about 70 μ in diameter containing a small, centrally located haploid nucleus and numerous small, functional chloroplasts. Soon after fertilization by the minute sperm, the hitherto naked egg begins to secrete an adhesive, rigid wall; and in a few hours it becomes tightly stuck to the substratum. About 12 hr after fertilization (at 15°C), the zygote *germinates*, that is, it starts to elongate by bulging at one pole (Figs. 1B and 2). After it becomes pear-shaped, it divides into a *rhizoid*, or holdfast, cell, which includes the protrusion, and another, larger cell, the thallus cell. The rhizoid marks the basal pole of the organism from then on.

The point of sperm entry tentatively determines the future rhizoid pole, but the subsequent action of other agents such as unilateral (unpolarized) light can readily redetermine the polarity until the time that germination begins.³ Indeed, the pattern of determination prior to germination is so labile that a high percentage of "twin" forms, i.e., cells which develop two antipodal rhizoids, can be induced by plane-polarized light.⁴

Methods.—It was considered essential to make the electrical measurements upon normally developing cells and hence upon cells surrounded by sea water. However, in this highly conductive medium, the voltage drop across one cell proved to be comparable to the minimum attainable electrode noise. Hence, the signal was multiplied by arranging the transeellular voltages of many

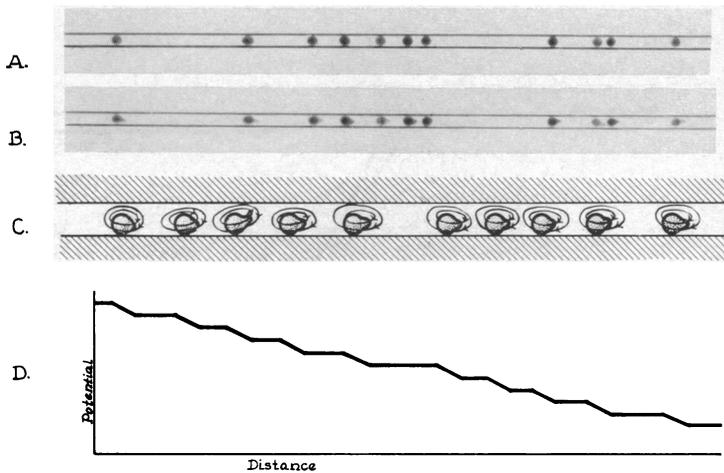


FIG. 1.—Eggs in series. (A) Photograph of eggs in part of a 100- μ bore capillary, 6 hr after fertilization and well before germination. Eggs are 75 μ in diameter. (They seem to be about 12% shorter across the capillary because of its lens action.) (B) Photograph of same eggs 26 hr after fertilization. All eggs have germinated in the same direction. Half have divided into a rhizoid and a thallus cell. (C) Highly schematic view of inferred current pattern in a tube. (D) Schematic graph of inferred change of potential along the tube.

cells in series. This was done by lining up many eggs in a long loose-fitting capillary, inducing parallel development with light from one end, and then measuring the voltage between the tube's ends. Division of the tube potential by the number of eggs gave the potential drop per egg. This, divided by the measured resistance past an egg, yielded a lower estimate of the polar current per egg (Fig. 1).

Each experiment was carried out with a precision bore capillary, about 10 cm long and 102 or 127 μ wide (compare the egg diameter of about 72 μ). About 100–200 recently fertilized *Fucus* eggs were distributed along a well-spaced file in such a tube and left undisturbed until 4 hr after fertilization, when electrical measurements were begun. At this time, the eggs had secreted a sticky wall and were well stuck to the tube. In experimental tubes, the eggs were exposed (at least until their polarity was irreversibly determined) to a large diffuse, wide-angle source of light placed at one end of the tube. This induced almost all eggs to form their basal poles, as expressed by outgrowth or rhizoid formation, away from the light and hence toward one end of the tube. In certain control tubes, near-random development was obtained by placing this same light source on one side of the tube with a mirror on its other side.

Tube potentials were led off via long sea-water-filled plastic tubes which contacted the capillary's ends. These leads went to large, isothermal, commercial calomel electrodes which in turn connected to a Keithley model 153 microvolt-ammeter. Both the noise and the sensitivity of this instrument are about 0.1 μ v on its most sensitive voltage range with the input shorted. The effects of spontaneous changes in electrode potential were reduced by manually reversing the attachment of the leads to the capillary about once a minute.

The average resistance past each egg was extracted from measurements of the change in tube potential produced by passing known small currents through the tube.

Results.—Representative data: Two tubes were each loaded with about 200 eggs (Fig. 2). In one, the *oriented* tube, the eggs were induced by light coming steadily from one end to grow toward the other; in the second, a control tube, continued lateral illumination let the eggs develop in nearly *random* directions. A third tube contained no eggs and also served as a control. No voltage developed until 12 hr after fertilization, when the eggs began to germinate. As those in the oriented tube germinated, there was a parallel rise in the voltage across this tube; the end

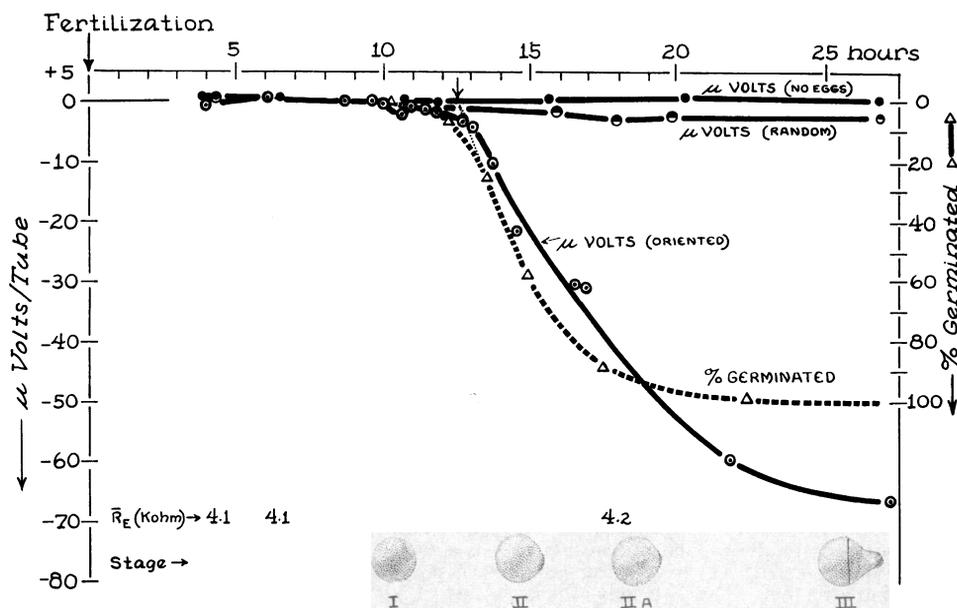


FIG. 2.—The course of representative tube potentials: oriented tube #4, random one #10, and a typical egg-free one. The germination curve is for the oriented eggs; germination in the random tube followed a similar course. At stage I, none of the eggs have germinated; at II, half have barely begun to elongate; at IIA, the *initial growth stage* (defined as 6 hr after the rise time), more than 90 per cent have done so; at III (photographed in Fig. 1B), half have reached the two-cell stage sketched. The arrow on the abscissa marks the rise time.

toward which the outgrowths formed, or rhizoid end, became increasingly negative. Germination of the eggs in the random tube, which followed a similar time course was accompanied by a voltage of only about 5 per cent of that in the oriented one; while the egg-free tube developed no significant voltage.

For quantitative consideration of such data, it is helpful to consider a tube stage indicative of the currents through eggs that have just germinated, i.e., just begun to elongate. In this particular oriented tube, at $18\frac{1}{2}$ hr after fertilization, 92 per cent of the eggs had reached or passed this stage, but none had yet progressed to the two-cell stage. Let us call this the *initial growth stage* of the population. At this stage, the tube potential was $45\ \mu\text{v}$ (4.5×10^{-5} v). Toward one end (usually quite exactly toward this end), 169 eggs had germinated, one egg toward the other; 19 eggs had not yet germinated. So 168 net eggs pointed one way and the potential per net oriented egg was 270 nanovolts (2.7×10^{-7} v). The resistance past each egg, \bar{R}_E , showed very little change as development progressed; at $18\frac{1}{2}$ hr, it was 4.2 kohm (4200 ohms). So, assuming that the tube voltage resulted from nearly pole-to-pole current loops as in Figure 1, one calculates the transcellular current through these eggs, as they began to elongate, to be $45\ \mu\text{v}/(168\ \text{net eggs}) \times (4.2\ \text{kohm}) = 64\ \text{picoamps}$ (6.4×10^{-11} amps) per cell.

Reproducibility: Similar results were obtained from all of the tubes studied. To summarize them, Table 1 shows the values found at the initial growth stage.⁵

Despite variations in conditions (to be discussed below), it can be seen that current (calculated on the pole-to-pole assumption) varied remarkably little, particularly

TABLE 1

CONDITIONS, DEVELOPMENT OF EGGS, AND ELECTRICAL OUTPUT OF MULTI-EGG TUBES AT THE INITIAL GROWTH STAGE

A. Tubes with Oriented Eggs

Tube no.	Bore (μ)	Light	Time of Flow	Total	No. of Eggs			μ v per Tube	Kohm per egg	Picoamp per net + egg
					-	+	NG			
1	102	Steady	None	163	160	1	2	-44	4.5	62
2	214	203	5	4	-62	4.6	67
3	Until 3.4	79	71	0	8	-18	3.9	59
4	None	190	169	1	19	-45	4.2	64
5	127	...	Alternate measurements	175	164	1	10	-28	2.5	69
6	After 4.9	276	235	5	36	-32	2.4	58
7	...	Until 0.1	After 0.1	164	143	8	12	-27	2.4	83

B. Tubes with Random Eggs

9	127	Steady	After 6.7	210	75	86	49	-0.5	2.4	(21)
10	102	...	None	196	93	68	35	-2.5	4.7	21

In the oriented tubes, the indicated number of minus and plus eggs are those which had started to form rhizoids away from and toward the light, respectively, by the initial growth stage; whereas in the laterally lit random tubes, minus eggs are those forming rhizoids toward the end of the tube from which it was loaded. NG means not yet germinated. Most of the eggs recorded as not germinated in the random tubes were probably germinated ones whose slight bulge was missed since it formed almost vertically rather than horizontally.

In all cases, a negative tube potential indicates that the end toward which most rhizoids formed was negative. Kohm/egg indicates the resistance past each egg. Times are in hours after the rise time.³ The average flow rate used was about 200 μ /sec through the tube except in #3 where it was about 50 μ /sec.

among the six tubes with well-oriented eggs subjected to steady light from one end; among these, the current averaged 63 ± 2 picoamps.

Proof that tube potentials arise from membrane-driven current loops: It is evident that the measured tube potentials were somehow produced by the developing eggs. However, it may well be asked whether—as has been assumed so far—they in fact arose from membrane-driven current loops or whether perhaps they arose from extracellular concentration gradients. Several lines of evidence serve to preclude the latter possibility.

The voltages were unaffected by rapid perfusion. They were unaffected by steady darkness, once the polarity of the eggs had been established. Moreover, they varied in the manner expected of current loop potentials upon substantial changes in the net number of oriented cells per tube (Table 1), and in resistance past each egg (Table 1), as well as a 16-fold change in the resistance of the measuring circuit.

The results yielded by tube 7 support the first two points above. After the first significant voltage appeared in it at 13 hr, it was both kept in darkness and steadily perfused at about 200 μ /sec, except for two brief observation periods.

Yet its potential developed normally during 16 hr of darkness despite perfusion fast enough to wash out the capillary every 4 min. Hence this potential could not have been produced by concentration gradients in turn somehow established by the light gradient along the capillary as a whole.

Furthermore, 200 μ /sec is fast enough to have largely destroyed any concentration gradients produced by, but outside of, each germinating cell; for even a typical small molecule, which has the relatively high diffusion constant of 1×10^{-5} cm²/sec, could have diffused less than 20 μ in the one third of a second it took the perfusion stream to move one cell diameter. Since tube 7 developed normal voltages despite such rapid perfusion during the voltage measurements, it follows that

its potential could *not* have arisen from concentration gradients produced by, but outside of, each germinated cell.

This last conclusion also follows from the results with tube 5. In it, measurements with and without perfusion at $200 \mu/\text{sec}$ were repeatedly alternated until 29 hr after fertilization. It was found that the measurements with and without perfusion fell on the same normal, rising voltage curve.

The small voltages found in both random tubes (Table 1B) are roughly accountable as current loop potentials. In each, the tube was electronegative on the end toward which most rhizoids pointed. In each, the calculated current per net egg was a third of that found in the oriented tubes. This low current figure is, at least in good part, explicable by the far larger fraction of eggs which grew out nearly across the capillary in these random tubes than in the oriented ones.

Later development: Beyond the two- to four-cell stage, the development of the embryos in all of the tubes was greatly inhibited. Few embryos ever divided into more than four cells, and the rate of elongation became increasingly subnormal. The best further development was found in the tube with the lowest cell concentration, #3. So, observations upon the later development and potentials for this tube are presented in Figure 3.

As the embryos progressed beyond the initial growth stage in this tube, the potential per embryo continued to rise. By 2 days after fertilization, when most embryos had cleaved to form three or four cells, this potential became four times higher than at the initial growth stage. Subsequently, however, practically no cell division occurred and the rate of elongation fell rapidly. As elongation slowed in these nondividing cells, the embryo potential showed a closely parallel decline. From 2 through 8 days after fertilization, the electrical output remained at about

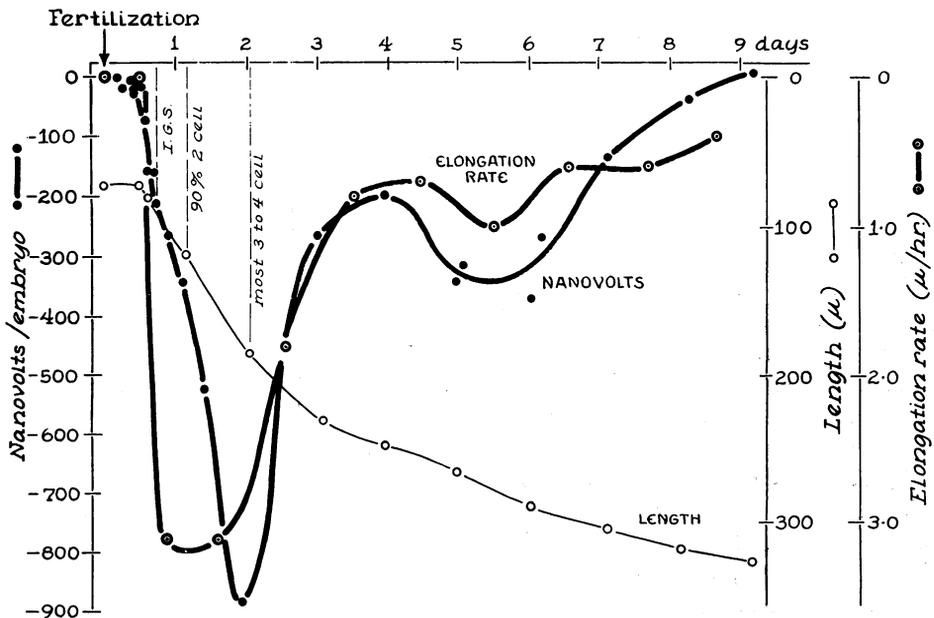


Fig. 3.—Later events in tube #3. IGS = Initial growth stage.

300 nanovolts per embryo per μ /hr elongation rate. The currents which generate these later potentials seem likely to be similar to or larger than the 60 picoamps estimated for the initial growth stage.

Cytoplasmic resistivity: A value of 230 ohm cm for the average cytoplasmic resistivity of 6-hr-old zygotes was extracted from measurements made with 1-8-Mc alternating currents upon closely packed cells at 15°C. This value is close to those which have been obtained for various other marine eggs by such methods.⁶

Discussion.—Nature and cause of the signals: The chief operations reported are measurements of the potentials across fine tubes bearing many developing egg cells in series. The detailed evidence establishes that these tube potentials are in fact indicators of currents which come to traverse each cell (Fig. 1). Therefore, they serve to measure at least the externally returned part of the intracellular currents; hence, together with internal resistivity measurements, a part of the intracellular electric forces imagined to act in developmental patterning.

The measured currents were associated with the inherent pattern of development in each egg rather than with the particular environmental vector, namely, unilateral light, used to induce parallel development of the population. This is shown, on the one hand, by the similar development of the current in light and in darkness (once parallel development has been induced) and, on the other, by the close correlation of the current's rise with the population's germination (i.e., start of elongation), as well as of its subsequent fall with the slowing of elongation within the confines of the capillary. Indeed, the association of current flow with the process of localized expansion or tip growth which results in elongation, suggests that these currents be referred to as "expansion currents."

The potential external to each cell is relatively negative in the region of expansion. Since plasma membrane potentials are always positive externally, this means that the membrane in the expansion region is relatively depolarized. It is certainly easy to imagine mechanisms whereby expansion would in fact *cause* depolarization.

Possible effects of the expansion currents: (1) To consider the effects of the currents, one must estimate their density in the cytoplasm and the resultant potential gradients. Their density through the initial outgrowth is estimated by dividing the 62-picoamp current inferred from the tube potentials by the outgrowth's cross-sectional area of 1000 μ^2 . This gives 6 μ amp/cm².

Then multiplying this by the measured cytoplasmic resistivity of 230 ohm cm gives 1.3 mv/cm as an estimate of the potential gradient in the initial outgrowth. This is believed to be a marked underestimate since these current and resistivity values are both likely to be well below the true ones.

(2) The persistence of stratification in the centrifuged *Fucus* egg during germination indicates that mixing through streaming does not occur.⁷ Hence, the trans-cytoplasmic potential gradients produced by the expansion currents must, to some degree, concentrate some negatively charged components at the growth pole.

An inevitable limitation, the only calculable limitation, and quite possibly the actual limitation upon such electrophoretic segregation is the leveling action of diffusion. To appraise this limitation, the cell interior can be considered to be a closed system subjected to a steady, homogeneous, potential gradient. Such a system will approach what may be called thermoelectric equilibrium.

The diffusion constant of a particle is inversely proportional to its diameter but its electrophoretic mobility, m , is independent of its size. Hence, at such an equilibrium, the larger a component the more concentrated it will be. Calculation, using a 1.3 mv/cm gradient and a zeta potential corresponding to the typical mobility of 1 μ /sec per v/cm in water, indicates that some particles one-half μ or larger should be substantially concentrated at the growth pole at equilibrium. However, consideration of the factors which make 1.3 mv/cm an underestimate and of the fact that some bacteria, at least, have zeta potentials more than four times the value assumed⁸ suggests that some particles as small as 0.01 μ might be so concentrated.

(3) If different ion species traverse the membrane where the current enters and leaves the cell, then the expansion current may directly create large qualitative ion gradients. For even if one takes the conservative estimate of 6 μ amp/cm², one obtains ionic concentration changes of 0.07 M per hour in a 30- μ long cell. The possible significance of such changes is shown by recent evidence that ion concentrations may be immediate controls of gene activity.⁹

(4) The germination site in the *Fucus* egg is not irreversibly determined until about the time of germination itself.³ Hence, one may wonder whether this final determination involves a positive feed-back loop which has transcellular current as a component. In fact, this possibility receives some support from a study which shows that *Fucus* eggs subjected to K^+ gradients tend to germinate toward the high K^+ end.¹⁰

(5) A lower estimate of the fraction of the egg's metabolic power used in driving these expansion currents is obtained as follows: In the dark, during the initial growth stage, at least 83 picoamps, or 8.7×10^{-16} equivalents/sec, traversed the egg and hence were pumped through at least one membrane. A previous study showed the germinating egg to consume about 1.5×10^{-15} moles O_2 /sec at 15°C.¹¹ The most efficient ion pump known, which is in the vertebrate kidney, pumps 28 ions per O_2 .¹² If the *Fucus* egg were that efficient, it would use $8.7 \times 10^{-16}/28 \times 1.5 \times 10^{-15} = 2.1\%$ of its respiratory power to pump the expansion currents.

The literature: This paper reports a measurement of transcellular currents likely to play a role in patterning. No clearly comparable information now exists. However, Scott *et al.*¹³ have reported measurements of current loops through the growing bean root which may be related. Local densities¹³ can be about 1 μ amp/cm². Moreover, the large and rapid effects of low auxin concentrations on these root currents suggests some role for them in controlling the rate of elongation.¹⁴

Sonneborn has referred to self-assembly and macrocrystallinity as two established principles underlying cellular differentiation.¹⁵ This report would seem to support Spek's self-cataphoresis as a third such principle.

Summary.—(1) At least 60 picoamps (6×10^{-11} amps) begins to flow *through* the normally developing *Fucus* egg when it begins to elongate, or "germinate." This current, considered as a flow of positive ions, enters the egg at its basal or elongation pole. It continues to flow as the embryo elongates, at a rate which seems to be proportional to this elongation rate.

(2) Calculation indicates that such a current may generate a sufficient potential gradient across the cytoplasm to concentrate significantly some relatively large and negatively charged particles at the expansion pole, and also may directly produce substantial ion gradients through the embryo.

This paper is dedicated to my friend and fellow scientist, Dr. Albert Tyler, on the occasion of his sixtieth birthday.

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⁵ The time when germination began varied between 10 and 14 hr after fertilization. Hence, it proved most practical to define the initial growth stage as the time 6 hr after the rise time, when germination and the voltage rise began. This, in turn, was defined by the intersection of the linear portions of the S-shaped germination and/or voltage curves with the X-axis.

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