

THE PULSE CURRENT PATTERN GENERATED BY DEVELOPING FUCOID EGGS

RICHARD NUCCITELLI and LIONEL F. JAFFE

From the Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907

ABSTRACT

Using a newly developed extracellular vibrating electrode, we have made the first study of the spatial distribution of the growth currents around a single developing egg. This pattern was studied during the current pulses which traverse two-celled *Pelvetia* embryos. These pulses can be stimulated to occur with a periodicity of 70 min by mild acidification of the sea water medium. Current enters only at the growing rhizoid's tip while leaving both the base of the rhizoid cell and the whole outer membrane of the thallus cell. The field in front of the rhizoid cell falls off as the inverse cube of the distance from the rhizoid cell's center in the manner of a dipole field. The total inward and outward currents are equal, agreeing with theory. The current density at the rhizoid cell's base is twice that at the top of the thallus cell and this probably represents a change in the outer membrane's properties. There are no significant differences in the current density over the thallus cell. These results suggest a model in which the pulse current leaks in through newly opened channels in the growing tip and leaks out elsewhere due to the resultant fall in the membrane potential.

This is the second paper reporting on the transcellular current pulses generated by the fucoid alga *Pelvetia*. 9 yr ago it was found by using a multicellular method that the developing egg of this common West Coast seaweed drives an electrical current through itself along its growth axis (1, 2). Since the spatial resolution of the multicellular method was poor, the detailed current pattern around a single egg could not be determined. To improve resolution we have developed a new ultrasensitive vibrating electrode system which measures slowly changing extracellular fields (4). Using this system we have made the first measurements of the current-generated field around a single developing fucoid egg. Here we determine the pulse current pattern generated by a two-cell embryo by measuring this field in many positions around the embryo.

MATERIALS AND METHODS

Zygotes of the seaweed *Pelvetia fastigiata* were obtained as previously described (3), and cultured at 15°C in natural sea water. 250 foot candles of unilateral white light were applied to orient growth in the horizontal plane needed for the current measurements.

The sensor of our extracellular current measuring system is a vibrating probe with a spherical, 30- μ m platinum-black electrode at its tip which measures voltages with respect to a coaxial reference electrode (4). The probe is vibrated at about 200 cycle/s in a horizontal plane between two extracellular points 30 μ m apart. Vibration between these points converts any steady voltage difference between them into a sinusoidal output measurable with the aid of a lock-in amplifier tuned to the vibration frequency. Since the electric field will be nearly constant over this small distance, it is approximately equal to the voltage difference divided by this distance. The current density in the direction of vibration

and at the center of vibration is then given by this field multiplied by the medium's conductivity.

We have found that the current pulses can be stimulated during the two-cell stage by slight acidification of the sea water with HCl from the natural pH of 8.2 to between 6 and 6.5. By using this technique we could systematically study the pulse field at various positions near the embryo as well as investigate the field's fall off with distance.

The initial procedures for these two types of experiments were identical. *Pelvetia* eggs were fertilized and allowed to develop in natural sea water until the first cell division around 24 h after fertilization. The culture dish was then moved to an inverted microscope and the vibrating probe was positioned outside the embryo at the desired point. The medium was exchanged with the lower pH sea water over a 2-min period, using a coupled, two-syringe system so that the amount of new medium added was exactly equal to the amount of liquid withdrawn. This was done to maintain a constant fluid level which minimized probe disturbance.

RESULTS

During the two-cell stage the pulses account for about one-fourth of the total spontaneous current and occur at an average frequency of 5 per hour (8). Some typical current pulses are illustrated in Fig. 1. They normally occur quite sporadically, often in large groups with very few pulses for hours in between. This makes it difficult to carry out a systematic study of the current pattern, so we sought ways to stimulate pulsing. After unsuccessful attempts with bursts of light and externally applied current, we found that slight acidification of the medium often stimulated pulses within a few minutes of the change and once or twice thereafter at 70–80-min intervals as shown in Fig. 2. This periodicity was observed for both the inward pulses at the rhizoid tip (Fig. 2 a), and the outward pulses at the rhizoid base and the thallus cell (Fig. 2 b).

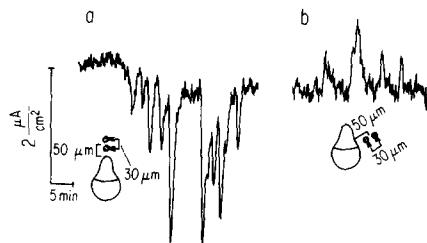


FIGURE 1 (a) A group of inward current pulses recorded in front of a 1-day old growing *Pelvetia* embryo. (b) A group of outward current pulses recorded normal to the base of the rhizoid cell. Indicated current densities are at the point of measurement.

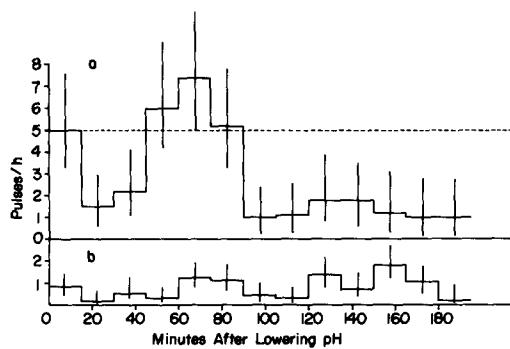


FIGURE 2 Pulse frequencies at various times after lowering the pH. (a) A total of 118 inward pulses were measured at the rhizoid tip. The dotted line represents the average pulse frequency at this stage under natural conditions. (b) A total of 100 outward pulses were measured leaving the thallus cell and rhizoid cell's base. Bars in both histograms indicate the 90% confidence limits for the expectation of a Poisson variable (9).

This suggests that the pulses seen entering the rhizoid are the same ones leaving the rest of the embryo rather than representing independent events at each position.

We also find that these stimulated current pulses have the same shape and average amplitude as the spontaneous pulses. The average frequency for the stimulated pulses is actually lower than the spontaneous frequency but the pulsing is much more regular and predictable. Thus, during the 0.5-h interval around the 70-min mark we could expect about three pulses to occur.

In order to determine the pulse current pattern we have studied 80 two-celled embryos with a total observation time of 236 h in acidified sea water. The stimulated pulses were measured at seven different positions around the embryo (shown in Fig. 3) while the probe was vibrated as close to the embryo as was safe.¹ We usually vibrated the probe normal to the embryo's surface to measure the component of the current crossing the membrane in that area. We have found that positive current enters only at the growing tip of the rhizoid cell and leaves the embryo at all points below the rhizoid cell's center. Fig. 3 summarizes the results of this study, indicating the number of pulses and the current direction in seven positions around the

¹ The smallest safe gap between the probe and the plasma membrane is about 20 μm. If the probe gets much closer it may hit the intervening cell wall and yield artifactual signals.

Probe Position	No. Eggs observed	Total h observed	No. of Pulses	Pulse Direction
	14	42	118	inward
	10	30	10	4out;6in
	14	29	72	towards tip
	16	33	40	outward
	8	33	17	outward
	8	36	15	outward
	10	33	28	outward

FIGURE 3 Current pulse data summary.

embryo. The largest number of pulses per unit time was recorded entering the growing tip where the current density is the highest. The larger the current density, the larger the number of pulses which will be above the noise level of our vibrating probe. We recorded fewer pulses per unit time at other points around the embryo because the current densities were smaller and more pulses fell below the noise level, this becoming undetectable. The peak-to-peak noise level in sea water is typically $0.3 \mu\text{A}/\text{cm}^2$ over a 2-min interval using a 10-s time constant. Only pulses with amplitudes greater than this noise level were counted.

When vibrating normal to the center of the rhizoid cell only 10 pulses were observed in 30 h, and of these 10, four were outward and six were inward pulses. Since the pulse frequency is so low here and the net current is essentially zero, the center of the rhizoid cell appears to be the center of this stimulated pulse *current dipole*.²

In order to plot this current pattern we must determine the relative current density during a pulse at many points around the embryo. Since all the current enters at the rhizoid tip, the current pattern can be described by expressing the outward current density at each position as a fraction of the inward rhizoid current. However, we had only one vibrating probe and could not move it fast enough around the embryo to observe both an entry and an exit position during a single pulse. We solved this problem by recording the pulses in each position

for many hours and plotting the pulse amplitude distribution for each position. By then comparing this amplitude distribution with that at the rhizoid tip we could determine the attenuation factor for each point. Fig. 4 illustrates this method of comparing amplitude distributions. The amplitudes are plotted on a log scale so that the multiplicative attenuation factors can be determined by shifting one curve over to coincide with another (analogous to the slide rule principle) and then taking the ratio of amplitudes. The relative current densities determined in this way are listed in Fig. 6 as they would appear during an average current pulse. This procedure is based on the fact that an inward current pulse at the rhizoid tip must result in a corresponding outward pulse along the lower area of the embryo to complete the current loop and the assumption that the probe is not disturbing the embryo (the basis for this assumption is discussed later).

The results presented thus far are based on measurements of current densities $50 \mu\text{m}$ from the embryo's surface. Since we want to know the current density crossing the cell surface we must extrapolate to this surface based on the field's fall off with distance. We determined this fall off in front of the rhizoid tip with the second set of experiments.

The basic approach of the fall off study was to measure the field during the same pulse³ at two different distances from the rhizoid tip. This was possible because we could move the probe between

² This should not be confused with an electrostatic dipole formed by two opposite charges. Here we have an ion *current* entering one end of the rhizoid cell while leaving the opposite end, and thus forming a *current* dipole with its center at the center of the rhizoid cell (6).

³ Pulses complete their initial rise in about 20 s and have an average duration of 100 s. For about 30 s after the initial rise, their amplitude is large enough for easy measurement while falling slowly.

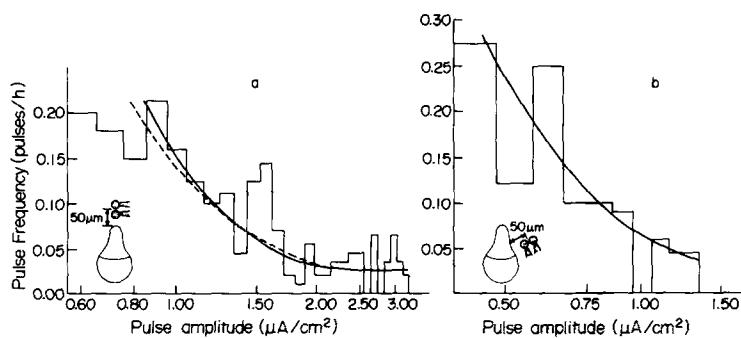


FIGURE 4 A comparison of the pulse amplitude distributions at two positions. (a) The amplitude distribution of 118 pulses entering the rhizoid tip. The solid line is an approximate fit to the histogram. The dotted line is the curve in Fig. 4 b shifted over to estimate the relative current density at the rhizoid base. The frequency is the number of pulses in the indicated amplitude range divided by the total observation time. Indicated amplitudes are at measuring point. (b) The amplitude distribution of 40 pulses measured leaving the base of the rhizoid cell. Both Figs. 4 a and b are plotted on a semilog scale.

two points in 2 s while recording with a 3-s time constant. So within 9 s after moving to a new position we could measure 95% of the signal. After a pulse had completed its initial rise we moved back and forth between two positions in front of the rhizoid (as illustrated in Fig. 5), spending 11 s for each measurement. This was done a number of times and the ratios of the current densities in the two positions were averaged resulting in one point in Fig. 5. We did this for three pairs of positions 13, 23, and 37 μm apart with a total of 52 measurements on 12 different two-celled embryos. Since the center of the rhizoid cell is the center of the current pattern, the probe's position in this field was considered as the distance between that point and the center of vibration. The starting position was always 100 μm from the rhizoid cell center and we then moved back and forth between this reference position, \bar{r} , and one of the other positions, r , farther from the tip. The solid lines represent the theoretical inverse square, cube, and fourth power field fall off.

$$i/\bar{i} = (r/\bar{r})^{-n} \text{ for } n = 2, 3, 4.$$

The experimental points agree quite well with the theoretical inverse cube fall off as expected for this current dipole field (6).

This result made the extrapolation at the rhizoid tip straightforward. The average distance from the rhizoid cell's center to its tip is 50 μm and it is another 50 μm to the center of vibration. Consequently, the pulses measured outside the tip would be expected to be $(100/50)^3 = 8$ times larger at the surface. To determine this extrapolation factor for

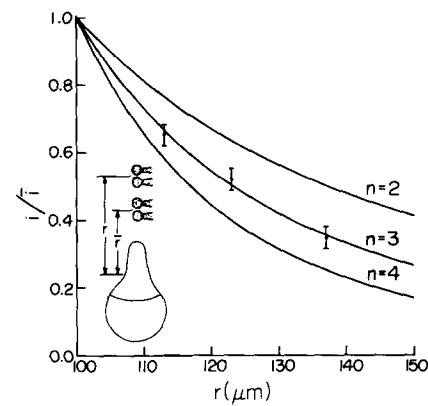


FIGURE 5 Relative current density vs. distance from the rhizoid cell's center. Reference current density, \bar{i} , was measured at a distance $\bar{r} = 100 \mu\text{m}$ in front of this center. Bars indicate standard errors. Solid lines are plots of $i/\bar{i} = (r/\bar{r})^{-n}$ for $n = 2, 3, 4$.

the other positions studied we could not determine the fall off empirically because the pulses were generally much smaller. Instead we have based the extrapolation on the observed field pattern. Since the rhizoid cell has current both entering the tip and leaving at the base, the pattern resembles a dipole, and indeed falls off like a dipole in front of the tip. Therefore, as a first approximation to the fall off at the base of the rhizoid cell we again used the inverse cube relation.

However, only outward currents were measured at the positions around the thallus cell which are relatively far from the current sink at the rhizoid tip. The current pattern here thus resembles that surrounding an isolated current source. This field

is known to fall off with the inverse square of the distance. Therefore in calculating the extrapolation factors for the thallus we have assumed that the field falls off with the inverse square of the distance from the center of this cell. These extrapolation factors are all listed in Fig. 6.

The final current density pattern at the surface is arrived at by multiplying the measured current

density at the center of probe vibration by the corresponding extrapolation factor. This pattern is illustrated in Fig. 7. There are large changes in the membrane current densities between the tip and rhizoid center, between this center and the rhizoid base, and between the rhizoid base and the thallus cell. No net current crosses the membrane at the center of the rhizoid cell. This is a membrane

Probe Position	Current density at point of measurement $\mu\text{A}/\text{cm}^2$	Extrapolation factor	Current density at embryo surface $\mu\text{A}/\text{cm}^2$	Surface area which current crosses $\text{cm}^2 \times 10^3$	Current crossing membrane pA
	-1	8 ± 3	-8	3.8	-304
	0	-	0	-	0
	+0.8	-	-	-	-
	+0.6	3.2 ± 2	+1.9	8	+152
	+0.3	3.5 ± 1	+1	10.5	+105
	+0.2	3.5 ± 1	+0.7	9.3	+65
	+0.3	3.5 ± 1	+1	3.8	+38
				total current	+56

FIGURE 6 Current densities measured around the two-celled embryo during an average current pulse. The extrapolation factors listed are extrapolations to the embryo's surface \pm the maximum estimated error.

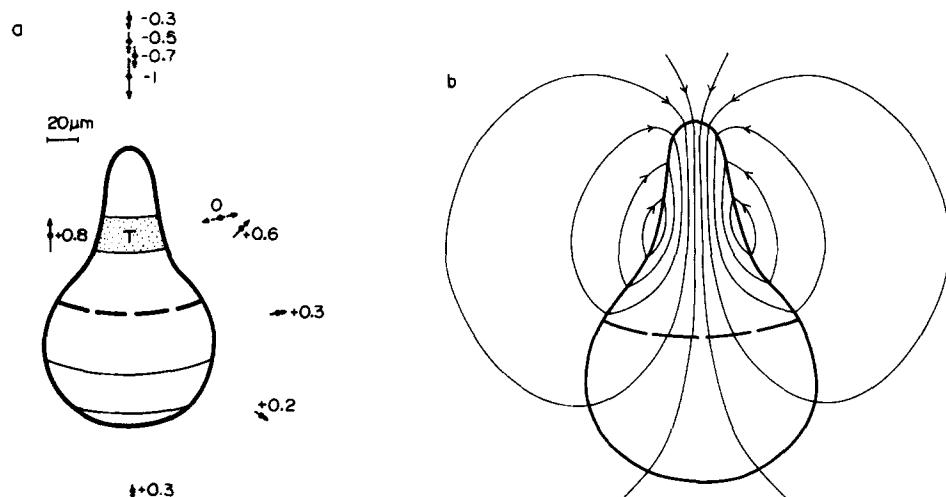


FIGURE 7 Current pattern generated by the two-celled *Pelvetia* embryo. (a) Numbers indicate the current densities ($\mu\text{A}/\text{cm}^2$) at the indicated measuring positions during an average pulse. The arrows represent the magnitudes and directions of the currents along the measurement axes. The surface area zones used in the calculation of the total current are drawn to scale. *T* marks the membrane transition zone. The dashed line represents the cross wall between the two cells and is also a zone boundary. (b) The spatial current pattern inferred from the current density measurements. Relative line densities indicate relative current densities (line densities are to be read per unit length normal to the lines).

transition region over which the current crossing the membrane reverses direction. There is no significant change in the current density along the thallus cell, but the current density at the rhizoid base is twice that at the thallus, suggesting a change in the membrane properties over that region.

One check on the credibility of this current pattern would be to determine if the total inward and outward currents were equal. We have made this calculation by multiplying the embryo's surface area corresponding to each probe position by the current density crossing that area. The area of current entry was found to extend 30–50 μm below the rhizoid's tip. We somewhat arbitrarily assumed that the inward current density over the terminal 40 μm is the same as that at the tip and is negligible thereafter. The outward current region was divided into four zones as indicated in Fig. 7a. The areas of all of the zones and their corresponding current densities are listed in Fig. 6. The calculated total inward and outward currents differ only by 18%. While the integration procedure is somewhat arbitrary, this result still helps to verify the current pattern shown in Fig. 7b.

DISCUSSION

These extracellular current measurements give the first detailed picture of the electrical current pattern around a single growing embryo, and show that the field in front of the growth tip falls off as the inverse cube of the distance from the center of the rhizoid cell. This field fall off agrees well with the theory for a dipole field pattern and thus lends support to the reliability of the measurements. This reliability is also supported by the near equality of the total inward and outward currents calculated from the measured current densities.

The largest current density in this field is found where current enters the rhizoid tip. This is also the area with the greatest morphological change and the site where new membrane and cell wall appear. The evidence for this tip growth is partly based on observations of embryos after coating them with 0.1- μm anion exchange beads. After some further growth one sees a strikingly bald, bead-free anterior region which is about as long as the total increase in length after coating the wall. Tip growth is also indicated by more exact demonstrations in a variety of similar systems, e.g., growing *Chara* rhizoids (10). It therefore appears that current entry is restricted to the growth region of

the embryo. This fact leads to a simple model of membrane control.

The simplest assumption is that this current entry at the tip during a pulse is a result of the opening of leaks or channels in the growth region. The current would then leave elsewhere only because of the resultant few millivolt fall in the membrane potential (12). There is therefore no need to assume any change in the membrane's properties other than at the growth point. We will see that this simple model is also supported by the homogeneous current density leaving the thallus cell.

The current densities around the rhizoid cell vary a great deal suggesting very sharp resistance changes along the rhizoid membrane. The first large membrane change appears between the active tip and the rhizoid center where no current passes at all. This suggests that the membrane resistance of the transition region at the rhizoid center is much larger than at the tip where there appears to be a localization of ion channels. The second change appears between the center and base of the rhizoid cell membrane. At the base there is a large outward current density which is one-fourth of the inward current density at the tip. The third sharp change is across the cross wall between rhizoid and thallus cells. The current density falls in half in this region, suggesting either that the membrane between the two cells poses a considerable resistance to the current or that there is a concentration of channels at the rhizoid base. We believe that the latter is more probable for two reasons. First, intracellular measurements using a two-electrode method indicated that no very gross electrical barrier could be normally present between these cells (11). Secondly, most plant cells are known to have plasmodesmata between them which should allow easy ion movement between cells. Hence, the change in current density in this region is more likely to be due to a concentration of channels at the rhizoid base.

The pulse current densities near the thallus cell membrane at the positions studied show no significant variation along the membrane. This, along with its unchanging and uniform appearance, suggests that the thallus cell's outer membrane presents a fixed and uniform resistance to current "injected" into this cell during a pulse. This conclusion is also supported by intracellular potential measurements which record the pulses as relatively small membrane depolarizations of only

2–6 mV (12). If the membrane resistance changed during a pulse, we would expect much larger membrane voltage changes. This fixed and uniform thallus resistance is consistent with the simple model presented in which the growth tip is the active membrane region.

A comparison of the magnitude of these voltage changes with the predicted depolarizations based on resistance and extracellular current data leads to a further insight concerning the membrane's properties. Measurements of the fucoid embryo's *average* membrane resistance when it is not pulsing yield a figure of $1.2 \text{ k}\Omega/\text{cm}^2$ (12). The larger depolarizations of about 6 mV presumably correspond to the larger pulses in which a peak of about $1 \mu\text{A}/\text{cm}^2$ leaves the thallus cell. $6 \text{ mV}/1 \mu\text{A}/\text{cm}^2$ yields a value of $6 \text{ k}\Omega/\text{cm}^2$ for the thallus cell's membrane resistance during a pulse. If we reject the unlikely possibility that this resistance increases during a pulse, the discrepancy between these two values (6 and $1.2 \text{ k}\Omega/\text{cm}^2$) suggests that even during nonpulsing times when only the steady component of the current traverses the cell, the thallus cell's membrane resistance is much higher than average. This implies that the rhizoid membrane resistance is probably an order of magnitude *lower* than average. This inference is easily consistent with our working model in which current leaks into the growing region of the embryo.

The systematic study of the current pattern was possible due to the improved control over pulse occurrence which followed the sudden increase in hydrogen ion concentration. This sudden increase imposed a 70-min periodicity on the pulsing and considerably increased the pulse frequency around the 70-min mark. Such a response suggests that the pH change is affecting some internal control mechanism which somehow influences the likelihood of pulse occurrence. This may be similar to the slowly changing global control factor suggested in the first paper (8) to explain the occurrence of consecutive pulses with the same amplitude.

We think that this apparent internal control is strong evidence that the vibrating probe is *not* stimulating the electrical activity. There are two further points supporting this conclusion. First, intracellular measurements indicated the same number of pulses per unit time in the absence of vibration (12). Secondly, both pulse frequency distributions in Fig. 2 show the same three peaks at the same times regardless of probe position. So we find the same pulse frequency pattern even when the probe is far from the active rhizoid tip region.

There does seem to be a small discrepancy in the magnitudes of the pulse frequencies in the third peak which may suggest slight probe interference after several hours, but there is no suggestion of probe stimulation.

The description of this extracellular field naturally raises the question of cell interactions by way of the field. Can one embryo influence the growth direction of another through its extracellular voltage gradient? We think that this is quite unlikely. We have confirmed Lund's 1923 report that at least 10 mV must be imposed across a developing fucoid egg to initiate rhizoid growth at the egg's positive pole (7, 5). However, even if one egg were touching the rhizoid tip of another, the voltage gradient across the egg due to current entering the tip of the other would be only about $1 \mu\text{V}$ during the large pulse and less on the average. Thus, the natural extracellular field is at least 10,000 times too small to orient a neighboring egg.

Finally, we want to emphasize that this current pattern is based on current *pulse* observations and may not represent the pattern for the steady current. We have not yet studied the steady pattern because this current is much smaller and very near the vibrating probe's noise level. However, when the average steady current decreases, the current in the pulses increases under natural conditions (8), so these two components of the current complement each other and probably have the same general function. We are not certain of this function, but the pulse current pattern is consistent with our working hypothesis that the current provides an electrical force to pull vesicles to the growth point. The pattern shows that the internal current (and thus the internal field) is concentrated in the region beneath the growth tip. This hypothesis is also supported by the earlier findings that the growth rate is proportional to the steady current and that this current begins before germination (1, 8). Since pulse function would be clearer if we knew the ionic composition of this current, we plan to determine the main ions involved by studying the pulses while varying extracellular ionic concentrations.

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