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Large electrical currents traverse developing Cecropia follicles

(pattern formation/oogenesis/Lepidoptera/vibrating probe/germ plasm)

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ABSTRACT **An intense (up to 20 $\mu A/cm^2$) steady electrical current enters the anterior or nurse cell end of the growing follicle (or oocyte–nurse cell complex) of the Cecropia moth and is balanced by a more diffuse current leaving elsewhere. In late growth stages, the total transfollicular current is about 100 nA. Moreover, a separate small current, of about 1 nA, seems to leave the furrow between the oocyte and the nurse cells. After the nurse cells collapse, but before shell formation, the transfollicular current is redistributed so that a second relatively localized inward current appears at the posterior pole of the follicle. Thus, at this later stage currents enter both poles of the follicle and leave its sides. Previous measurements, with intracellular microelectrodes, seem to imply a very large (order of 1000 nA) back current across the cytoplasmic bridge between the oocyte and nurse cells. A simple model is presented that attributes the apparent bridge current, and the more directly measured transfollicular and furrow currents, to the action of an ion pump lying within the nurse cell face of the furrow membrane.**

The mechanisms that establish pattern are a central problem of developmental biology, and an important division of this question concerns the mechanisms that polarize developing eggs or spores. In fucoid zygotes (1, 2) and in lily pollen (3) a relatively steady electrical current enters the prospective growth pole hours before this region is irreversibly fixed.‡ The early current that enters the prospective growth pole of fucoid eggs (4) and at least the functioning growth tip of growing pollen tubes (5) consists, in significant part, of calcium ions, and there is evidence to support the hypothesis that this inward current, particularly its calcium component, is part of the polarizing or localizing mechanism itself (ref. 6, pp. 464–465).

These early currents were in part measured and analyzed with the aid of a new tool, the socalled ultrasensitive vibrating probe for measuring steady extracellular currents (7). Because these currents are one of the few clues we have to the polarizing mechanisms and because the vibrating probe should be applicable to most developing eggs, it was of obvious interest to extend this exploration to animal eggs. We elected to begin with the oocytes, or rather the follicles, of the Cecropia moth because of some observations by one of us, by conventional electrophysiological techniques, that seem to indicate a flow of significant steady electrical currents through the follicle (8, 9). The Cecropia egg, like that of most insects, forms in a sort of assembly line called an ovariole (Fig. 1). The developing oocytes move down much of the ovariole while connected by broad cytoplasmic bridges to an anterior cap of seven siblings called nurse cells. During this prolonged phase, the highly polyploid nuclei of the nurse cells make masses of RNA which pour through the front end of the oocyte (10). Late in the process of yolk deposition and of growth, when the follicles are about 1.5 mm long, the nurse cells atrophy and collapse.

Woodruff and Telfer inserted conventional microelectrodes into follicles 0.9–1.2 mm long, a stage towards the end of yolk deposition or vitellogenesis but well before collapse of the nurse cells. They found the oocytes to be about 9–11 mV positive to that of the nurse cells (8) and recorded a resistance between them of about 5–20 kΩ (9). These measurements would imply the steady flow of current through the bridge(s) from oocyte to nurse cells. Furthermore, they imply a bridge current of the order of 10 mV/10 kΩ or 1 μA in this direction. On the other hand, when follicles of this stage were dissected away from neighboring follicles and squeezed into a 0.6-mm-wide capillary tube, the outside of the nurse cell end was generally found to be about 5–10 mV negative to the outside of the oocyte end. This would imply the steady flow of some current, of unknown size, along the outside of the follicle from oocyte to nurse cell and, hence, a return of this transfollicular current somewhere through the inside of the follicle from the nurse cell end to the oocyte end. Thus, a transfollicular current of unknown size seemed to flow opposite to a bridge current of about 1 μA at this stage (9).

These findings are of obvious interest. Yet, because of the real possibilities of injury currents produced by puncture damage (particularly of the oocyte) or by dissection or compression damage (particularly at the nurse cell end) or both, even the existence of one or both of these currents remained rather uncertain. We have thus explored the currents through developing Cecropia follicles with a vibrating probe for measuring steady extracellular currents. This method should be relatively reliable, since follicles are not dissected apart, squeezed, punctured, or even touched by the vibrating probe. Furthermore, it yields quantitative information about most of the pattern of electrical currents traversing the developing follicle's surface. Except for a preliminary report on currents through the medaka zygote (11), this is the first such report on an animal system. A preliminary report has appeared earlier (12).

MATERIALS AND METHODS

Ovariole sections were dissected out of *Hyalophora cecropia* pupae on day 17–19 of the pupal–adult molt and freed of the ovariole sheath. Such isolated ovariole sections consist of a series of follicles connected by interfollicular stalks of follicle cells. A follicle consists of an oocyte–nurse cell complex surrounded by a relatively thin layer of follicle cells. They were dissected into a dissecting solution developed by Anderson and Telfer (13) for studying the mechanism of vitellogenesis (40 mM KCl/15

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‡ We follow the standard convention that current moves in the direction of positive charge movement.

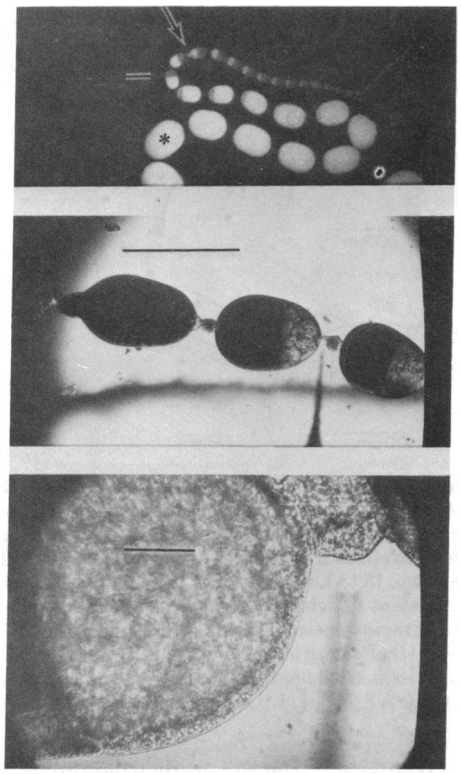

FIG. 1. Cecropia follicles with vibrating probe in test positions. Scale: *Top* and *Middle*, 1 mm; *Bottom*, 0.1 mm. The nurse cell cap is to the right (and the oocyte to the left) of the follicles in the bottom two figures and in the arrowed one above. Asterisk indicates a follicle after collapse of the nurse cells.

mM $MgCl_2$/5 mM $CaCl_2$/110 mM Tris succinate, pH 6.2).§ They were maintained (and examined with a vibrating probe) in Anderson's medium supplemented with about 10% of blood from diapausing pupae. This measuring medium had a resistivity at 20°C of 98 Ω·cm. The vibrating probe system used has been described (7, 15).

RESULTS

Vitellogenic follicles

Fig. 2 shows a typical recording of current densities normal to a Cecropia follicle during a vitellogenic stage. The most prominent feature of this recording is a relatively intense current flowing into a region close to the anterior pole of the follicle, while the most intense outward current measured leaves close to its posterior pole. (Measurements could not be made exactly at the poles of a follicle within an intact ovariole because of the interfollicular stalks.)

Fig. 3 *A–C* and Table 1 summarize the results of many similar recordings. These included about 100 current density measurements on 15 follicles at various stages of vitellogenesis. The pattern of current normal to the follicles is essentially the same throughout vitellogenesis, from early vitellogenic follicles about 0.3 mm long to late ones about 1.2 mm long. There is always a strong peak of inward current density at the most forward point that is accessible for measurement and two smaller peaks of outward current density, one at the most posterior accessible point and the other at the furrow. The transition from inward to outward current occurs somewhere towards the rear of the nurse cell cap, and (with one exceptional data point) current always leaves the whole accessible surface of the oocyte.

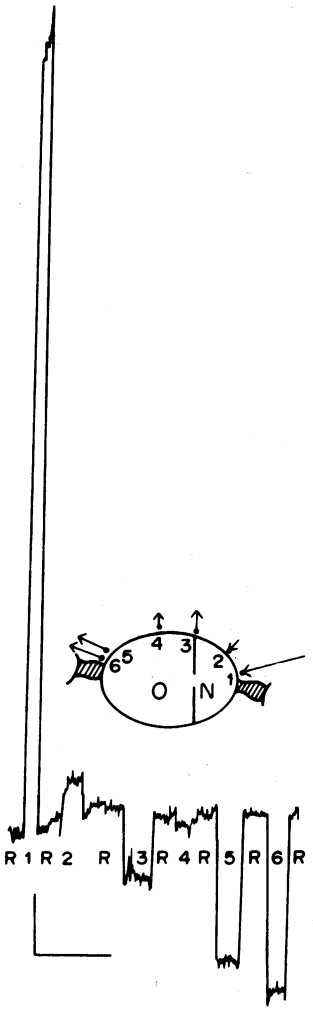

FIG. 2. Tracing of a representative record of current densities normal to a midvitellogenic (900 μm long) Cecropia follicle. Scale: 1 μA/cm² against 5 min. R indicates probe is in a reference position about 500 μm away from follicle; 1–6 indicate probe is in one of the positions indicated by the dots on the sketch. O, oocyte; N, nurse cell cap. Follicle studied remained attached to ones on either end of it by interfollicular stalks indicated by crosshatching on the sketch. Follicle 12 was studied on 4/19/1977. A control scan around a 200-μm glass bead showed no detectable changes with position.

In mid and late vitellogenic stages, where the measurements are most reliable, the calculated *net* current that enters a follicle is only about half of that which leaves it. We would suppose that the missing inward current enters the unexplored front polar regions of the follicles. Moreover, the true net transfollicular current should only slightly exceed the net measured outward current since little current is likely to leave the unexplored rear polar region. Thus a bit more than 50 nA of steady current flows through a late vitellogenic follicle.

We would suppose that the peak of current leaving the furrow originates well inside of it and should be thought of as a current separate from the main transfollicular current. One can estimate the size of this furrow current in late vitellogenic fol-

§ Anderson's medium was developed in part because of its similarity to the inorganic composition of the hemolymph of Cecropia pupae (14) and in part because it supports prolonged uptake of trypan blue *in vitro*, an uptake which is considered to indicate continuation of the normal uptake of yolk precursors via endocytosis. However, whereas most of the major ionic concentrations in Anderson's medium are close to the natural level, Cl^- and Na^+ ions are not. Cl^- ions are 10 times natural (80 mM compared to 8 mM), while Na^+ ions are missing (which compares with the 7 mM natural level).

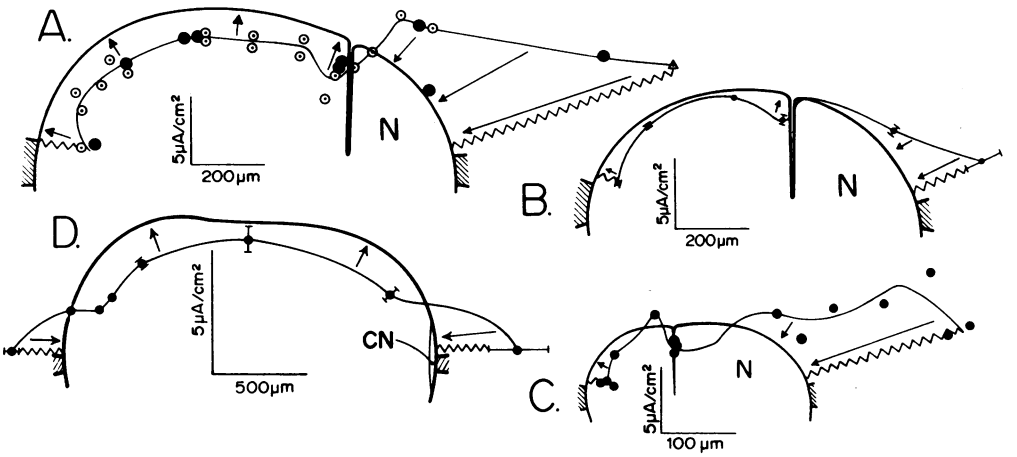

FIG. 3. Current patterns normal to the surface of Cecropia follicles. Heavy line indicates half the outline of a follicle. N, nurse cell cap; CN, collapsed nurse cells. Each data point is plotted so that its distance from the nearest section of the follicle surface indicates the current density measured near this section. As the arrows suggest, points outside of the follicle indicate inward current while ones inside indicate outward current. Serrated lines show boundaries of the explorable regions. (*A*) Late vitellogenic pattern. Graph is a composite of measurements made on both sides of three different follicles, nos. 16 and 17 (⊙ and ●), which were 1000 μm long, and no. 18 (▲), which was 1125 μm long. The curve ignores one anomalously low value. (*B*) Midvitellogenic pattern. Composite data from nine different follicles, 700–900 μm long. Each value plotted is the average of 7–10 measurements (except for the value 150 μm behind the fold, which averages 3 measurements). Bars indicate SD of the mean. (*C*) Early vitellogenic pattern. Composite data from follicles 1–3, each about 300 μm long. (*D*) Pattern after collapse of the nurse cells, but before chorion formation. Composite of measurements from nine different follicles each about 1800–2000 μm long. Starting at the nurse cell end, the following number of measurements were averaged or used to give each plotted data point: 9, 6, 3, 3, 1, 1, 1, and 8. Error bars indicate SD of the mean.

licles by multiplying the local increase in current density (of 1–2 $\mu A/cm^2$) by the area of the band around the mouth of the furrow. The radius of the band at this stage is about 340 μm, while the width of the band of extra outward current is of the order of 30 μm. Together these yield a band area of the order of 10^{-3} cm^2 and thus a net furrow current of the order of 1 nA, two orders smaller than the transfollicular one.

Fig. 4 shows the tangential rather than the normal component of current flow near the surface of a late vitellogenic follicle. Current flows forward over the whole accessible surface of the follicle. Moreover, the intensity of this forward current grows steadily as one moves forward, with much of the change occurring near the furrow. Thus it rises slowly from a density of 0.4 $\mu A/cm^2$ near the rear pole to 1 $\mu A/cm^2$ at a point 80 μm behind the furrow, jumps to 6 $\mu A/cm^2$ at a point 40 μm in front of the furrow, then rises further to 17 $\mu A/cm^2$ at the most forward point explored. Altogether, this tangential pattern is fully consistent with the inferences drawn from the normal one: current flows forward from the oocyte and furrow sources towards the nurse cell sinks; the forward current jumps near the furrow as current from the furrow joins it; it is quite concentrated and still rising en route to the inferred main sink at the front pole.

Most of our measurements were made with the probe as close as practical to the follicle surface. For measurements of the tangential component, this means that the probe center was about 25 μm from this surface (as the drawing indicates). However, we explored the decline in current density as one moves away from the follicle. The results of one such study are also shown in Fig. 4. When the probe center reached a point about 80 μm away, the tangential current density fell to half of the value recorded 25 μm away and was still easily measurable at a point 170 μm out.

Fig. 5 shows the results of an experiment in which we poisoned a late vitellogenic follicle with 0.5 mM dinitrophenol and followed the resulting changes in current density normal to the anterior pole of the follicle as well as to its furrow. The drug effected a striking reversal of both currents, and this reversal was at least partially reversible by removing the drug.

Follicles after nurse cell collapse

Fig. 3*D* shows the pattern of current normal to the surface of follicles in the period between nurse cell collapse and chorion formation. This figure pools measurements made on nine separate "post-collapse" follicles. Like follicles in earlier stages, these post-collapse follicles show a relatively large current entering their most anterior accessible point. By this stage, however, the anterior pole current enters a region over the shrunken remnants of the nurse cells, which are supposed to be disconnected from the oocyte (16, 17). Moreover, as before, less intense

Table 1. Average transfollicular current values

Fig. and stage	No. of follicles	Peak densities, $\mu A/cm^2$ In	Peak densities, $\mu A/cm^2$ Out	Net current, nA* In	Net current, nA* Out
3*A*: Early vitellogenic	3	12, (1)†	1.3	1.5	1.1
3*B*: Midvitellogenic	9	8	2	4	9
3*C*: Late vitellogenic	3	18	4	30	50
3*D*: After collapse	9	3, (2)†	1.3	7	90

* Net currents were obtained by integrating over the surfaces of revolution implicit in the follicle sections shown in Fig. 3. (Note that integration by inspection can be grossly misleading here since one cannot easily weigh the very different areas of relatively polar and relatively equatorial zones in this way.)

† Secondary peak.

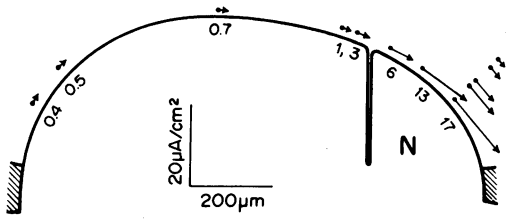

FIG. 4. Current pattern tangent to the surface of a late vitellogenic follicle (no. 16). As before, the heavy line indicates half the outline of the follicle while N indicates the nurse cell cap. The tangential arrows indicate the tangential current densities through the heavy points. Arrow lengths are proportional to current density for densities of 2 $\mu A/cm^2$ or more, but not for smaller ones. Numbers also indicate current densities (in $\mu A/cm^2$). Note 4-fold difference in current density scale between this figure and the previous one.

currents leave most of the rest of the oocyte. However, an important new feature appears. A second inward current is now found near the posterior pole. Thus, the post-collapse follicle has current entering *both* of its poles and leaving its sides.

Integration of the data shown in Fig. 3*D* yields a total outward current leaving the follicle's sides of about 90 nA, an apparent anterior inward current of 4 nA, an apparent posterior one of 3 nA, and a total apparent inward current of only 7 nA. Again it seems probable that the apparent deficiency in inward current is accounted for by the currents entering the two extreme poles, which are covered by the stalks and thus inaccessible for measurement. Thus, the total transfollicular current does not seem to change very much after the nurse cells collapse; only its distribution does.

DISCUSSION

Current patterns through Cecropia follicles

Our main finding is of a large steady current that enters the anterior or nurse cell end of the Cecropia follicle throughout the extended developmental period investigated; i.e., from a stage early in yolk deposition when the oocyte is only about 100 μm long and shorter than the cap of nurse cells feeding it (Fig. 3*C*) to a stage late in yolk deposition when the oocyte is about 2000 μm long and the nurse cells have atrophied and collapsed (Fig. 3*D*). A second important finding is of a current that enters the posterior pole of the oocyte during the relatively late stage after nurse cell collapse and before chorion formation (Fig. 3*D*). During this relatively late stage, both a posterior pole current and an anterior one enter the oocyte. The relatively intense and long-lasting anterior pole current may be considered to conform to the general tendency of steady current to enter the animal pole of various zygotes (table 2 of ref. 6).¶ The anterior inward current is also consistent with the negative potentials (discussed above) which are generally found in front of follicles that are isolated and squeezed into a capillary tube at a stage before the nurse cells collapse (9). The Cecropia follicle pole currents, particularly the anterior one, are both relatively large and intense compared to those entering the growth poles of fucoid zygotes (2) and of lily pollen (3). These plant cell currents are of the order of only 100 pA in total size (compared to 100 nA in Cecropia) and of 1 $\mu A/cm^2$ in peak density (compared to 20 $\mu A/cm^2$ in Cecropia).

Before the collapse of the nurse cells, the overall direction of current through the follicle is toward the rear; but intracellular microelectrode measurements seem to show steady current

¶ It is true that the term "animal pole" is rarely applied to insects, but it is generally defined as the site of germinal vesicle dissolution, and in Cecropia this site is clearly at the anterior end, albeit towards one (probably dorsal) side of this region (16, 18).

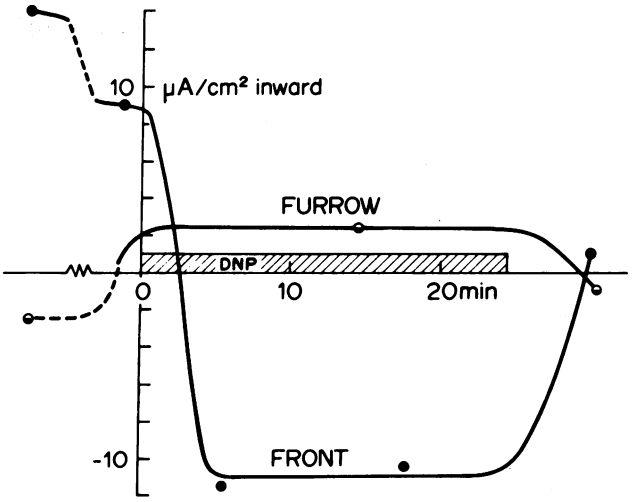

FIG. 5. Reversal of nurse cell current by 0.5 mM dinitrophenol in late vitellogenic follicle no. 18. Graph shows measurements of the normal current density in front of the nurse cell cap and at the furrow or at the boundary between this cap and the oocyte. Measurements before the break in the graph were made about 30 min before dinitrophenol (DNP) was added to the medium.

flowing the other way through the intercellular bridge(s)—i.e., towards the front (8). One might resolve this paradox by attributing the apparent bridge current to oocyte injury produced by impalement. However, the published data do not show as much variability as one might expect from an injury current. For example, the oocyte potential averaged -29.0 ± 1.1 mV, where 1.1 mV is the standard error of the individual values. We would therefore like to consider a model (Fig. 6) that accommodates both main currents through the oocyte–nurse cell complex. The essence of the model is an internal current loop. The transfollicular current directed towards the posterior would cross the furrow while the purely internal current returns through the bridge. Both of these large currents (and the smaller furrow current) would be driven by a battery that pumps charges out of the nurse cells and into the furrow. In addition to explaining the directions of the three observed currents in a simple way, this model would also explain the size of the transfollicular one, I_{TF}. If the only battery that drives current through the follicle lies in the furrow, then I_{TF} will be given by:

$$I_{TF} = E_B/(R_o + R_n), \qquad [1]$$

in which E_B is the measured 10-mV bridge potential (9) and $(R_o + R_n)$ are the peripheral resistances of the oocyte and the nurse cell parts of the follicle. According to some measurements

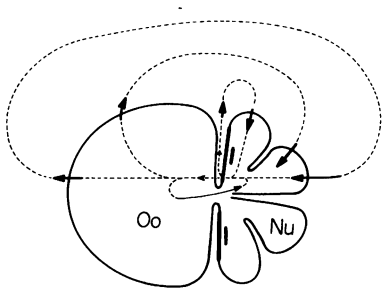

FIG. 6. Model of the steady current pattern through a Cecropia follicle. Oo, oocyte; Nu, nurse cells. Battery symbol indicates the hypothetical main ion pump; heavy arrows indicate currents that were measured directly with the vibrating probe; light arrow, one inferred from intracellular measurements of voltages and resistances. The lengths of these arrows suggest the relative sizes of these currents.

of Woodruff and Telfer (9), it would appear that ($R_o + R_n$) is of the order of 100 kΩ. Then according to Eq. 1, the transfollicular current should be about 10 mV/100 kΩ or 100 nA, a value which fits our present independent estimate from extracellular measurements.

Woodruff and Telfer reported that 0.5 mM dinitrophenol reduced the membrane potential of the nurse cell from 40 mV down to 8 mV within 10 min (8). We now find a reversal of the anterior pole current by 0.5 mM dinitrophenol within this period (Fig. 5). Considering the large size of the oocyte and the speed of the response, these observations suggest that most of the membrane potential is generated by an electrogenic pump. The reversal of the anterior pole current might be explained if much of it consists of a Cl^- ion leak. A striking reversal of at least the pulsatile component of the inward current at the growth pole of fucoid eggs can be produced by depolarizing the membrane by 50 mV (with a 10-fold rise in external K^+ ion), and this reversal is believed to result from a large Cl^- leak at the growth pole (19). Moreover, the 10-fold higher than natural [Cl^-] present in our culture should additionally favor the inward leak of Cl^- ions, which would correspond to an outflow of current.

Developmental significance of the currents

Relatively little is known about the localization process in the eggs of Cecropia and other Lepidoptera. Nevertheless, it seems reasonable to assume that the process in Cecropia is similar to that in other higher insects. There is evidence for the very early localization or concentration of three or four different morphogenetic factors at the poles of such higher insect eggs: one at the anterior pole and two or three others at the posterior pole (20, 21). The anterior pole factor, which might be very crudely referred to as a "head factor," favors the development of relatively anterior structures (ref. 20, pp. 126–132). It has been best studied in the dipteran *Smittia*, where its ultraviolet inactivation spectrum, photoreactivability, and specific sensitivity to RNase indicate that it consists of ribonucleoprotein (22). Perhaps this material is introduced into the anterior pole as part of the RNA that enters the oocyte through the bridge from the nurse cells. In any case, one might speculate that the anterior current system in the Cecropia follicle somehow acts to localize this head factor.

A functionally comparable posterior pole factor, which favors the development of posterior structures and might be crudely called a "tail factor," has been best studied in the homopteran *Euscelis* and is internally transplantable (in *Euscelis*) but otherwise uncharacterized (ref. 21, pp. 165–168). The well-known germ plasm is also localized at the posterior pole of various insects (23). It has been best studied in *Drosophila*, where it is associated with granules having a characteristic fine structure and is externally transplantable (24). It first becomes effectively transplantable at stage 13 of *Drosophila*, a stage that is shortly after nurse cell collapse and just before shell formation (24), while the symbiotic bacteria associated with the tail factor of *Euscelis* are seen to "assemble in a cup-shaped depression at the posterior pole of the oocyte . . . and become almost completely engulfed by the egg cell" at a (comparable?) stage shortly before formation of the *Euscelis* egg shell (21). Since the posterior pole current first appears in the Cecropia oocyte at what appears to be a comparable stage, one may speculate that this current somehow activates or completes the germ plasm (its fine structure does not change at stage 13, only its transplantability) or induces the aggregation and uptake of the tail factor in insect eggs or both.

Note Added in Proof. Since submission of this manuscript, Robinson has published a study of the electrical currents through *Xenopus* oocytes (25).

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