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Patterns of Ionic Currents around the Developing Oocyte of the German Cockroach, *Blattella germanica*

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The development of patterns of current around vitellogenic oocytes of the cockroach, *Blattella germanica,* was examined by means of a two-dimensional vibrating probe. Previtellogenic oocytes exhibited small unstable currents. Shortly after vitellogenic uptake began (oocytes 0.6–0.8 mm anterior to posterior) currents were either all inward or all outward at the plane of measurement. A dorsoventral pattern of currents was first observed around oocytes a little larger than 0.8 mm. Current exited dorsally (source) and entered ventrally (sink). In these oocytes source and sink were small, less than half the anterior–posterior length. As oocytes grew, relative sizes of source and sink increased until they extended across the major part of dorsal and ventral surfaces. Many late vitellogenic oocytes had a pattern of dorsal outward current with a bimodal distribution. At the onset of chorionation measured currents were again small, unstable, and exhibited no well-defined pattern. Current density was greatest during midvitellogenesis. © 1990 Academic Press, Inc.

INTRODUCTION

The establishment of pattern is a fundamental issue throughout development. One approach to examining the development of cell polarity has been to measure extracellular ionic currents around developing oocytes by the use of a noninvasive vibrating probe (Jaffe and Nuccitelli, 1974). By means of this technique stable ionic currents have been observed to enter and leave specific regions of both amphibian and insect oocytes (Robinson, 1979; Jaffe and Woodruff, 1979; Dittmann *et al.*, 1981; Overall and Jaffe, 1985; Bohrmann *et al.*, 1986a; Huebner and Sigurdson, 1986; Kunkel, 1986; Verachtert and De Loof, 1986). The polarity demonstrated by these patterns of ionic currents suggests that there is a segregation of pumps from leaks or channels and that these macromolecules are localized.

Most insect oocytes which have been examined by this technique exhibit ionic currents with an anterior–posterior pattern. Almost all of these oocytes, however, were from meroistic ovaries. In this type of ovary the oocyte is associated with nurse cells either directly (polytrophic) or indirectly by way of trophic cords (telotrophic). Moreover, the trophic region is anterior, the oocyte posterior. Thus in these follicles or ovarioles observed anterior–posterior current pattern may be due to activity of the nurse cells. The ionic currents may also represent a complex interaction between nurse cells and oocyte and/or the cells between or surrounding them. In contrast ionic currents observed around oocytes from panoistic ovaries may have a less complex origin since these oocytes are not associated with nurse cells. Ionic currents measured are therefore most probably generated by the oocyte itself, by the surrounding follicle cells, or by some combination of the two. They are likely then to be rather directly related to the physiology of the developing oocyte and perhaps may be a part of the mechanism of polarization of the future embryo.

The ovariole of the cockroach *Blattella germanica* is panoistic. During oogenesis terminal oocytes exhibit ionic currents which enter and leave the oocyte with a dorsoventral pattern (Kunkel, 1986). The present work examines the ontogeny of patterns of currents, and thus of one aspect of polarity, by measuring currents around oocytes at different stages of vitellogenesis, from 0.5 mm when vitellogenin uptake has just begun, to 2.3 mm, the onset of chorionation. The ions which contribute to these currents are not considered in the present work. An abstract of this work has been previously published (Bowdan and Kunkel, 1989).

MATERIALS AND METHODS

Forty-one oocytes, ranging in size from 0.5 to 2.3 mm, were examined using the two-dimensional vibrating probe at the National Vibrating Probe Facility (NVPF; Woods Hole, MA).

Animals

In insects, strings of oocytes develop within ovarioles. In *B. germanica,* only the terminal oocyte (the one closest to the oviduct) undergoes vitellogenesis in a given reproductive cycle. Oocyte size depends upon the num-

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ber of days the female has been feeding at 30°C (Kunkel, 1973). Subterminal oocytes, i.e., oocytes anterior to the terminal oocyte within the same ovariole, remain previtellogenic. A 0.5-mm oocyte will come from a female which has been feeding for 1 to 2 days, whereas a 2.3-mm oocyte (beginning of chorionation) comes from a female which has been feeding 6 to 7 days. Thus an oocyte of a particular stage was available by dissecting a female which had been feeding for the appropriate number of days. For convenience oocytes have been considered as though their anterior to posterior length is the equivalent of their physiological age. Doubtless the two are well correlated but they are not necessarily identical.

Chamber

Measurements were made around oocytes lying in a custom-made chamber. This chamber consists of a 35-mm-diameter perspex dish with an oval trough 22 mm long and 8 mm wide cut out of the center. The bottom of the dish is formed by a 25-mm-diameter circular glass cover slip held in place with a ring of vaseline mixed with silicon grease.

Oocyte Preparation

Ovarioles were dissected out in a simple salt medium containing 4 m*M* $CaCl_2$, 14 m*M* KCl, 5 m*M* $MgSO_4 \cdot 7H_2O$, 145 m*M* NaCl, 11 m*M* H_3PO_4 (to buffer the medium at pH 6.8) 110 m*M* sucrose (to give an osmolarity of 390 mOsm), 12.5 mg/100 ml penicillin, and 5 mg/100 ml streptomycin. Several were transferred to the exchange chamber, examined, and all except one discarded. Polylysine treatment of the cover slip base of the chamber held the oocyte securely in place. A drop of 0.01% poly-L-lysine was placed on the base, the excess was carefully removed, and the remaining thin layer of solution was allowed to dry. Before the oocyte was added the chamber was rinsed off with medium. The medium was kept at 30°C by means of a Peltier regulated heating coil. A layer of mineral oil over the medium prevented evaporation. Most oocytes were positioned for examination with a lateral surface adhering to the base of the measuring chamber and thus probing was along dorsal and ventral surfaces in a midsagittal plane. A few oocytes were positioned so that either the dorsal or the ventral surface adhered to the base and probing was along the lateral surfaces in a frontal plane. The vertical position of the probe was such that the probe was in focus with the connective between terminal and subterminal oocytes. Observations were made with the dish on the stage of an inverted microscope (Zeiss, IM35). The oocyte was oriented and the probe positioned in the x–y plane with the help of a reticule in the eyepiece of the microscope. A video camera (Dage-MTI series 68) was attached to the camera port of the microscope and an image of the oocyte displayed on a monitor.

Vibrating Probe

The two-dimensional vibrating probe (Scheffey, 1988) measures the potential difference between the two extreme positions of vibration in the x and y dimensions. It cannot detect any current in the third (z) dimension. Platinum chloride coated platinum wires embedded in the experimental chamber acted as ground wires. Measurements made at the NVPF are fed into a DEC PDP 11/23 computer for preliminary analysis. Data then collected consist of the x,y,z location of the probe, the x, y, and total, current (I_x, I_y, I_t) and the angle of the resultant current vector. X and y coordinates of the outline of the oocyte can also be collected. Computer graphics hardware from Matrox Corp. allowed vectors to be displayed on the monitor superimposed on the image of the oocyte. All computer software used in the initial data analysis and vector display were developed at the NVPF. Data from the NVPF system were captured on an MSDOS computer. Further data analysis was made with an MSDOS computer using commercial spreadsheets and custom-written software.

Currents

Oocytes were not defolliculated thus the currents measured may have been produced by the oocyte, by the follicle cells, or by both. All currents measured represent the total flux of ions. Outward current represents a net total flux either of positive ions flowing out or of negative ions flowing in to the follicle. Similarly, inward current represents a net total flux of negative ions flowing out or of positive ions flowing in. Orientation of the oocytes is such that I_y is most nearly the current normal to the oocyte surface. Thus I_y is a better representation of ionic flow through the region of membrane closest to the probe than either I_x or I_t. Those follicles with obvious injury currents or with decaying currents were discarded. In most instances the currents were stable for hours. If currents were variable but did not show a continuous decline the data were not rejected.

RESULTS

Changes in Oocyte Shape during Oogenesis

Previtellogenic and very early vitellogenic oocytes (those less than ~0.8 mm) are ellipsoid and the germinal vesicle sits either in the center of the cell, or against one of the longer surfaces (Wheeler, 1888, de-

scribes its movements). Older, but still early vitellogenic oocytes (0.8–1 mm) are still ellipsoid but the germinal vesicle always lies close to one of the long surfaces. As the oocytes accumulate more vitellogenin they become larger and banana shaped with one long surface convex, the other flat to concave. The germinal vesicle lies at the center of the flat/concave surface. As the oocytes become larger they become more and more opaque due to the accumulated vitellogenin, and the germinal vesicle can no longer be seen. Just before chorionation the oocyte is 2 mm or larger, cylindrical, and completely opaque. At all sizes the width (dorsal-ventral and right lateral–left lateral) is approximately four-tenths the anterior–posterior length.

The orientation of the oocyte can be recognized by means of its shape and by the position of the germinal vesicle. The ventral surface of the embryo will develop in the region which is the flat/concave face of the mid-vitellogenic oocyte. Thus this region is the ventral, the convex face the dorsal, surface of the oocyte. The anterior is that end to which the subterminal (previtellogenic) oocytes are attached and the oviduct extends from the posterior end. Since the surface where the germinal vesicle comes to lie becomes concave that position of the germinal vesicle indicates the ventral surface in early vitellogenic oocytes (0.8–1 mm) which are not yet banana shaped. It cannot be used as a marker of the future ventral surface in oocytes smaller than 0.8 mm, however. Thus, when these small oocytes are placed in the dish for examination the dorsal and ventral surfaces are not known. Dorsal and ventral surfaces are also difficult to discern in the large, cylindrical, opaque, oocytes.

Medium

During preliminary vibrating probe studies (Kunkel, 1986) currents could be maintained for hours only when oocytes were bathed in cockroach serum. Thus it seemed that some serum component was necessary for the maintenance of the currents. Experiments being conducted at that time on *in vitro* vitellogenesis in a related cockroach, however, (Kindle *et al.*, 1988), could be performed in an artificial saline. This medium was therefore tested for its utility in vibrating probe experiments. It proved to support stable electrical survival of oocytes for several hours. Substitution of sucrose for glucose proved to be the necessary crucial change. A simple version of this medium is that which is now used. Values for current density reported in Kunkel (1986) are an order of magnitude greater than those reported in this work. Values in the earlier work were overestimated due to an error in the computer program being developed at the time.

Effects of Injury

It was necessary to examine the effects of known injury to reject data from damaged oocytes. Some oocytes were therefore deliberately injured to examine the resulting currents. Mild injury produced by touching the oocyte resulted in a localized area of small inward currents. If the injury was a little greater the region of inward current slowly enlarged. Prodding or poking the oocyte resulted in an intense, highly focused, inward current that decayed over a period of 1 or 2 hr.

Changes in Current Patterns

Currents measured around subterminal (previtellogenic) oocytes were small (at or barely above noise level) and erratic. Vitellogenic oocytes smaller than ~0.8 mm had vectors which all pointed either in or out without any focus and without regard to the position of the germinal vesicle. Current loops must be completed but the observed vectors did not demonstrate this. It is reasonable to assume, however, that, since the observed currents were either inward or outward, current loops were completed at the inaccessible portion of the oocyte.

The earliest evidence of dorsoventral pattern was seen in an 848 μm oocyte. Only two oocytes from 0.8–1 mm were probed midsagittally and both are illustrated in Figs. 1A, 1B. In these early vitellogenic oocytes the regions of inward and outward current were small and central. Vectors measured from anterior and posterior regions were parallel to the oocyte surface. They probably represent current loops between the small dorsal source and the equally small ventral sink. Oocytes positioned so that either the dorsal or the ventral surface was attached to the measuring chamber and so that probing was in a frontal plane had current patterns which would be predicted from the midsagittal measurements. That is, if the ventral surface was attached to the dish, the dorsal surface therefore facing outward, and the probe was high enough to be closer to the dorsal than the ventral surface then all the currents measured fanned out symmetrically from the oocyte (Fig. 1C). Similarly, when the dorsal surface was attached to the dish, ventral surface facing out, vectors focused tightly inward (Fig. 1D).

At the beginning of midvitellogenesis (oocytes ~ 1 mm, e.g., Fig. 2A) outward current was spread across the dorsal surface whereas inward current was focused in the midventral region. In slightly larger oocytes (e.g., Fig. 2B, 2C) the region of inward current spread to occupy a larger portion of the ventral surface. Probing of oocytes of these sizes in a frontal plane produced current patterns which matched the midsagittal pattern. That is, when the ventral surface faced up vectors focused inward centrally and fanned outward anteriorly and posteriorly (Fig. 2D). It is thus reasonable to pro-

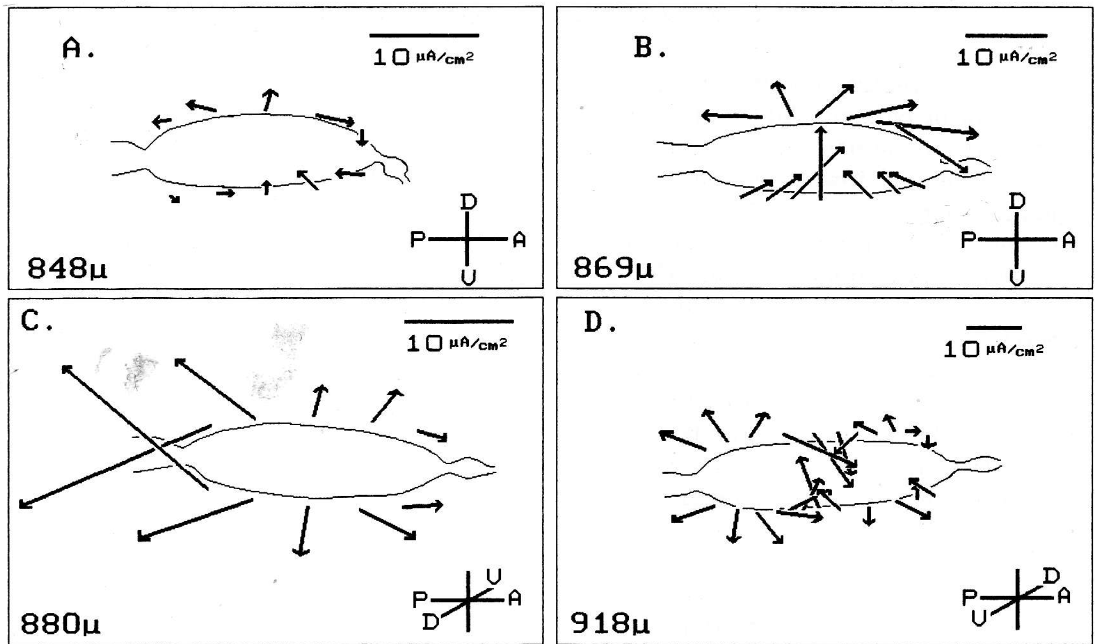

FIG. 1. Current vectors measured around early vitellogenic oocytes. (A, B) Dorsal outward and ventral inward currents sharply focused. (C, D) The oocytes were rotated 90° so that probing was along a frontal plane, at a dorsolateral aspect in C, a ventrolateral aspect in D. Oocyte outline and vector positions, angles, and length drawn by the use of computerized values collected at NVPF. Arrows represent total current density, the vector calculated from measured I_x and I_y; length of arrow is proportional to current density; angle is vector angle; base of arrow is position of probe during measurement. Orientation of oocyte shown at lower right of each example; size of oocyte given in micrometers at lower left; calibration of vector lengths given by scale bar in the upper right corner of each example.

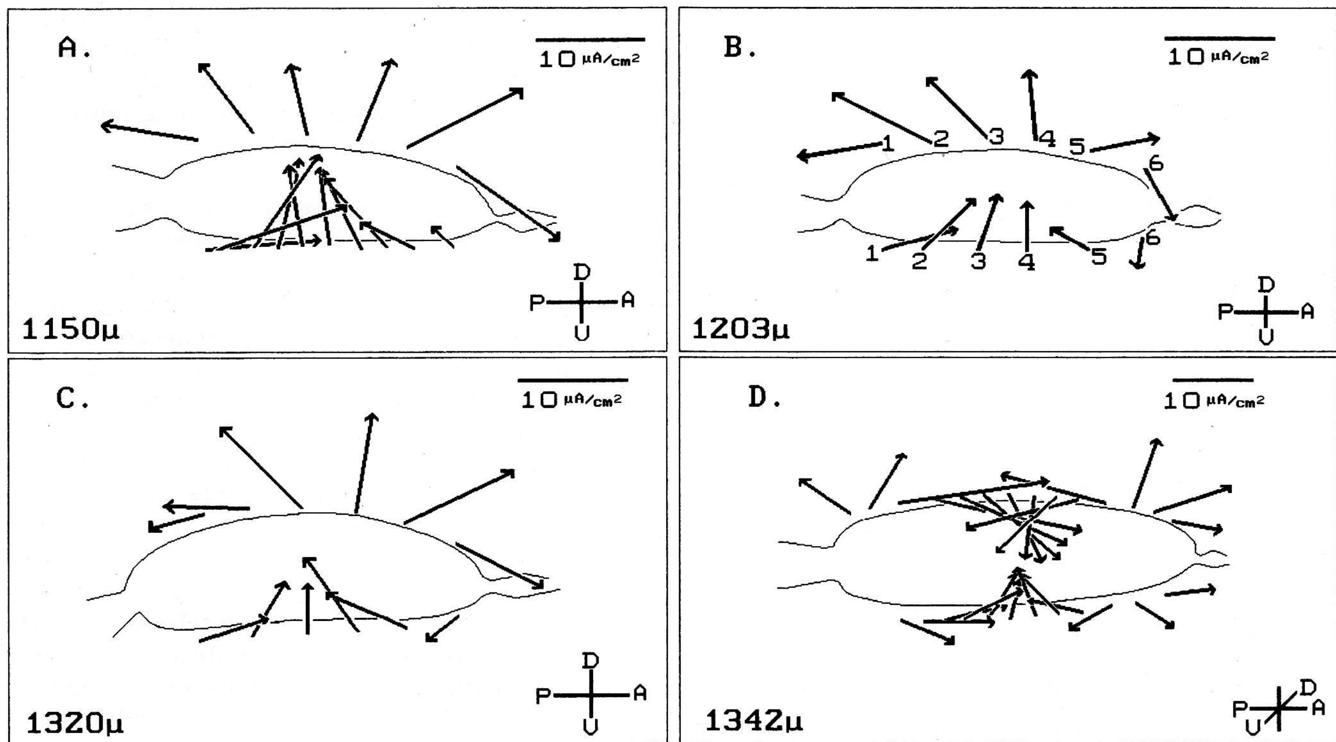

FIG. 2. Current vectors around midvitellogenic oocytes. (A–C) changes in current patterns as the oocytes become older (larger). The sink of ventral inward current is at first focused but then spreads across most of the ventral surface. The source of dorsal outward current covers the greater part of the dorsal surface. Numbers at bases of arrows in B represent numbering system for probe positions used in Fig. 5. Posterior dorsal vectors may include a contribution from the injury current of the oviduct. It is unlikely that anterior vectors include a contribution from the penultimate oocyte because I_t decreased as the probe was positioned closer to this previtellogenic oocyte. (D) Oocyte rotated 90° so that ventral surface faces out and probing is in a frontal plane. Symbols as for Fig. 1.

pose that the dorsal and ventral regions are the only major source and sink of current. Added support for this proposal comes from the observation that dorsal outward current and ventral inward current are approximately equal and opposite.

Larger oocytes tended to exhibit a pattern of dorsal outward currents which was bimodal (e.g., Fig. 3A). What was observed as the oocytes increased in size was not a gradual decrease in current density recorded from the middorsal surface but rather an increase in the number of oocytes exhibiting a bimodal pattern. This bimodal distribution of dorsal current may be because (1) there are two dorsal foci of outward current, one anterior and one posterior, or (2) the region of outward current forms an annular ring. Evidence for the second possibility comes from oocytes from which measurements were made with the probe in more than one vertical plane. In one plane current densities were similar across most of the dorsal surface. In the second there was a clear bimodal distribution of current density. One such set of measurements is presented in Fig. 3B.

At about the time chorionation begins oocytes are >2 mm long. More than 10 oocytes of this size were examined and all exhibited small current vectors which were erratic in both direction and magnitude. The data from most of these oocytes were therefore discarded. Since no more consistent data were ever collected, however, a decision was made that this kind of activity was typical for oocytes of this size.

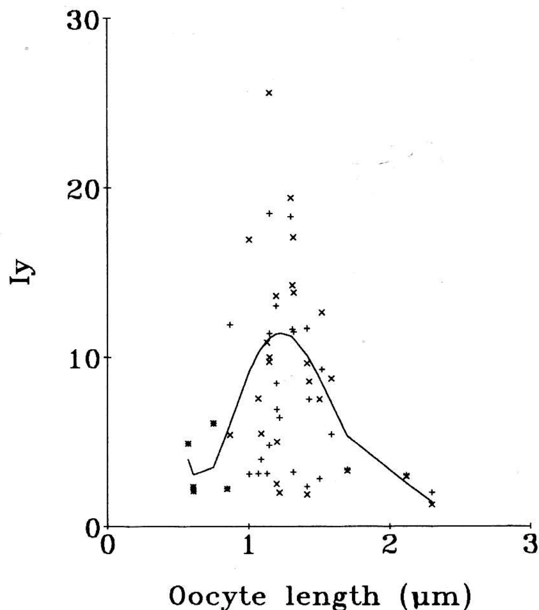

FIG. 4. Peak dorsal (×) and ventral (+) absolute I_y from all oocytes probed midsagittally. Line calculated to give best fit—third order polynomial—demonstrating that current density is greatest in midvitellogenic oocytes.

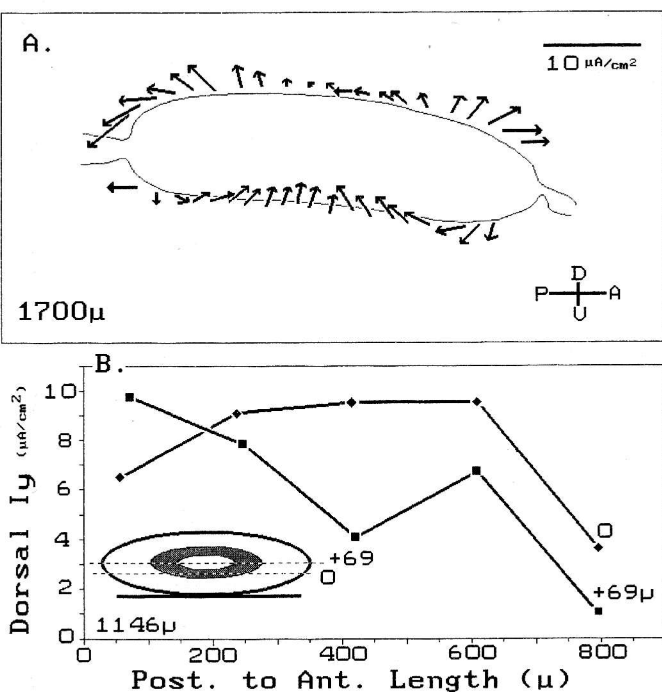

FIG. 3. (A) Late-vitellogenic oocyte. Current vectors are small and dorsal outward vectors have a bimodal distribution. Symbols are as for Fig. 1. (B) Evidence that the source of the observed bimodal dorsal current is an annular ring rather than two independent regions. At probe position $z = 0$, I_y is uniform across major part of dorsal surface. At probe position $z = +69\mu$, I_y distribution is clearly bimodal. Inset diagrams dorsal face of oocyte with proposed ring of outward current; dotted lines, planes of probe movement.

It is not only the pattern of currents which changes during oogenesis. Current density also changes. Current approximately normal to the oocyte surface is measured in the y direction. Absolute maximum y current (I_y) recorded dorsally and ventrally from each oocyte is shown in Fig. 4. These data demonstrate (a) much variability, and that (b) current density is greatest in midvitellogenic oocytes.

Inter- and intraoocyte variability is illustrated and compared in Fig. 5. The 23 mid- to late-vitellogenic oocytes (1–1.7 mm) which were probed midsagittally were divided into four approximately equal groups, groups b–e. (Group c includes the oocytes of Fig. 2, group e the oocyte of Fig. 3A.) The two early vitellogenic oocytes constituted a group of their own, group a. I_y recorded at each probe position along the oocytes was averaged for each size group. Fig. 5A shows means, standard deviations and standard errors of I_y recorded at specific positions along the dorsal and ventral surfaces.

I_y measurements from nine individual oocytes which were scanned at least four times were averaged for each oocyte to examine intraoocyte variability, Fig. 5B. Only one of these scans from each oocyte was also used in the

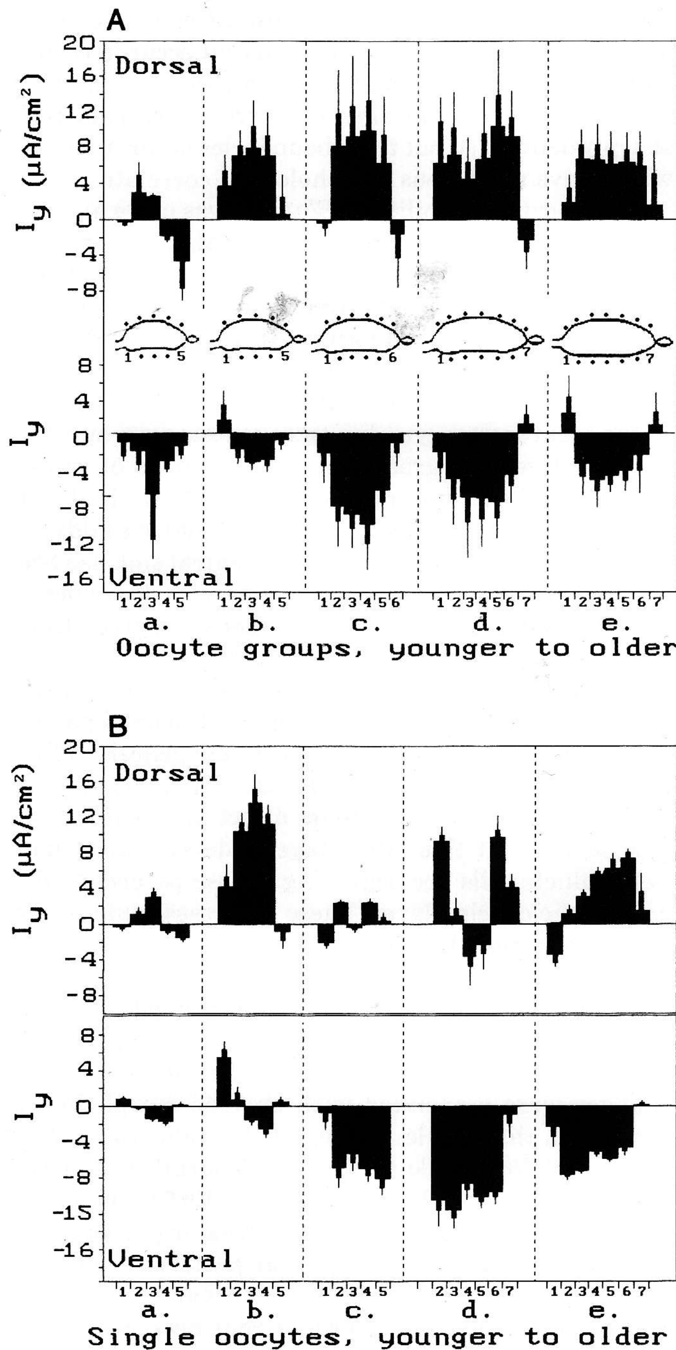

FIG. 5. Comparison of Inter- and Intra-oocyte variability at similar midsagittal dorsal and ventral positions for different size class oocytes. (A) Means and errors among several oocytes of I_y measured at each position along oocytes; bin numbers identify measuring positions, in order, posterior to anterior (example of numbering system shown in Fig. 2B). Positions are separated by ~200 µm along x axis for oocytes of all sizes. Early to late vitellogenic oocytes divided into groups: *group a*, (848, 869 µm) $N = 2$; *group b*, (1005–1146 µm) $N = 5$; *group c*, (1149–1220 µm) $N = 6$; *group d*, (1300–1414 µm) $N = 6$; *group e*, (1430–1700 µm) $N = 5$ for dorsal, 6 for ventral. (B) Means and errors of I_y for individual oocytes scanned at least four times. Each graph, a–e, contains data from one oocyte which is a member of a corresponding group, a–e, of A. Oocyte sizes in micrometers left to right: *a*, 848 µm, number of scans (K) = 4, test period span (T) = 1 hr, (the data from one of these scans illustrated in Fig. 2A); *b*, 1005 µm, $K = 4$, $T = 1.5$ hr; *c*, 1198 µm, $K = 4$, $T = 1$ hr; *d*, 1414 µm, $K = 7$, $T = 1.5$ hr; *e*, 1430 µm, $K = 4$, $T = 1.5$ hr. Thick bars = ± one standard error of the mean; thin bars = ± one standard deviation.

examination of interoocyte variability of Fig. 5A. Time between scans ranged from 10 min to 2 hr with most interscan-intervals being about 20 min. Total duration of the scanning periods ranged from 50 min. to more than 3 hr. The means and standard deviations for five of these oocytes, one from each group, are presented in Fig. 5B. Comparison of the standard deviations of Fig. 5B with those of Fig. 5A demonstrates that intraoocyte variability was significantly less than interoocyte variability ($P < 0.001$; overall F test). The small intraoocyte variability also demonstrates the stability of the oocytes over quite substantial periods of time (more than 3 hr in the case of one oocyte not illustrated). Despite the averaging of the interoocyte data, bimodality is still evident in the dorsal currents of Fig. 5A (d.).

DISCUSSION

The present experiments were performed using a two-dimensional probe which measures current in both x and y directions. When the probe is close to the center of a source or sink it measures current projecting straight out of (or in to) the source (sink) and thus the vector consists mostly of a y component. The more the probe is moved laterally away from the axis of the source (sink) the more the direction of the current curves and so the smaller the y component, the greater the x component. Thus the angle of the calculated vectors immediately indicates whether the current being measured is directly associated with a source, sink or whether it is a portion of a current loop between source and sink. It is possible to estimate the rough area of source and sink rapidly and to show that in *Blattella* source and sink are localized. There is an area of the dorsal surface which is a current source and an area of the ventral surface which is a current sink. It also seems clear that these are the only major source and sink of current, although there are undoubtedly additional minor sources and sinks. Thus pumps and channels are localized and there is therefore a dorsoventral polarization which is evident at a very early stage in development. The patterns of currents and their changes throughout oogenesis led to a model of the changing regions of inward and outward currents (and thus of localized regions of channels and/or pumps) on the oocyte surface which is illustrated in Fig. 6.

If the oocyte is not positioned perfectly during probing the plane of probe movement will intersect the re-

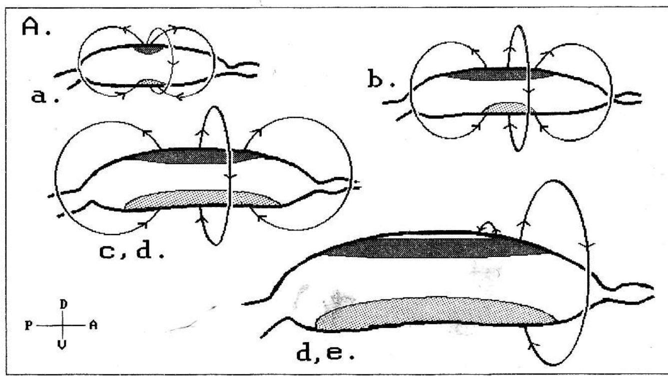

FIG. 6. Model of changing regions of sources and sinks of currents (i.e., regions of channels, pumps) on the oocyte surface throughout vitellogenesis, plus associated current loops deduced from observed current vectors. Oocytes labeled with letters to indicate size group of Fig. 5 such a current pattern would be associated with. Two of the diagrams are labeled "d" because oocytes of group d may have either a unimodal or a bimodal distribution of dorsal outward current.

gions of source and sink at an angle. This will distort their apparent areas and positions and increase the apparent interoocyte variability. The oocyte depicted in Fig. 2C, for example, was visibly tilted so that in the two most posterior dorsal measuring positions the probe was no longer midsagittal. It was therefore no longer immediately adjacent to the source and consequently the vectors from those two posterior positions were parallel to the oocyte surface. All measurements made which did not depict symmetrical patterns around oocytes could be interpreted as originating from the sources depicted in the model of Fig. 6 if the assumption was made that the oocyte had not been positioned orthogonal to the probe, that it was tilted or rolled a little. The fact that all observed patterns of currents can be explained by this simple assumption is also further support for the model.

Most insect ovarioles or follicles which have been examined exhibit a pattern of currents, the sole exception being the megalopteran *Sialis* (Huebner and Sigurdson, 1986). No other insect follicle, however, has a dorsoventral pattern of currents. Most of the oocytes that have been examined were meroistic. By and large currents entered the nurse cell region and exited the oocyte and the region of current reversal was marked by some morphological feature. In polytrophic follicles the region of current reversal was either at or near the nurse cell border (*H. cecropia*, Jaffe and Woodruff, 1979; *Drosophila*, Overall and Jaffe, 1985; Bohrmann *et al.*, 1986a) or at the junction between anterior flattened and posterior cuboidal follicular cells (*Sarcophaga*, Verachtert and De Loof, 1986). In telotrophic ovarioles there are two current circuits. One enters the tropharium and exits from anterior previtellogenic oocytes. The other is around the terminal oocyte. In all of these cases the source of outward and the sink of inward current occur in regions which are morphologically different. In *Blattella*, however, the source and sink of current are relatively sharply delineated but their boundaries occur in regions which have no obvious morphological correlate.

Interoocyte variability in *Blattella* was quite high due in part to positioning errors. In general, however, there was less than an order of magnitude difference between the smallest and the greatest I_y measured at a given position within an oocyte group. Intraoocyte variability was significantly less than interoocyte variability.

The changes in current pattern which were observed in the cockroach oocyte during oogenesis were substantial but developed gradually. Small regions of inward and outward current appeared during early vitellogenesis and gradually expanded. Increase during midvitellogenesis of the relative size of the ventral sink has been mathematically modelled in the case of two oocytes (Kunkel and Bowdan, 1989). The dorsal source of outward current spread so that eventually it left a central region which was either an active or a passive sink. The largest oocytes apparently had no well-defined pattern of current. At earlier stages in vitellogenesis follicle cells are separate from one another (patent) thus allowing serum and its contents direct access to the oocyte surface. At this later stage in development, however, follicle cells are beginning to lose patency and to deposit the chorion layer. These processes could lead to partial electrical barriers creating local unpredictable current circuits.

Other oocytes which have been examined also show gradual changes in current pattern during oogenesis. Unlike the changes seen in *Blattella*, however, these changes were associated with obvious morphological changes in the follicle. In polytrophic follicles of *H. cecropia* and *Drosophila* (Jaffe and Woodruff, 1979; Overall and Jaffe, 1985; Bohrmann *et al.*, 1986a) the oocyte grows to constitute a greater and greater proportion of the follicle and since the general pattern of currents remains the same, the area of outward current increases while that of inward current decreases. Similarly the boundary between flattened and cuboidal follicle cells in *Sarcophaga* becomes relatively more anterior so that the area of outward current increases and that of inward current decreases (Verachtert and De Loof, 1986). A posterior sink of current develops at chorionation in *Drosophila* (Overall and Jaffe, 1985; Bohrmann *et al.*, 1986a). In the telotrophic ovariole of *Rhodnius* the major change observed during oogenesis was an increase in the outward current recorded from the connective between the terminal and subterminal oocytes and from the anterior region of the terminal oocyte (Huebner and Sigurdson, 1986).

The increase in current density observed in midvitellogenic cockroach oocytes has also been seen in some, but not all, of the other oocytes which have been examined. Huebner and Sigurdson (1986) found that the outward currents recorded from the anterior end of the terminal oocyte and the connective between the subterminal and terminal oocytes in *Rhodnius* increased at midvitellogenesis and remained large until chorionation. Bohrmann *et al.* (1986b) treating the *Drosophila* follicle as a dipole, found that the apparent dipole length changed dramatically from 2 to 160 μm during stage 10. There is thus a major change in the electrophysiological properties of the follicle at midvitellogenesis.

With which physiological role might ionic currents be associated? Bohrmann *et al.* (1986a) suggested that ionic currents might not play a role in development in part because the currents they observed around the *Drosophila* follicle were so variable. Nevertheless, the currents are so ubiquitous that possible roles must be carefully examined. Major activities of the follicle, in addition to general metabolic processes, are (1) an increase in the amount of oolemma; (2) activities of follicle cells; (3) the generation of RNA by nurse cells and/or germinal vesicle; (4) uptake of vitellogenin; and (5) redistribution of macromolecules and/or organelles.

Oocyte plasma membrane increases throughout oogenesis and this activity may contribute to the observed ionic currents. Ionic currents have been found associated with growing membranes in such diverse systems as goldfish retinal ganglion cell filopodia (Freeman *et al.*, 1985) and fungal hyphae (Takeuchi *et al.*, 1988). In some systems ionic currents were not only associated with growth but preceded and predicted the region where growth would occur (e.g., Nuccitelli and Jaffe, 1974). In all instances charge entered the growing region.

Follicle cells are highly active during vitellogenesis (reviewed in Huebner, 1984) and a number of observations suggest that the follicular epithelium may make an important contribution to the observed ionic currents. Jaffe and Woodruff (1979) found an inward current over the nurse cells and an outward current from the oocyte of *H. cecropia.* But Woodruff *et al.*, (1986) removed regions of the follicular epithelium and discovered an exit current from denuded nurse cells. The telotrophic ovariole of *Ips perturbatus* has a "loose" follicular epithelium, an entry current into the anterior region and an outward current from the posterior region of the tropharium (Huebner and Sigurdson, 1986). In contrast, these workers found only an inward current around the tropharium of *Rhodnius,* an ovariole with a tight follicular layer. Verachtert and De Loof (1986) found that the region of current reversal lay at the junction of flattened and cuboidal follicular cells in *Sarcophaga.* In cockroach oocytes follicle cells are patent during vitellogenesis (Anderson, 1964), and thus the layer is loose suggesting that the observed currents emanate from the oocyte. A role for follicle cells in the generation or modification of ionic currents in the cockroach cannot be ruled out, however.

Huebner and Sigurdson (1986) suggested that inward currents might be associated with regions of highest RNA synthesis. The primary sites of active RNA synthesis in insect meroistic follicles are the nurse cells, in panoistic follicles the germinal vesicle serves this function. In support of Huebner and Sigurdson's thesis currents are directed inward toward the nurse cells in many meroistic follicles (Jaffe and Woodruff, 1979; Dittmann *et al.*, 1981; Overall and Jaffe, 1985; Bohrmann *et al.*, 1986a; Huebner and Sigurdson, 1986; Woodruff *et al.*, 1986) with the beetle, *Ips perturbatus,* being an exception (Huebner and Sigurdson, 1986). In those panoistic follicles which have been examined currents are inward at the position of the germinal vesicle (Verachtert and De Loof, 1986; Kunkel, 1986, and this work). Even in oocytes of the amphibian, *Xenopus,* this general pattern is seen. Inward currents were observed at the animal pole, the site of the germinal vesicle (Robinson, 1979). However, the lack of a correlation between germinal vesicle position and direction of currents in young *Blattella* oocytes (those smaller than 800 μm), the observation by Woodruff *et al.* (1986) of an outward current from denuded nurse cells, and the exception mentioned above of *Ips* would seem to contradict the universality of this thesis. Measurement of RNA synthesis in these different situations would test the hypothesis more directly.

The increase in ionic currents seen in midvitellogenesis in both *Blattella* and *Drosophila* suggests that perhaps the observed ionic currents may be associated with the process of vitellogenin uptake. Ferenz *et al.* (1981) found that the rate of vitellogenin uptake in the locust oocyte was greatest during midvitellogenesis. This, in turn, may be related to activity of follicle cells since Oliviera *et al.* (1986), examining *Rhodnius,* found that both follicle cell patency and vitellogenin uptake were greatest at midvitellogenesis. Even though there was no vitellogenin in the bathing medium in either the present experiments or in those of Bohrmann *et al.* (1986a) cycling of receptors and membranes associated with vitellogenin uptake probably continued since endocytotic processes actively continue in the absence of ligand (see, e.g., Braulke *et al.*, 1987).

The observation of external ionic currents indicates the likely presence of internal voltage gradients. This then leads to the possibility that electrophoresis may be occurring (Jaffe, 1981). It is possible that this is the

mechanism by which macromolecules (e.g., rRNA) move from nurse cells to oocyte in meroistic follicles (Woodruff and Telfer, 1980) although there is some question in the case of *Drosophila* (Bohrmann and Gutzeit, 1987; but see Woodruff *et al.*, 1988). Such movement could lead to the establishment of an anterior–posterior gradient.

If electrophoresis plays a role in the movement of charged macromolecules from one cell to another in a syncytial system it may also play a role in the distribution of molecules within a single cell. The cockroach oocyte exhibits the localization of source and sink necessary for the development of a voltage gradient. It is not clear, however, that this gradient would be steep enough for electrophoresis to occur. In polytrophic follicles the crucial gradient is probably confined to the small intercellular bridges between nurse cells and oocyte (30–40 μm in *H. cecropia*, Telfer *et al.*, 1981). In the *Blattella* oocyte there are no obvious constrictions, the distance between dorsal source and ventral sink in a 1200-μm oocyte would be approximately 480 μm and a voltage gradient would therefore probably be shallow and thus insufficient for effective electrophoresis across the oocyte.

A voltage gradient may be involved in other processes leading to localization or the establishment of gradients, however. One postulated effect is lateral electrophoresis or electroosmosis of macromolecules (e.g., receptors) within the plane of the plasma membrane. This has been shown to occur as a result of an imposed DC electrical field (reviewed in Robinson, 1985). A second postulate (Robinson and Jaffe, 1975) is the establishment of an ionic gradient such as the free calcium gradient which has been demonstrated in fucoid eggs (Brownlee and Wood, 1986). Third, voltage gradients may affect cytoskeletal organization. Luther *et al.* (1983), for example, have demonstrated that microfilament bundles in cultured *Xenopus* epithelial cells become aligned perpendicular to an applied DC electrical field. Each of these postulated effects may be active in the cockroach oocyte to produce the observed dorsoventral polarity.

Observations on the *Xenopus* oocyte may provide evidence for a cytoskeletal role for ionic currents during oogenesis in oocytes. Extracellular currents measured about the *Xenopus* oocyte are inward at the animal pole and outward at the vegetal pole (Robinson, 1979). Yolk platelets move from the animal to the vegetal half of the *Xenopus* oocyte (Danilchik and Gerhart, 1987). It is suggested that this movement is guided by oriented cytoskeletal elements.

In the *Blattella* oocyte vitellogenin enters the oocyte by endocytosis and is incorporated into transitional yolk bodies. Those bodies which are near the ventral surface (inward current) may then migrate toward the dorsal surface (outward current) guided by cytoskeletal elements which have become oriented by the observed steady currents. Telfer *et al.* (1982) have demonstrated that coated pits are associated with microfilaments in *Rhodnius* and this may also be true in *Periplaneta* (Anderson, 1964). As a result of this movement the amount of yolk in ventral regions will be depleted thus contributing to the characteristic banana shape of the oocyte.

A plausible case can be made that ionic currents play a role in a number of physiological activities for all oocytes studied, including those of *Blattella*. The observed changing patterns of currents may therefore be indicative of underlying patterns of distribution both of ion channels and/or pumps and of physiological processes.

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