

the embryos. HCB has been shown to cause circulatory defects common to several other aromatic compounds: toluene, carbaryl, parathion, tolbutamide, dinitrophenol, and 2,4,5-trichlorophenoxyacetic acid (4, 5, 6, 7). The abnormalities observed in the developing mcdaka heart are similar in several respects to those seen in zebrafish embryos treated with retinoic acid (8). The anterior-posterior axis of the heart is truncated, especially affecting the anterior region, as in zebrafish (and *Xenopus*) heart development (8). These effects seem both dose-dependent as well as stage-dependent. Because the circulatory defects uncovered in our studies are lethal, resident aquatic species of Devil's Swamp that are exposed to HCB during embryonic development may experience appreciable early mortality. These aquatic populations are currently being followed.

Supported by grants from the Department of Energy and the Department of Defense.

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Pattern of Potassium Ion and Proton Currents in the Ovariole of the Cockroach, *Periplaneta americana*, Indicates Future Embryonic Polarity

Joseph G. Kunkel and Ellen Faszewski (National Vibrating Probe Facility, Marine Biological Laboratory)

Ionic currents are associated with developing patterns in various organisms (1) and are ascribed to the movement of various ions. The function of these currents in each system is still unclear. We previously reported a pattern of ionic current about the vitellogenic follicles of cockroaches and termites (2). This group of insects is particularly interesting because of the simplicity of their ovarian follicles: a large oocyte surrounded by a single cell layered follicle epithelium. The observed pattern of currents, which we investigated with the older wire probe technology, indicates the location of the future embryonic germ band. We now report the identity of the ions involved, which we investigated using the recently developed non-invasive ion selective electrode technology at the National Vibrating Probe Facility, MBL, Woods Hole (3). Microelectrodes with tips of 2 μm were filled with 15 μm columns of liquid ion exchanger (LIX) cocktails. Potassium-sensitive LIX (Fluka Potassium Ionophore I-cocktail A) and proton-sensitive LIX (Fluka Hydrogen Ionophore I-cocktail A) were used. The microelectrodes, oscillated 10 μm in the X-, Y-, and Z-directions to measure μV gradients in those dimensions, were propelled by stepper motors controlled by computer software, 3DVIS, designed to measure 3-D patterns. Total flux was calculated by vector addition of the measured X-, Y- and Z- μV difference components. The efficiency of the K^+ electrode to measure K^+ flux was 80%; that of the proton electrode is also assumed to be high, but the effects of buffering in physiological salines are unclear. We therefore report our proton flux in terms of μV drop over a measured distance which can be interpreted as pH difference. Ovaries of the cockroach were dissected into cockroach Ringer, and the individual ovarioles were separated from connective tissue. Single ovarioles were transferred to a measurement chamber bathed in an appropriate saline. For measuring potassium, the *Periplaneta* Ringer of Smith was used (157 mM Na^+ , 3 mM K^+ , 2 mM Ca^{++} ,

2 mM Mg^{++} , 165 mM Cl and 8.6 mM HEPES, pH 7.2). For protons, the same Ringer, but with a weaker buffer (HEPES, 0.96 mM) was used to prevent the dampening of proton fluxes.

We measured substantial outward K^+ and proton gradients at the anterior end of each vitellogenic follicle within an ovariole, Figure 1A. The pattern of both the proton and K^+ gradients were largely identical, outward about an anterior polar cap, with the exception that a generalized lower level outward proton current was observed about the entire follicle. This low level outward current may reflect a generalized respiratory secretion of CO_2 from the tissue in general. No ion gradients were detected around previtellogenic follicles or around follicles close to, or after, chorion formation (Fig. 1B). The major gradients of ions exit the follicle through a tight epithelium of follicle cells that form a cap over the anterior pole of the follicle. The follicle anterior pole can be thought of as the vegetal pole of the *Periplaneta* oocyte; this is because the embryonic germ band will develop at the posterior pole. The location of the germ band can be considered the animal pole. Aside from the vegetal polar cap of 'tight' epithelium, the remainder of the follicle cell epithelium around the vitellogenic follicle is 'patent' (4), allowing the bathing medium to reach the oocyte surface. The extent to which the observed currents are electrically coupled between the follicle cells and oocyte is unknown; but TEM sections show that all follicle cells are morphologically coupled to the oocyte via gap junctions. We suggest that the tight cap of follicle cells at the anterior pole act as a polarized epithelium, responsible for the pumping of ions, which we see (Fig. 1). In many insects the V-type ATPase is responsible for pumping of protons. This pump is sensitive to the inhibitor Bafilomycin A1 from *Streptomyces griseus*. In several oocytes, which we treated with 1 μM Bafilomycin, the peak proton flux seen at the anterior cap of the follicle was inhibited by up to 60% over a period of 15 min. This finding

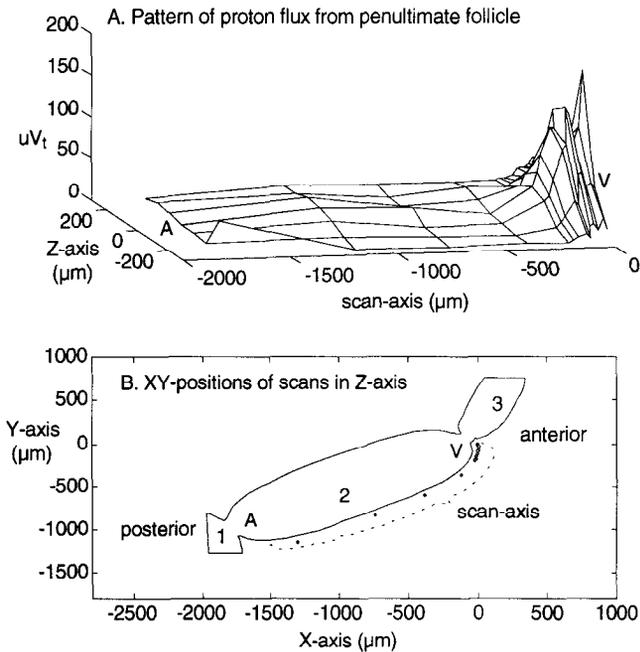


Figure 1. Pattern of proton movement about a vitellogenic ovarian follicle of *Periplaneta americana*. The animal (A) and vegetal (V) pole positions of the penultimate follicle are indicated in both 1A and 1B. A. Total- μV drop (μV_t) over the oscillation distance ($10 \mu m$) in three-dimensions. The scan-axis represents the linear additive distance from the (0,0) XY-location at the anterior end of the penultimate follicle to other locations along the scan route as depicted in Figure 1B (58 mV represents 1 pH unit of proton concentration difference with a measured background pH of 7.1). B. Outline of a section of ovariole including the anterior tip

suggests that a portion of the observed gradients are generated by the V-type proton pump and the established proton gradient might be further utilized by a K^+/H^+ exchange pump to produce the outward K^+ flux at the anterior pole. If a gradient of ions is being created through or around the enclosed oocyte by electrical coupling with the follicle cell layer, a force to influence the polarity of the future embryo may be at play.

The hospitality and advice of Peter J. S. Smith at the National Vibrating Probe Facility was a deciding factor in our progress on this project. We also thank Jeffery R. Demerest for sharing his experience with proton LIXs. This research was supported by grants to the National Vibrating Probe Facility and from the Biotechnology Program of the University of Massachusetts at Amherst.

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of the terminal follicle (labeled 1, chorionated and scheduled to ovulate in 1 day), the entire penultimate follicle (labeled 2, vitellogenic and to be ovulated in 4 days), and a portion of the pen-penultimate follicle (labeled 3, as yet non-vitellogenic and to be ovulated in 7 days). The 14 XY-positions of 7 3-D scans in the Z-axis are indicated. These positions were transformed into scan axis locations corresponding to the 14×7 mesh of points plotted versus measured μV -difference in Figure 1A. The anterior and posterior polarity of the ovariole is labeled.

Reference: *Biol. Bull.* **189**: 198–199. (October/November, 1995)

Chemotaxis, Aggregation Behavior, and Foot Formation in *Dictyostelium discoideum* Amoeba Controlled by Microbeam Uncaging of Cyclic-AMP

Yoshio Fukui and Shinya Inoué (Marine Biological Laboratory)

At a certain stage of development, amoebae of the cellular slime mold *Dictyostelium discoideum* signal to each other by secreting c-AMP [cyclic-3',5' adenosine monophosphate (1, 2, 3)]—and then aggregate. We analyzed the responses of aggregation-competent amoebae to brief applied pulses of c-AMP under high-resolution video DIC (differential interference or Nomarski contrast) microscopy.

Miniature sources of c-AMP pulses were generated by illuminating caged c-AMP (4) with a 366-nm-wavelength UV (ultraviolet) microbeam delivered as 3-ms flashes repeated every 0.65 s; we had added the caged c-AMP to the buffer and agar layer overlying the amoebae (5). A Zeiss Ultrafluor (UV- and visible light-transmitting, 100 \times /1.25 NA, glycerol immersion objective) lens equipped with a DIC prism replaced a conventional condenser to focus a highly reduced image of a first-surface micromirror, placed in front of the field diaphragm, superim-

posed with the DIC image of the specimen. The UV-reflecting micromirror was located at the focus of the UV source, an auxiliary 100-Watt Hg-arc lamp with quartz collector, 366-nm band-pass filter, and electrically activated shutter. The $2.2 \times 3.0 \mu m^2$ /UV image can be seen as a bright rectangle at the tip of the dark shadow of the mirror support in Figure 1A and B, slightly off center from the visible (546 nm) light image of the specimen in DIC. Moving the micromirror or specimen carrier placed the source of c-AMP in different locations relative to one or more amoebae.

Migrating aggregation-stage amoebae responded to the c-AMP pulses by turning towards the source (Fig. 1A) and migrating it. The first amoeba to reach the source engulfed it, and the others spiraled and aggregated around this first amoeba, which remained at the source (Fig. 1B). When the artificial source of c-AMP was removed by shutting off the UV flashes, all the amoebae dispersed