

Models of Pattern Formation in Insect Oocytes

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*Abstract. Pattern formation in early insect development is dominated by coordination of the germ line polarity with the polarity of the follicle cell layer. The production of an elaborate protective chorion, covering the ovulated oocyte, has made establishing parallel polarity of germ line and soma absolutely essential. Genetics and molecular biology, particularly on *Drosophila melanogaster*, have identified numerous signals passed from follicle cell to oocyte and vice versa. The physiological basis of this communication is beginning to be established with the identification of several membrane receptors and potential signal transduction steps. The contributions of three physiological models of pattern formation are discussed as they relate to the growing genetic model. Evidence for and against ionic currents as factors in polarity determinations is particularly emphasized.*

The origin of polarity and pattern in living organisms has been of general interest for over a century (Hallez, 1886; Wilson, 1896; Jaffe, 1981, 1985; Meinhardt, 1982; Brenner *et al* 1981; Steen, 1988; Cooke, 1988; Cummings, 1990). Pattern development in oocytes has been of particular interest because in some sense it reflects starting from scratch, a ground state in terms of pattern. This is more or less true depending on the group of organisms involved. Oocytes of some algae start out as newly fertilized zygotes with a spherical symmetry and no apparent poles; polarity is determined in response to environmental cues which will optimize the alga's orientation with the substratum (Jaffe, 1981). Different degrees of regulation of pattern formation are seen with other groups. Many oocytes do not establish one or both axes of polarity until after fertilization. In insects, however, both the antero-posterior (A-P) and the dorsal-ventral (D-V) axes are established early, in the ovary prior to ovulation and fertilization. The tradition of the insect embryo's A-P axis paralleling that of the maternal A-P axis, part of the Law of Hallez (Hallez, 1886; cf. Gutzeit and Sander, 1985), has been

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formalized by many evolutionary inventions. The elaborately sculptured egg chorion layer (Margaritas, 1985) is secreted by a follicle cell layer which at some point must become aware of or impose its own polarity, A-P as well as the D-V, on the oocyte. The sculpturing and secretion of the chorion layer includes details such as points of sperm entry and hinged openings from which the larva hatches. All insect oocytes are ovulated with their presumptive anterior pole pointing anterior in the females oviduct. While this is clearly fact it does not eliminate the job of discovering the details of how that polarity is transmitted or imposed by the maternal tissues on the germ cells that become oocyte, or *vice versa*. Several models of pattern formation are discernable in the developmental biology literature. These models can each be envisioned to apply to insect oocytes but the substantial differences in morphology between the three major types of insect ovary require a brief introduction to their differences.

Insect ovaries are distinct in morphology and somewhat in physiology. While the organization of ovaries into follicles, oocytes surrounded by follicle cells, is found broadly in the animal kingdom, the organization into strings of polarized follicles, the ovariole, is characteristic of insects (cf. Aizenstadt, 1988). I will focus on the relationship of oocyte to follicle cell in my discussion of polarity determination in insects. Three types of insect ovariole exist: panoistic, meroistic polytrophic and meroistic telotrophic, Figure 1 (Mahowald, 1972; Gutzeit and Sander, 1985). In all three types follicle cells surround the germ cell and interact with it intimately during oocyte development. The panoistic ovariole is the simplest in morphology, consisting of an oocyte surrounded by a follicle cell layer. The two meroistic ovaries have more complicated cytological derivations and physiologies. Oogonia divide to form a cluster of sister cells, cystocytes, which remain connected by cytoplasmic bridges. One of the cystocytes becomes the oocyte and the remainder become nurse cells. Nurse cells directly contribute cytoplasm and macromolecules to the developing oocyte during vitellogenesis and pattern formation.

The simpler morphology of the panoistic ovariole may allow certain physiological aspects of the pattern formation

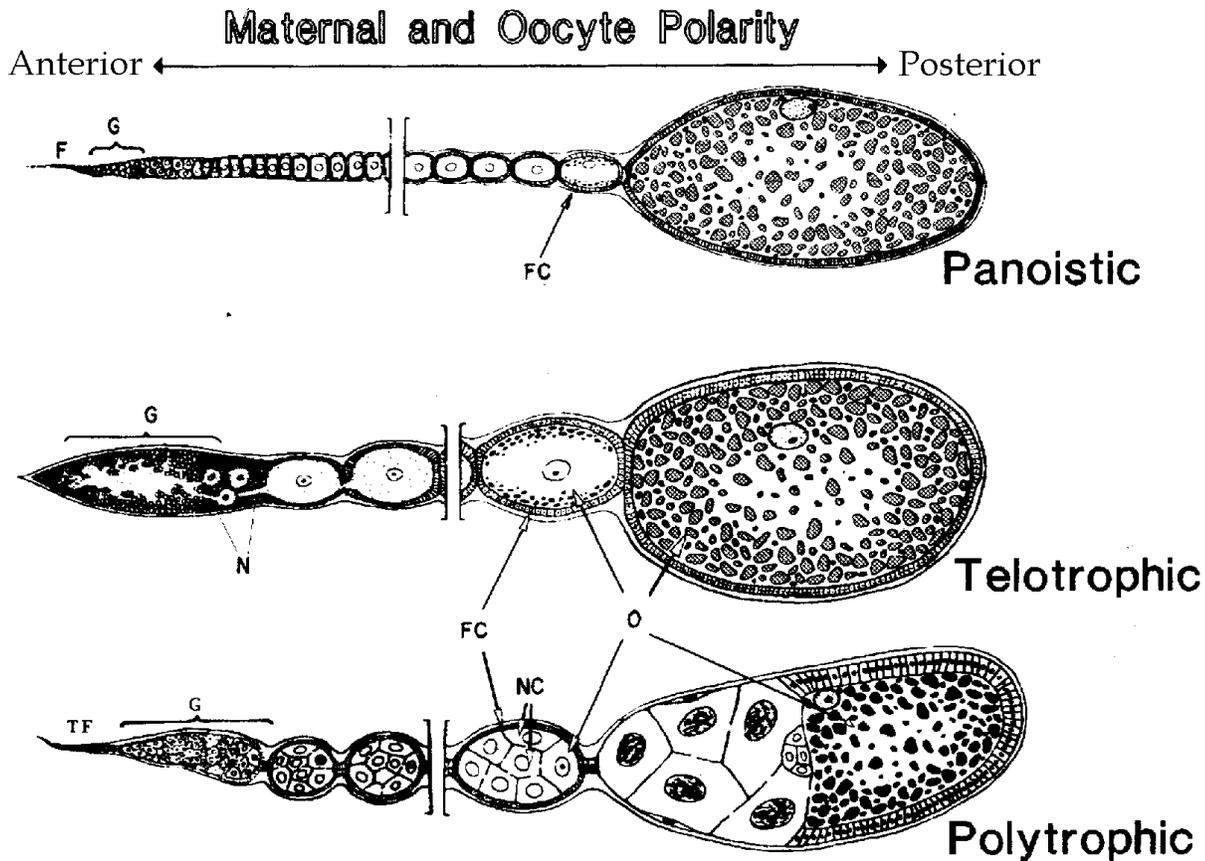


Figure 1. Three basic types of insect ovariole as they relate to the maternal antero-posterior axis. FC follicle cells. G germarium. N nutritive chord. NC nurse cell. O oocyte. TF terminal filament. (Modified from Mahowald, 1972)

process to be addressed more directly. In particular, the short germ band type panoistic oocyte may focus communication between oocyte and follicle cell layer in a pattern parallel to the varied location of the germ band, Figure 2A, B, allowing the communication to be visualized and studied.

The timing of egg polarity determination changed to ovulation, in a heterochronic sense (Gould, 1977), during the evolution of land animals. Internal fertilization and extended embryonic development created several storage and protection problems that had to be resolved. Among the solutions to these problems were storage forms of cell machinery *i.e.* ribosomes and mitochondria, and nutrients, yolk, and a physically impervious covering over the developing egg, in insects the chorion. These physiological needs were met in insects, more or less, by follicle cell specialization. The follicle cell layer participates in provisioning the oocyte and eventually secretes a protective chorion. This structure is secreted prior to ovulation and fertilization and thus includes stereotyped entry point(s) for the sperm, the micropyle, and predetermined weak points through which the larva will hatch. The differentiation of follicle cells into at least eleven different cell types (Margaritas, 1985) reflects the diversity of chorion structures and sculpturing that decorate the insect egg. For the larva to hatch through the eggshell its axes must be in parallel with its surrounding chorionic sculpturing. Establishing oocyte polarity is a particularly poignant topic in insect development.

Several helpful reviews and critiques of insect embryonic polarity exist (Gutzeit and Sander, 1985; Sander *et al* 1985; Jaffe, 1986; North, 1986; Woodland and Jones, 1986; Anderson, 1987; Melton, 1991) but I hope to add a new dimension based on discussing the need for coordinating follicle cell and oocyte polarity.

There are four major models of pattern generation for the oocyte, Figure 3: 1) The Molecular-Genetic Pattern Formation Model. 2) The Toothpaste Model. 3) The Electrophoretic Model. 4) The Polar Coordinate Model. These distinct models may each have useful contributions to our understanding of the origins of polarity.

1) *The Molecular-Genetic Pattern Formation Model.* The genes controlling pattern determination in *Drosophila* and other metazoans are slowly but surely yielding to genetic and molecular analysis (Anderson and Nusslein-Volhard, 1984a; Melton, 1991). Passing on polarity to the fertilized oocyte is a complex process which does not allow simplification to a single bottleneck in polarity as is experienced in the *Fucus* egg (Jaffe, 1981). Interactions between several maternal and zygotic genes produce the antero-posterior and dorsal-ventral axes, Figure 3. The maternal genes include both somatic genes expressed in the follicle cell layer and germ line genes expressed in the nurse cells (Wieschaus 1979; Frey and Gutzeit, 1986; Schuepbach and Wieschaus, 1986a). Gastrulation on the appropriate surface and activation of the embryo's

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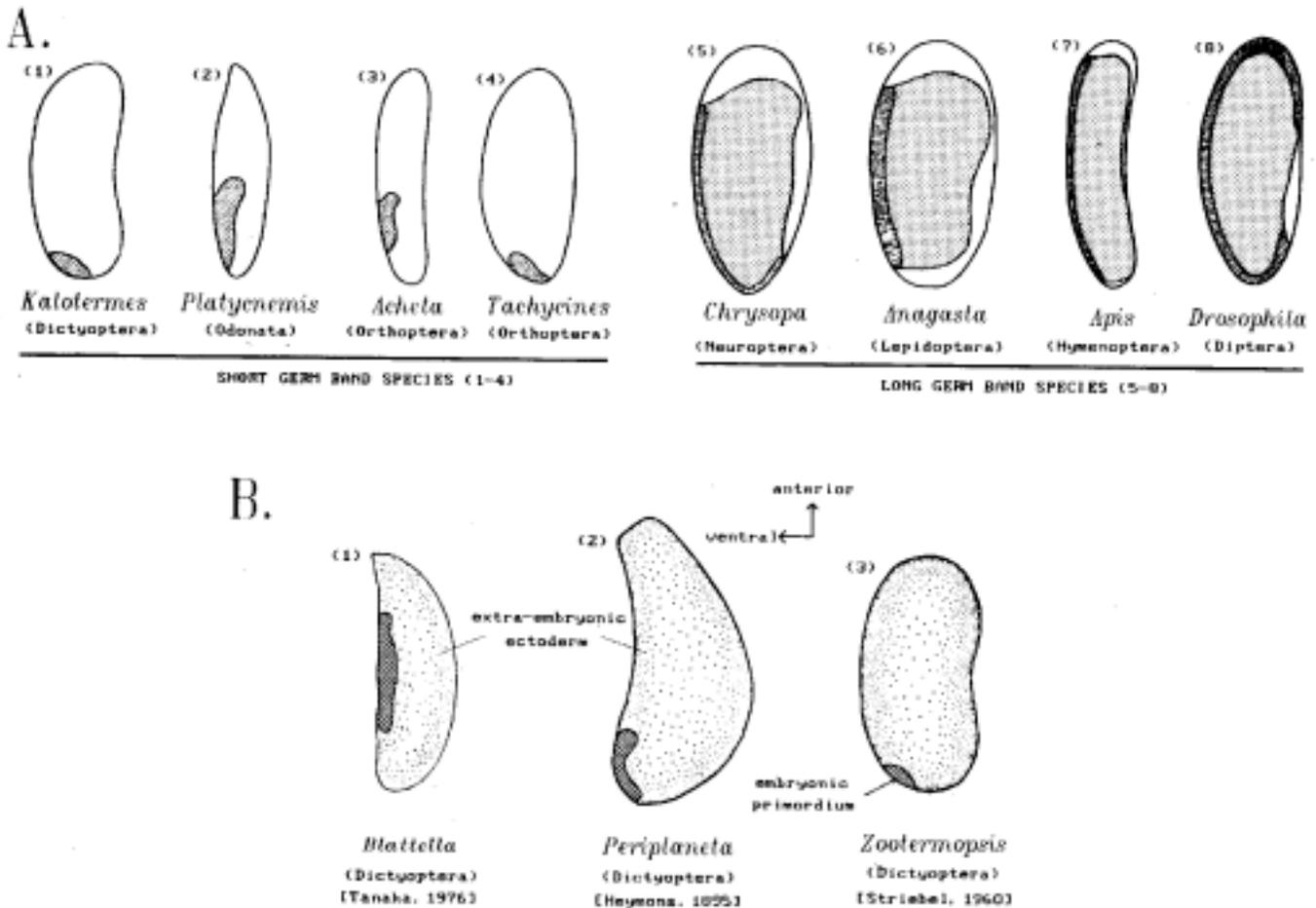


Figure 2. Placement of the embryonic primordium or germ band in various insect oocytes. A. Contrast between the short germ band and long germ band follicles. *Drosophila* is an extreme long germ band type as its germ band almost completely surrounds the oocyte. The stippled area represents the extent of the germ band. B. Variation of the placement of the germ band within the insect order Dictyoptera. The crosshatched area represents the extent of the early embryonic primordium or germ band. (After DT Anderson, 1972a, b).

zygotic segmentation genes at the appropriate locations represents an end point in the pattern formation process. At that point we can say that the torch of polarity has been correctly passed to the next generation. Several stages or levels of gene interaction have been discovered which may involve communication between follicle cell and oocyte in *Drosophila's* pattern formation.

Figure 3 is a schematic and polyglot-eclectic version of several previously published schemes of pattern formation in *Drosophila* (North, 1986; Woodland and Jones, 1986; Manseau and Schuepbach, 1989a; Melton, 1991). An attempt has been made to catalogue the several developmental stages of gene interaction known to result in proper embryonic pattern formation in *Drosophila*.

Pattern in *Drosophila* embryos is controlled largely separately in two axes, the A-P axis and the D-V axis. There are some germ line maternal affect genes *sp* and *cap*, however which interact in both A-P and D-V axis determination (Manseau and Schuepbach, 1989b). The origin of the A-P axis can always be argued to be a historical inheritance from the

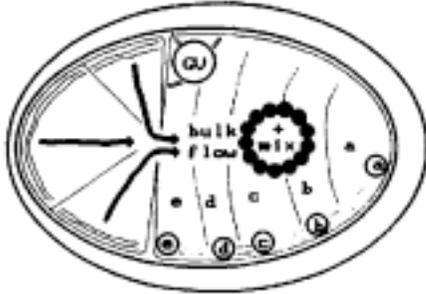
asymmetric cleavage of a stem cell in the germarium of the, ovariole. However, geneticists have, largely correctly, insisted on finding mutants associated with the sequence of steps the germ cell takes in the ovariole. The only caveat to that approach is the existence of gene products, such as *caudal* mRNA, which has been identified as a gene product based on containing a homeo-box and which expresses itself in a localized way by *in situ* hybridization, but for which there are no known mutants (Mlodzik *et al* 1985). This type of gene represent a hidden class of genes which will have to be characterized and contended with in new ways.

The A-P axis is the first axis to be determined and one of the first genes that must act properly to establish the oocyte's A-P axis is *egalitarian* (Mohler and Weischaus, 1986). This is a loss of function mutation which results in all 16 cystocytes in the germarium being equivalent, *i.e.* no cell is determined as the oocyte. Selection of the posterior most cystocyte to be the oocyte is critical to determining the eventual A-P axis. An early sign of polarity, in the absence of mutant *egal*, is the deposition of *Oskar* mRNA in the posterior-most cell of the

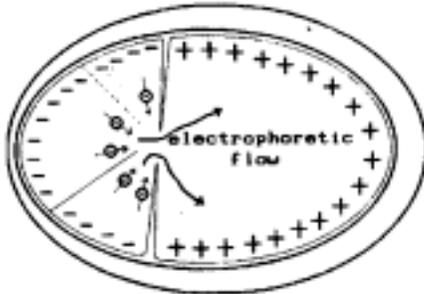
Epigenetic Models:

Genetic Model:

Toothpaste Model:



Electrophoretic model:



Polar coordinate model:

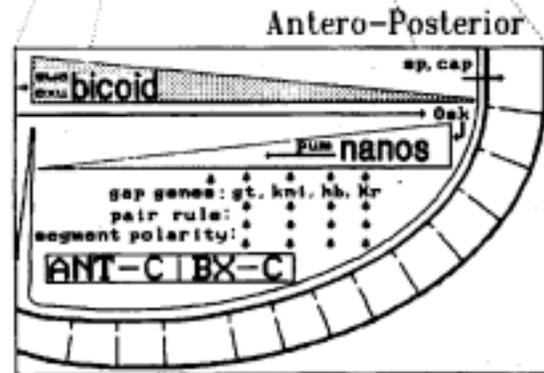
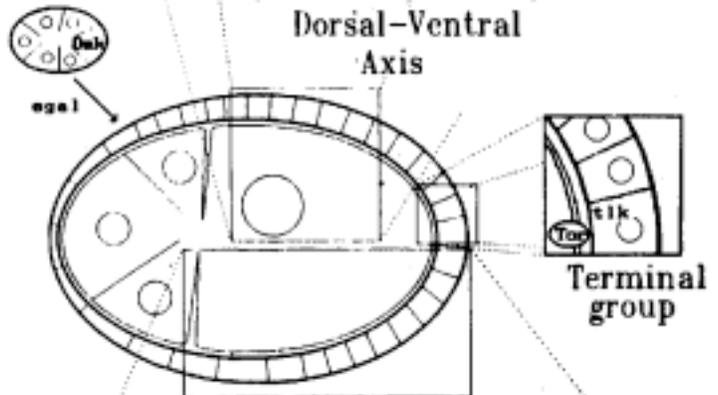
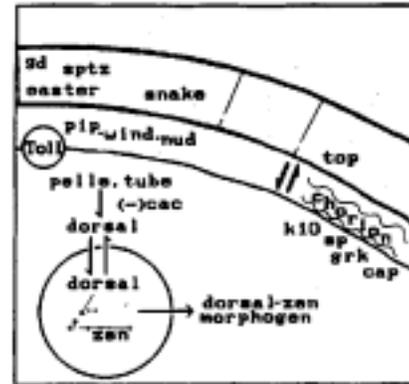
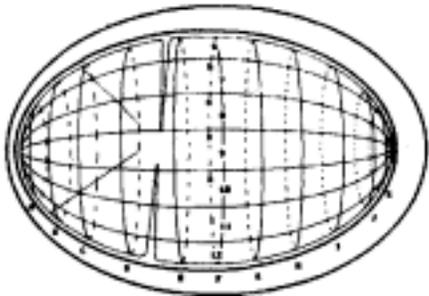


Figure 3. Models of pattern formation as applied to the polytrophic meroistic follicle. Three models based on physiological and experimental embryology of insect oocytes which may be applicable to explain the phenomenology associated with mutations which contribute to the Genetic Model of pattern formation. Five insects to the Genetic model are (clockwise from 10:00): 1) An early follicle at the stage that the posterior cystocyte is beginning to accumulate *Oskar* mRNA. 2) Dorsal-ventral axis determinants including two groups, the Toll associated follicle cell and germ line components, and the torped (top) associated germ line modulators of chorion formation genes. 3) Terminal-group of the A-P axis determinants. 4) The bicoid and nanos gradients and their response cascade of gap, pair rule and segmentation genes.

cystocyte cluster. *Osk* mRNA is produced by the 15 nurse cells but continues to accrete and becomes localized at the posterior pole of the oocyte (Lehmann and Nüsslein-Volhard, 1986). It requires a directed migration of the mRNA from its point of synthesis in the nurse cells and its anchoring at a specific location in the presumptive oocyte. The *Osk* gene product is responsible, along with other posterior group maternal effect genes, *tudor*, *vasa*, *staufen*, *valois*, *spire* and *cappuccino* in determining the localization of

nanos (*nos*) mRNA (Lehmann and Nüsslein-Volhard, 1986). *Nos* is the posterior morphogen protein, a transcription factor. The distribution of this transcription factor in a gradient decreasing toward the anterior is aided by the *pumilio* gene (Lehman and Nüsslein-Volhard, 1987a). An analogous gradient of the *bicoid* (*bcd*) gene product from the anterior region of the oocyte, based on *bcd* mRNA localization (Berleth *et al* 1988), creates a second measurable morphogen signal. Localization of *bcd* mRNA is regulated by the

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two genes *exuperantia* and *swallow* (Frohnhofer and Nüsslein-Volhard, 1987; Manseau and Schuepbach, 1989h). Theoretically the combined levels of *bcd* and *nos* provide an antero-posterior coordinate system, shortly after fertilization, which can activate appropriate zygotic genes specific to presumptive segments of the future embryo. A third A-P determining factor, *torso* (*tor*), proscribes the expression of terminal versus central elements. In this case *tor* codes for a putative receptor tyrosine kinase. The receptor is distributed uniformly over the oocyte surface but acts in response to an extracellular spatially restricted ligand originating from terminal follicle cells (Casanova and Struhl, 1989; Stevens *et al* 1990). The tyrosine kinase would be linked to gradient(s) of intracellular signal(s) as is the case for *Toll* (*Tl*). The A-P associate transcription factors call forth the activation or repression (Irish *et al*, 1989) of the gap genes, *giant*, *knirps*, *hunchback* and *Kruppel* (Lehmann and Nüsslein-Volhard, 1987b). These are the first in a hierarchy of zygotic genes (gap-> pair-rule-> segment-polarity-> homeotic genes) which refine the pattern designated by a cell's position in the gradient. The zygotic genes being affected are in nuclei which by 2.5 hours after fertilization of a *Drosophila* oocyte will be cellularized in a blastoderm layer.

The dorsal-ventral axis of *Drosophila* is established under control of a sequence of maternal gene expressions, the dorsal group of genes (Anderson and Nüsslein-Volhard, 1986). *Very few zygotic genes with global dorsalizing effects have been found despite saturation genetic screens* (Anderson and Nüsslein-Volhard, 1984a). The dorsal-group genes are represented primarily by loss of function alleles which result in dorsalized embryos (Anderson and Nüsslein-Volhard, 1986) i.e. the definitive role of the *dorsal* (*dot*) gene product, a sequence specific transcription factor (Ip *et al*, 1991), is to induce ventral structures and repress dorsal structures. Insight into the role of somatic and germ line cooperation is provided by the fact that the dorsal-group includes several follicle cell maternal genes as well as several germ line maternal genes. *Gurken* and *torpedo* for instance control ventral structure from germ line and soma respectively (Schuepbach, 1987). Communication of the oocyte with the follicle cells is necessary for proper follicle cell migratory and synthetic behavior in secreting an elaborate regionally diverse chorion structure (Weischaus *et al*, 1978; Weischaus, 1979; Margaritis, 1985). This set of dorsal and chorion gene expressions may represent our best opportunity to understand the role of somatic and oocyte interaction in the determination of oocyte polarity.

Perhaps a pivotal gene, if one can be thought to exist, of the dorsal group which has a role in determining the ventral side of the future embryo is *Tl* (Anderson and Nüsslein-Volhard, 1984a, 1986; Anderson *et al*, 1985a, b; Anderson, 1987; Hashimoto *et al*, 1988). The *Tl* gene product is a membrane protein, presumably a receptor, which may be central to coordination of the induction of the ventral side of the embryo in coordination with the dorsal chorion laid down by its overlying follicle cells. *Tl* protein is uniformly distributed in the D-V axis however it acts on what will be the

Table I. *Current measurements in oocytes.*

Order: Genus	Current type	References
Dictyoptera <i>Blattella</i>	D-V	(Kunkel, 1986; Kunkel et al, 1986; Kunkel & Bowdan, 1989; Bowdan & Kunkel, 1990)
<i>Nauphoeta</i>	VG-Ca ⁺⁺	(Sigel et al 1990)
<i>Periplaneta</i>	A-P	(Huebner & Sigurdson, 1986; Kunkel, unpublished)
<i>Zootermopsis</i>	A-P	(Kunkel & Stuart, unpublished)
<i>Locusta</i>		(O'Donnell, 1988)
Hemipteran <i>Dysdercus</i>	A-P	(Dittmann et al, 1981)
<i>Rhodnius</i>	A-P	(Huebner & Sigurdson, 1986, Diehl-Jones & Huebner, 1989)
	Ca ⁺⁺ AP	(O'Donnell, 1985, 1986)
Dipteran <i>Sarcophaga</i>	A-P	(DeLoof, 1983; DeLoof & Geysen, 1983; Geysen et al., 1988; Vcrachtet et al, 1986, 1988)
<i>Drosophila</i>	A-P	(Overall & Jaffe, 1985; Woodruff et al, 1988; Woodruff, 1989)
	no currents	(Bohrmann et al, 1986a, b; Bohrmann & Gutzeit, 1987; Bohrmann, 1991; Sun & Wyman, 1987)
Lepidoptera <i>Hyalophora</i>	A-P	(Woodruff & Telfer, 1973, 1990; Jaffe & Woodruff, 1979; Telfer et al, 1981; Woodruff et al, 1986)
Amphibia <i>Xenopus</i>	A-V	(Robinson, 1979; Miledi, 1982)
	A-V CI	(Barish, 1983; Miledi & Parker, 1984)
	cGMP&C ⁺⁺	(Dascal et al, 1984, 1987)

A-P = antero-posterior axis

D-V = dorsal-ventral axis

AP = action potential

VG = voltage gated current.

A-V = animal vegetal axis

presumptive ventral surface. Cytoplasm from *Tl*⁺ oocytes can induce a ventral pole wherever it is injected in *Tl*- oocytes. This once enigmatic fact belies a possible key to understanding induction of a D-V axis.

From a successfully activated *Tl* receptor, a cascade of ventralizing gene activities culminates in wild type *dor* gene product, a transcription factor, being distributed at 90 to 180 minutes after fertilization in a dorsal to ventral gradient (Steward *et al*, 1988) and localized primarily in the nuclei of the ventral blastoderm. This gradient of protein is found despite the fact that the maternal mRNA for *dor* is uniformly distributed throughout the oocyte at ovulation. Part of the cascade involves correctly inducing *Dor* uptake into ventral nuclei (Steward, 1989). *Dor* protein, in one of its regulatory roles, inhibits the production of the *zerknult* (*zen*) gene product. *Zen* is a morphogen which is involved in positively regulating the induction of dorsal structures (Doyle, Kraut

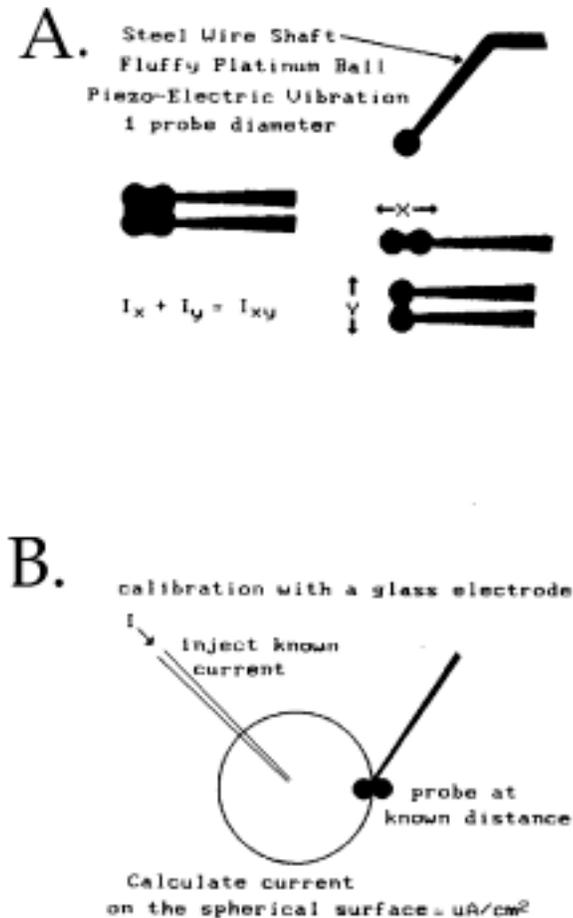


Figure 4. The modern two-dimensional vibrating probe for non-invasive measurement of ionic currents entering and leaving cells and tissues. A. The silhouette of a modern wire probe vibrating in two dimensions. B. Calibration of the probe is achieved by measuring an ionic current emanating from a glass electrode as it passes a tangent to a sphere of known diameter in a defined medium.

and Levine, 1989). One of the first ventral embryonic activities at the time of blastoderm formation is gastrulation, which is abnormal in dorsal-group mutants.

Of prime interest to our discussion of the origins of pattern is the mechanism by which *Tl* and *torso* action are localized respectively to the presumptive ventral and terminal oocyte surfaces. While other dorsal-group gene products can rescue dorsal mutants to some extent, only *Tl* can direct the position of a ventral focus of embryo formation. Several of the follicle cell somatic maternal effect mutants, *pipe*, *windbeutel* and *nudel*, produce products that are secreted into the perivitelline space between the follicle cells and oocyte. These products accumulate only in *Tl* embryos which do not express the *Tl* receptor (Stein *et al.*, 1991). They may play a role in transmitting a coordinating signal between soma and germ cell. *Torsolike* (*tlk*), a locally expressed follicle cell gene, plays a similar role in activating the *torso* receptor (Stevens *et al.*, 1990). How are the *pip-wind-nud* and *tlk* ligands presented to the receptors?

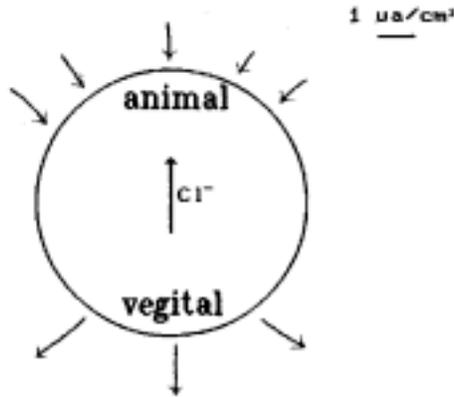
It is yet unclear how the *Drosophila* embryo gets its

patterning signals in toto. The nurse cells are clearly the source of important mRNAs which are involved from early germinative stages in the establishment of gradients of morphogens within the oocyte. The extent to which insect follicle cells are sources of important molecules other than yolk storage proteins and protective chorion layers that enter or directly communicate with the oocyte is currently of great interest but still somewhat obscure. Communication in both directions is implied by somatic genes which affect oocyte polarity and germ line maternal genes which affect follicle cell behavior. It is suggested that substances (*e.g.* proteoglycans [Ruoslahti and Yamaguchi, 1991]) are secreted by the follicle cells into the perivitelline space which are subsequently sensed during the pattern determination cascade (*e.g.* by *Tl* and *torso*) of the pre-embryonic female gamete and early embryonic zygote through the action of follicle cell and germ line maternal products. Whether these are definitive messages determining pattern or instances of 'touching base' in a more extensive pattern of communication is not clear. The history of pushing back the regulation of pattern formation further into the oocyte stage would suggest we have much more to learn about earlier communication.

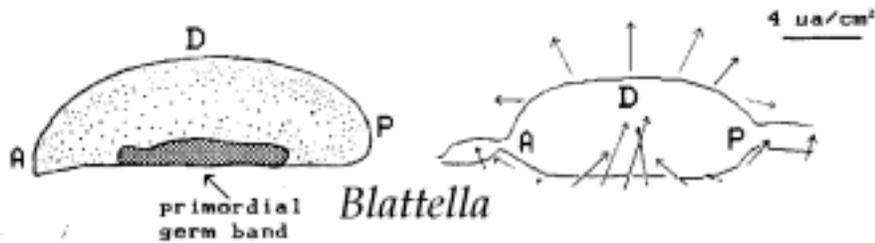
The extent to which physiological mechanisms can be placed on the above genetic model is somewhat limited so far. In some instances mechanisms have been suggested by the molecular biology. Thus the similarity between the ventralizing maternal affect locus *top* and the EGF receptor suggests a model that may parallel the EGF factor family of hormonal receptor mechanisms (Schejter and Shilo, 1989; Price *et al.*, 1989). Further progress may certainly be sought directly by tracing how an EGF-like receptor in the follicle cell layer is necessary for and results in a ventralizing effect on the future embryo. The epistatic relationship of *grk* and *top* over *fs(1)K10* (Schuepbach, 1987) combines with the EGF homology to suggest a communication signal that impinges on the follicle cell layer early, prior to the sealing off of the follicle cell layer from the oocyte by chorion secretion. The *Tl* and *torso* receptors on the other hand are uniformly distributed over the oocyte surface but are activated at or shortly after chorion formation, responding to perivitelline space components produced locally or at least anchored locally by overlying follicle cells. While the genetic studies have suggested the players in the process, they still do not address the modes-of-action and physiology of how and where polarity arises. The succeeding three models are the best developed physiological models of pattern formation. However, their relationship to the genetic model is less than clear.

2) *The Toothpaste Model.* Work on the ultrastructure of polytrophic merostic insect ovarioles focusing on microfilaments and microtubules suggests that mechanical forces are responsible for nurse cell provisioning of the developing oocytes (Gutzeit, 1986a). This process, as illustrated in Figure 3, could create onion-like layers of oocyte cytoplasm which originate as waves of extruded nurse cell cytoplasm. However, microtubules in *Drosophila* ovarian follicles (Gutzeit, 1986b) are observed to function in continually mixing oocyte cytoplasm such that no layering of the streaming

Xenopus type current pattern



Blattella-type current pattern



Periplaneta-type current pattern

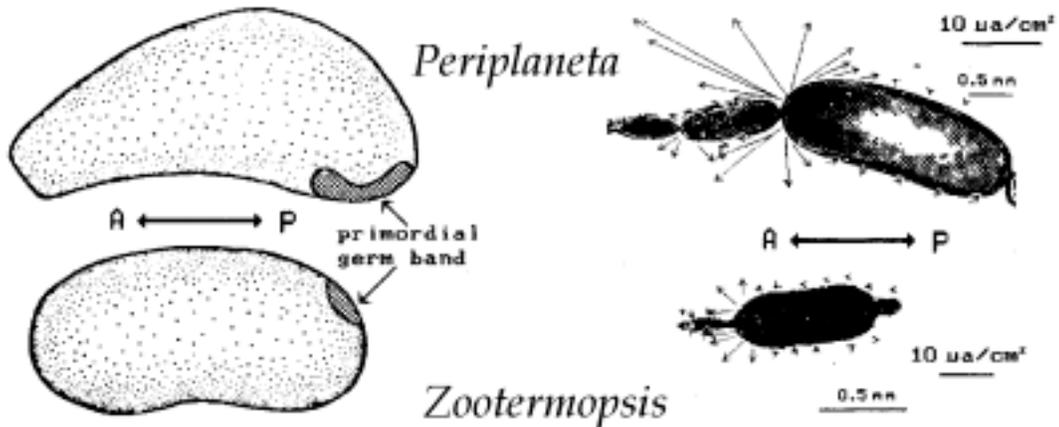


Figure 5. Current patterns about four species oocytes, each of which has a known animal vegetal orientation. *Xenopus* has been extensively studied and the observed current pattern is based on a vegetal to animal flow of chloride. The germinal band location (left) and the ionic current pattern about the terminal follicle (right) are displayed for three Dictyopterans. The *Blattella*-type pattern seen in *Blattella germanica* was originally described as a dorsal-ventral current (Kunkel, 1986; Kunkel and Bowdan, 1989) based on the primordial germ band localization by Tanaka (1976). The *Periplaneta*-type current pattern is seen in *Periplaneta americana* terminal and subterminal oocyte and a terminal oocyte of the termite *Zootermopsis angusticollis* (Kunkel and Stuart, unpublished). Current leaves the ovariole at the pole opposite the presumptive germ band location as identified by Heymon (1895) and Striebel (1960) respectively. **A, P** refer to anterior and posterior poles of the embryonic axis.

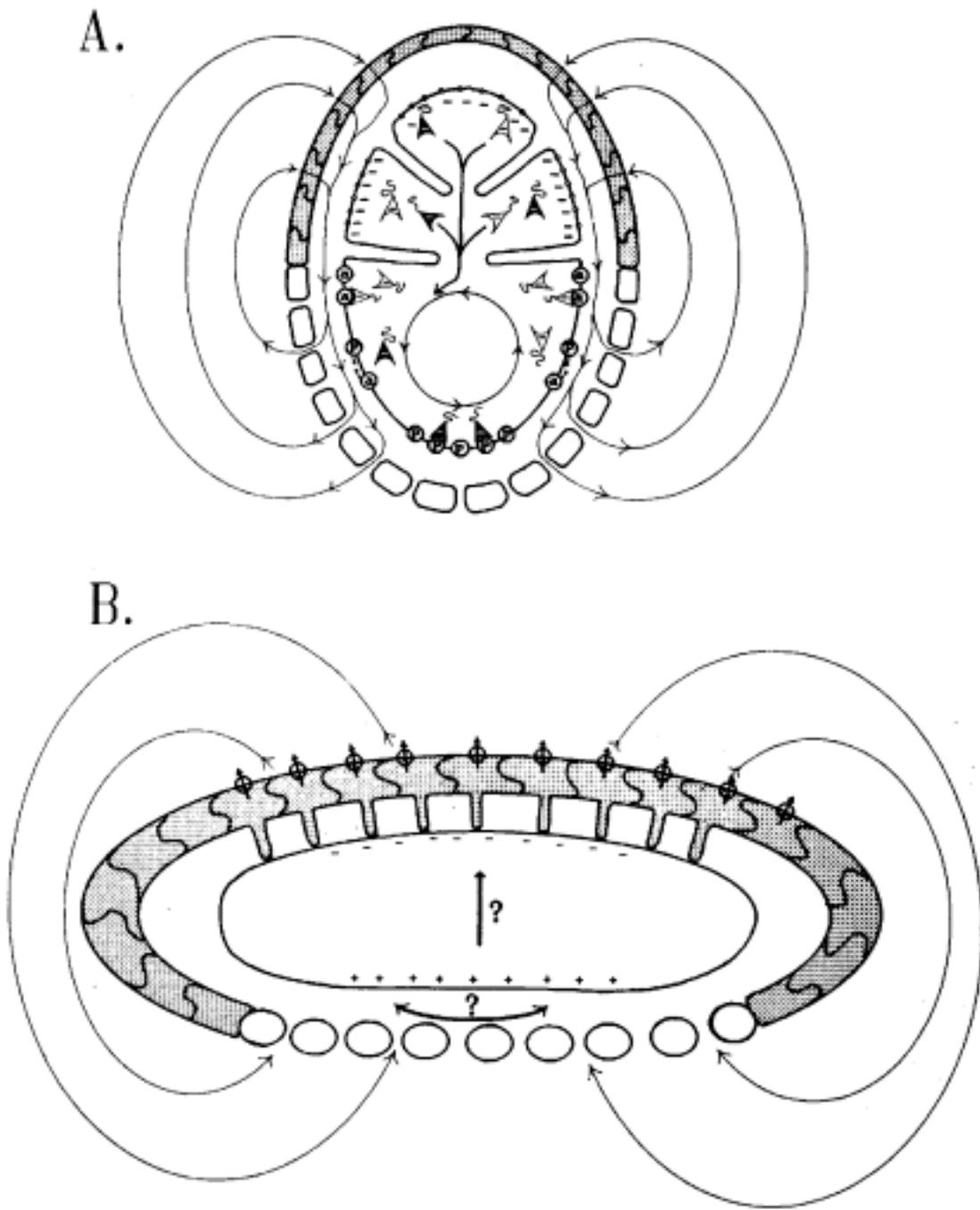


Figure 6. Hypothetical model, of ionic influences on pattern formation in polytrophic (A) and panoistic (B) oocytes. Current is driven in both models by an electrically tight follicle cell epithelium (stippled) in one region of the follicle. A region of patent follicle epithelium allows passive flow of current to complete the circuit. In both models a current loop can flow between the oocyte and follicle cell layer. In A the current is shown influencing the lateral electrophoresis of membrane anchors for important anterior (a) and posterior (p) pattern determinants. The flow of maternal mRNA from the nurse cells to the oocyte is affected by cytoplasmic extrusion and/or electrophoretic forces until it enters the oocyte where it is circulated by microtubule based cyclosis until it docks with its appropriate anchor in the oocyte cortex. In B the path of ion flow, through the oocyte or around the oocyte is in question in the strong current phase. Gap junctions connect the follicle cell layer with the oocyte. Both models create an asymmetric environment for the oocyte allowing for it to respond in a polarized fashion.

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contents would occur in the central region of yolk. The oocyte cortex of 5 to 10 microns thick and its overlying cell membrane are the only oocyte structures that could seemingly escape this mixing. This is the region that is potentially available to be organized prior to ovulation when some important communications are occurring between nurse cells, oocytes and follicle cells. After colchicine treatment, nurse cell cytoplasmic extrusion continues but oocyte mixing stops and the oocyte length becomes layered with aged strata, including the germinal vesicle, which becomes displaced posteriorly from its normal location. The normal location of the germinal vesicle, which is asymmetric on the D-V axis (Geysen *et al.*, 1988), also depends upon intact microtubules.

Strong evidence suggest that contractions in a cortical layer of microfilaments in nurse cells is responsible for the extrusion of nurse cell cytoplasm into the oocyte (Gutzeit and Huebner, 1986). This is visible as nurse cell cytoplasm streaming into the oocyte cytoplasm during late *Drosophila* oogenesis, stage 10B and 11, when the oocyte is growing at the expense of shrinking nurse cells (Gutzeit and Koppa, 1982). Such streaming was responsible for the classical illustrations of Bier (1963) demonstrating a physical flow of labeled nucleic acid from nurse cell to oocyte in *Musca*. Of some importance are the facts that the origin of *bicoid* and *Oscar* mRNA from the nurse cells and their deposition at the respective anterior and posterior of the growing oocyte initiates quite early and proceeds through the vitellogenic phase of the oocyte. Thus despite mixing of the central yolk plug, the ~7 μm thick cortex, anterior and posterior, continue to accrete these positionally important molecules (Gutzeit and Koppa, 1982). It is of some interest then whether the microtubule based mixing process is working for, or counter to, this positioning process? How do the localized substances get anchored and how do the anchors become asymmetrically distributed? There are clues to this in the story told above of the epistatic relations of germ line and soma maternal genes which communicate during oogenesis but which later affect the distribution of ligands produced by the follicle cells.

Other insects including other Diptera as well as Hemipter-ans (Huebner and Gutzeit, 1986; Geysen *et al.*, 1988) and Lepidoptera (Jarnot *et al.*, 1988) have been shown to have cortical microfilaments and microtubules which may play an important role in physical movements of ooplasm as well as patterning phenomena such as localization of maternal mRNA (Kastern *et al.*, 1990). It is clear that the presumptive D-V axis is already established for the oocyte of many insects prior to chorionation based on the anchored position of the germinal vesicle. What then is the role of the later communication of the follicle cells with the oocyte via the *Tl* and *torso* receptors? If the ventral and terminal follicle cells communicate the proper D-V and A-P axis signal to the ubiquitous *Tl* and *torso* receptors, when did the follicle cells learn their appropriate orientation relative to the oocytes germinal vesicle based D-V axis and *Oskar/bicoid* based A-P axis? These may result from axis communications occurring prior to chorionation to ensure the correct parallelism between germ cell and soma. These questions accentuate a broad time and space interface, alluded to in the introduction,

that must exist between soma and germ cells while their parallel polarities are developing. We may currently be learning piecemeal messages in a continued communication to ensure a coordination of polarities of the two generations, insight into the basis of what enthralled Hallez a century ago.

3) *The Electrophoretic Model.* Ionic currents entering and leaving oocytes, Table i, have been associated with various aspects of germ cell development and polarity of diverse species (Jaffe and Nuccitelli, 1977; Jaffe, 1981, 1986). Transient calcium currents have been observed in various amphibian and marine invertebrate oocytes associated with the more definitive events of maturation and fertilization. Voltage gated calcium channels have been observed in oocytes of many species (Hagiwara and Jaffe, 1979) including hemipter-ans (O'Donnell, 1985) and cockroaches (Sigel *et al.* 1990) but are largely missing from amphibian oocytes (Dascal *et al.*, 1986). These channels can result, experimentally at least, in action potentials and may be important for signaling important events such as fertilization or maturation. Excitability of insect oocytes has been demonstrated using K^+ channel blockers (O'Donnell, 1988).

These transient current functions are most likely separate from the phenomenon of the steady ionic currents, which have been measured with the vibrating probe, Figure 4. This instrument has lead to a burst of knowledge about ionic currents associated with oocytes and ovarioles. In the amphibian, *Xenopus*, the defolliculated 1 mm oocyte has been studied. Currents on the order of $1 \mu\text{A}/\text{cm}^2$ have been observed entering the animal pole and exiting the vegetal pole. These currents, by convention positive, have been identified to be negative ions (chloride) traveling in the opposite direction (Robinson, 1979). Calcium modulates the chloride channels (Barish, 1983; Miledi and Parker, 1984).

Insect oocytes have only rarely been studied in the absence of their surrounding follicle cell layer. They are more commonly studied with the follicle cell layer intact or partially removed. This has resulted in problems of interpretation of the ionic currents. Nonetheless, steady ionic currents have been reported surrounding all three types of ovarioles. The ovariole types will be dealt with separately since their currents may have substantially different origins and functions.

In the meroistic polytrophic ovarioles of Diptera (Woodruff, 1989a, b) and Lepidoptera (Woodruff and Telfer, 1973, 1990) currents are concomitant with the flow of materials between the nurse cell and oocyte, Figure 3B. The functions and virtual existence of these currents are controversial but could be involved in the polarized migration of maternal mRNAs such as *bicoid* and *oskar* in *Drosophila* which take place throughout the vitellogenic phase. These molecules can not be targeted to their respective anterior and posterior anchor sites by simple extrusion of the germ cell plasm from the oocyte, otherwise they would have similar distributions.

It is possible that the targeting could be achieved by appropriate charge characteristics of their respective nucleoprotein particles combined with appropriate binding characteristics of their target anchor sites. The anchor sites themselves could be inserted randomly in the cell membrane of the oocyte and obtain their localization in the A-P axis by lateral electrophoresis in the plane of the membrane. It is possible that such a polarizing current is derived from the follicle cell layer (to be dealt with below).

Not everyone agrees on the importance of ionic currents in oocyte physiology. In particular some laboratories have had difficulty measuring significant currents in *Drosophila* and emphasize other mechanisms of transport and pattern formation (Bohrmann *et al.*, 1986a, b; Bohrmann and Gutzeit, 1987; Sun and Wyman, 1987; Bohrmann, 1991). *Drosophila* oocytes are small on the scale of oocytes that have been examined with the vibrating probe and this may test the limits of the technology and be a factor in the negative results in some labs. The vibrating probe's resolution is limited by how close one can approach the source of current. Since the probe vibrates one probe diameter, it is impossible to get less than one probe diameter from the surface being measured. Typically, probe diameters are in the 10's of microns. The current from a point source falls off with the square of the distance while that of a disc source has more complex spatial kinetics (Kunkel and Bowdan, 1989). With a small source, such as a feature on the surface of *Drosophila's* ~ 150 μm diameter stage 10B oocyte, a difference in the position of the probe of 10 μm from that feature reduces the strength of the current substantially. This increases the variance of measurements of separate preparations and separate locations on the same preparation. Important currents may be operating at earlier stages when the follicle is even smaller. This size problem makes the location of the source of a current and its strength on such oocyte surfaces hard to estimate, particularly if there are currents short-circuited over relatively short dimensional distances with minute electrical fields detectable externally. The use of pairwise measurement and testing have been emphasized as necessary to obtain significant results (Woodruff, 1989).

Drosophila, the most critical of the pattern formation model systems, still has much controversy surrounding the role of ionic currents in any aspect of oocyte physiology. It has been suggested that the use of complete medium rather than physiological saline is necessary for *Drosophila* oocytes to exhibit their normal pattern of development and perhaps to exhibit 'normal' current patterns (Bohrmann, 1991). Abnormal media are suggested to result in imbalances between oocyte and nurse cell which promote artifactual currents. The bulk of the cytoplasm, it is argued, is extruded by mechanical forces involving cortical actin in the nurse cells, the toothpaste model above.

Meanwhile in larger polytrophic oocytes the ionic currents are technically less difficult to measure (*i.e.* *Sarcophaga*, DeLoof, 1983; DeLoof and Geysen, 1983; Geysen *et al.*, 1988; Verachtert *et al.*, 1988; *Hyalophora* Woodruff *et al.*, 1986a, b;

Woodruff and Telfer, 1973, 1990), but their exact significance is still unknown. Experiments with charge modified lysozyme have characterized the conditions under which molecules will pass in a charge dependent manner from nurse cell to oocyte and *vice versa* (Telfer *et al.*, 1981) however arguments about media composition effects inducing artifacts of potential are still valid irrespective of oocyte size.

In telotrophic meroistic ovarioles of *Dysdercus* (Dittmann *et al.*, 1981) and *Rhodnius* (Huebner and Sigurdson 1986) the ionic currents in the nutritive chord region were originally described as consistent with aiding polarized transport of material along the chord from nurse cell to oocyte. However the complexity of the telotrophic ovariole currents was emphasized when the two-dimensional vibrating probe was applied to *Rhodnius* (Diehi-Jones and Huebner, 1989). The complexity may also include changes in medium components with different effects in different regions, causing fluctuations of potential and resultant artifactual currents.

These possible and apparently realized complexities in both meroistic types of oocytes argue for further investigations in the simpler panoistic ovariole type. Steady currents have been measured surrounding the panoistic ovarioles of locusts and cockroaches, Table 1. Since these ovarioles lack nurse cells, that complication is not a basis of steady currents. The oocyte and surrounding follicle cells remain for debate and experimentation. Currents about intact cockroach follicles can be substantially larger than those seen in the defolliculated *Xenopus* oocyte (Figure 5). The polarity of the cockroach oocyte may be most closely analogous to the amphibians animal-vegetal axis with respect to the orientation of the ionic currents. The germ band can be analogized to the animal pole and the bulk of the yolk can be analogized to the vegetal pole. Steady ionic currents have been found associated entering the area of the future germ band (Figure 5), in three Dictyopteran ovarioles: *Blattella germanica* (Kunkel, 1986; Kunkel *et al.*, 1986; Kunkel and Bowdan, 1989; Bowdan and Kunkel, 1990) for which the germ band was localized by Wheeler (1898) and Tanaka (1976). *Periplaneta americana* (Huebner and Sigurdson, 1986; Kunkel, unpublished) for which the germ band was localized by Heymons (1895). *Zootermopsis nevadensis*, (Kunkel and Stuart, recent results) for which the germ band was described by Striebel (1960). It should be noted that the position of the germ band may vary substantially in the short band type oocytes even within insectan orders (Figure 2-A3, A4 and Figure 2B). Thus *Blattella's* germ band is located in the middle of the length of the oocyte while that of *Periplaneta* and *Zootermopsis* lay at the posterior end of their respective elongate oocytes. In the amphibian *Xenopus* and in these three Dictyopterans the outward current emanates from the yolky side of the oocyte opposite where the animal or germ band cytoplasm is situated. The A-V axis of amphibians may be analogous to the D-V axis of short band oocytes such as described for the cockroach, Figure 5. The first cleavage furrow, which demarcates the D-V axis of amphibians (Klein, 1987), is always parallel to the A-V axis. Thus the currents observed in both

amphibian and cockroach may be more appropriately thought of as A-V axis indicators rather D-V axis indicators.

It should be noted that it is defolliculated oocytes of *Xenopus* that exhibit the current patterns on the order of $1 \mu\text{A}/\text{cm}^2$ (Figure 5). The defolliculated oocytes of cockroaches exhibit currents on the same order of magnitude (Anderson *et al* 1990) as those of *Xenopus*. This small current may be the maximum intrinsic current that an oocyte exhibits. The larger currents seen in intact cockroach follicles may be derived from the follicle cell layer, Figure 6. This suggestion is based on observations that when the oocyte of a Periplaneta-type follicle is removed from the follicle cell layer the outward current at the anterior pole of the follicle (Figure 5) increases, even after the injury effect declines suggesting that the follicle cell layer is driving the current (Kunkel and Stavropoulos, unpublished).

Investigators who measure ionic currents about oocytes may be measuring, in part, the physiological process by which maternal genes communicate a portion of their pattern information between the follicle cells and oocyte and *vice versa*. While it is known that the follicle cells and oocyte cooperate in some ways to coordinate the follicle cell polarity with the oocyte polarity, the physiology behind that coordination is not known. Does the oocyte gain its polarity A-P (and D-V) and then signal the follicle cells to organize or *vice versa*. Several late links in the chain of events have been identified such as *nud-pip-wind* and *tlk* as the probable follicle cell products which stimulate the *Tl* and *torso* receptors. The *Tl* and *torso* connections may be only the last of the coordinating signals which guide the parallel axes of follicle and germ cell to their proper sympathy. Since these receptors are activated after the chorion is laid down it is unlikely that ionic currents are directly causal at that point. It is however possible that ionic currents are involved in earlier coordinating signals such as in the placement of the *nud-pip-wind* and *tlk-ligands* in their appropriate places in the perivitelline space or in the epistatic relationship of *gurken*, *torpedo* and *k10* or in the localization of the anchors for *Oskar* and *bicoid* mRNA.

4) *The Polar Coordinate Model.* The A-P axis of insects has been investigated in several insect systems and interpreted as a product of two longitudinal, interacting gradients (reviewed in chapter 8 of Meinhardt, 1982). Insect eggs have been approached using a variety of ligation and ablation experiments, which are more applicable to the A-P axis. Thus we have for instance the evidence for anterior determinants in *Smittia* (Ripley and Kalthoff, 1983). The physical difficulty in experimentally approaching the dorsal-ventral (D-V) axis have resulted in less experimental embryology on the D-V axis and less discussion of interrelation of axes in orthogonal gradient-models. The polar coordinate model has mainly been applied to limb morphogenesis (French, 1991) and insect imaginal disc development (chapter 9 of Meinhardt, 1982) but in principle can be applied to oocyte pattern development (Figure 3) (cf. Mohler and Weischaus, 1986).

Its major strength lies in its ability to predict the outcome of regeneration experiments in which regions of a developing or established field are ablated. Intercalation of missing pattern elements between two extremes occurs automatically based on this model. It lends itself to gradient hypotheses quite well and as such the genetic model based on the *bcd* and *nos* A-P gradient and *dor* D-V gradient could use the polar coordinate model and its principles as a framework. In addition experiments performed on *Drosophila* eggs could be interpreted and used to extend the polar coordinate model itself. In particular when an egg is pricked at an early stage after fertilization and it loses some of its localized cortex, the polar coordinate model predicts an outcome if the model is properly applied to the oocyte. If the pricked egg loses all or substantial amounts of its *bcd* or *nos* mRNA it would be predicted to exhibit defects which are phenocopies of the *bcd* and *nos* mutant. The regulation of the embryo to produce an abnormal phenotype fits a polar coordinate model in several respects but is lacking in some ways when evaluated relative to certain genetic experiments involving duplications (Gergen and Weischaus, 1986). The polar coordinate model predicts mirror image duplications through its corollary of regeneration of missing gradient levels through a *shortest distance* route. Clearly it would be beneficial if these inconsistencies could be resolved.

It is particularly interesting that many of the *Drosophila* zygotic pattern formation genes operate in early embryology and then again in later embryology. If they are behaving in a polar coordinate fashion in late functions, in which the polar coordinate model has been applied, then it may be possible to extend a revised polar coordinate model (revised by fitting to the polarity gene cascade process) to the earlier stages of *Drosophila* embryonic development. How far earlier in polarity formation might the polar coordinate model apply? Does it apply to the prezygotic germ cell or perhaps to the overlying follicle cell layer? It is entirely possible however that since pattern formation in the oocyte is dealing with passage or coordination of the pattern across generations via maternal effects that *this discontinuity* has forced mechanisms of maternal gene expression that preclude, or demand modification of, the application of the polar coordinate model. We perhaps must look to distinctly different models to explain prezygotic oocyte polarity.

Physiology of follicle cell interaction with oocytes

The interaction of follicle cells with developing oocytes has been studied extensively from an ultrastructural point of view (reviewed in Aizenstadt, 1988). Of particular note are the numerous examples of follicle cell connections to the oocyte via gap junctions between macrovilli and oocyte (cf. Anderson and Albertini, 1976) and several examples of physical passage of materials between follicle cell and oocyte. The materials passed in birds and fish include items as large as ribosomes and the possibility of packages of cytoplasm. The passage of small molecules in mammalian follicles includes

cyclic nucleotides which are involved in the hormonal regulation of maturation (reviewed in Gilbert, 1988).

Physical connections between follicle cells and oocytes have been reported in insects. Electrical connections between the follicle cells and oocyte of *Cecropia* silk moths are associated with a critical change in the physiological state of nurse cell and oocyte at the beginning of vitellogenesis (Woodruff and Teller, 1990). Of note with respect to follicle cell oocyte connectivity is an elaboration of an electrical model of the polytrophic ovariole proposed by Verachert and De Loof (1989), (Figure 6A), based on Lucifer yellow dye coupling and vibrating probe measurements on *Drosophila*, *Sarcophaga* and *Manduca*. This model suggests two independent circuits of current in the ovariole. One generated by the non-patent epithelium of the anterior squamous follicle cells overlying the nurse cells. The current from this epithelium travels posteriorly between follicle cells and nurse cells to exit the ovariole through the patent follicle cell overlying the oocyte. The oocyte nurse cell complex supports a parallel and independent circuit which enters the oocyte surface traverses the bridge to the nurse cells and exits the follicle cell surfaces. Verachert and De Loof suggest a variant on the separate circuit model in which a squamous non-patent epithelium of follicle cells covering the nurse cells drive a current directly into the nurse cells. I incorporated that idea into the panoistic oocyte model (Figure 6B).

An important insight of the Verachert-DeLoof model is the incorporation of a different physiology for the squamous follicle cells covering the nurse cells and the patent cylindrical follicle cells surrounding the oocyte. This is consistent with the growing respect for the variety of follicle cell phenotypes with respect to chorion secretion and the localization of substances responsible for induction of ventral and posterior poles.

In cockroaches the follicle cell layer goes through a cycle of actin filament organization and reorganization associated with vitellogenesis, chorion formation, ovulation and involution (Zhang and Kunkel, 1990). During the vitellogenic phase the actin bundles are found in the numerous macrovilli which reach from the follicle cells to the oocyte surface (Figure 6B). These macrovilli are suspected to be the avenue of dye movement into follicle cells seen when oocytes are injected with Lucifer yellow (Anderson *et al* 1990). The follicle cell ' layer in cockroaches, while it is not a source of vitellins as it is in Dipterans and Lepidopterans, has been recently demonstrated to be a source of the abundant calmodulin found in the cockroach oocyte (Zhang and Kunkel, 1988; Zhang, dissertation in progress).

Microtubules of the follicle cell layer were found to be important in maintaining the proper polarity of *Drosophila's* follicle cell layer (Gutzeit, 1986). Colchicine resulted in accumulations of yolk proteins in the follicle cells and of vitelline membrane products at both apical and basal sides of the follicle cells. While most functions of the cytoskeletal components have been related to the gross functions of yolk provisioning and chorion formation, the more subtle properties of follicle cell oocyte communication over pattern coordination are

clearly potential concomitant events which need organization during this critical phase of development.

The follicle cells about the panoistic oocyte are at least diverse as those of *Drosophila* in the types of chorion they must eventually produce to cover the oocyte. Recent observations on cockroach oocytes (Zhang and Kunkel, 1990 and in preparation) have demonstrated that the patency of *Blattella* follicle cells parallels the dorsal ventral currents which surround the oocyte (Figure 6B). This observation is consistent with the model of currents in which an electrically tight follicle cell epithelium on the dorsal aspect of the *Blattella* oocyte drives an outward current. The current, either through direct electrical coupling of follicle cells to the oocyte or through localized channels on the dorsal oocyte plasma membrane, is driven through the oocyte. The ventral aspect of the oocyte is covered by a patent follicle cell layer which allows the current to passively flow into the oocyte through channels in its ventral plasma membrane.

It is still a mute point whether ionic currents driven by oocyte or follicle cells are involved in communication between oocytes and follicle cell layer. However as genetic evidence for a broader communication between germ cell and soma accumulates, it would be wise to ask what cytological mechanisms might be involved in the communication? To what extent can mass action and simple diffusion of molecules from site of synthesis to site of action regulate cell and tissue interactions? The macromolecules of cytoplasmic motility, actin, myosin, calmodulin and microtubules, may play important roles in efficient movements of molecules such as maternal messenger RNAs or their anchors to their proper locations. Ionic currents may play a physical role of either coordinating signals or providing a potential gradient field in which other macromolecular systems orient. Establishing the extent, timing and mechanism of these physiological links between the maternal and offspring generation is a challenge.

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