

REVIEW

Structure and Function of the Egg Cortex from Oogenesis through Fertilization

Christian Sardet,¹ François Prodon, Rémi Dumollard, Patrick Chang, and Janet Chênevert

BioMarCell, UMR 7009 Biologie du Développement, CNRS/UNIV P et M Curie, Observatoire, Station Zoologique, Villefranche sur Mer 06230, France

A. WHAT IS THE CORTEX?

The Egg Cortex

The Scope of This Review

B. STORING INFORMATION AND BUILDING THE CORTEX DURING OOGENESIS

Localizing Maternal Determinants in the *Drosophila* Cortex

Structuring and Positioning Information in the *Xenopus* Cortex

Cortical Polarity and Storage of Information in Other Oocytes

The Cortex during Vitellogenesis

C. MATURATION PREPARES THE CORTEX FOR FERTILIZATION

Restructuring Cortical Endoplasmic Reticulum and Organelles during Maturation

Restructuring the Cytoskeleton during Maturation

Relocalization of Cortical mRNAs and Domains during Maturation

The Cortex as the Site of Attachment and Fusion of Sperm and Egg

D. FERTILIZATION TRANSFORMS THE EGG CORTEX

Sperm-Induced Calcium Waves and Membranes

Waves of Exocytosis and Endocytosis at Fertilization

Fertilization-Induced Microfilament Reorganizations

The Fertilized Egg Cortex

E. WHAT THE CORTEX IS

The Cortex as a Repository for Spatiotemporal Information

The Questions That Remain © 2001 Elsevier Science

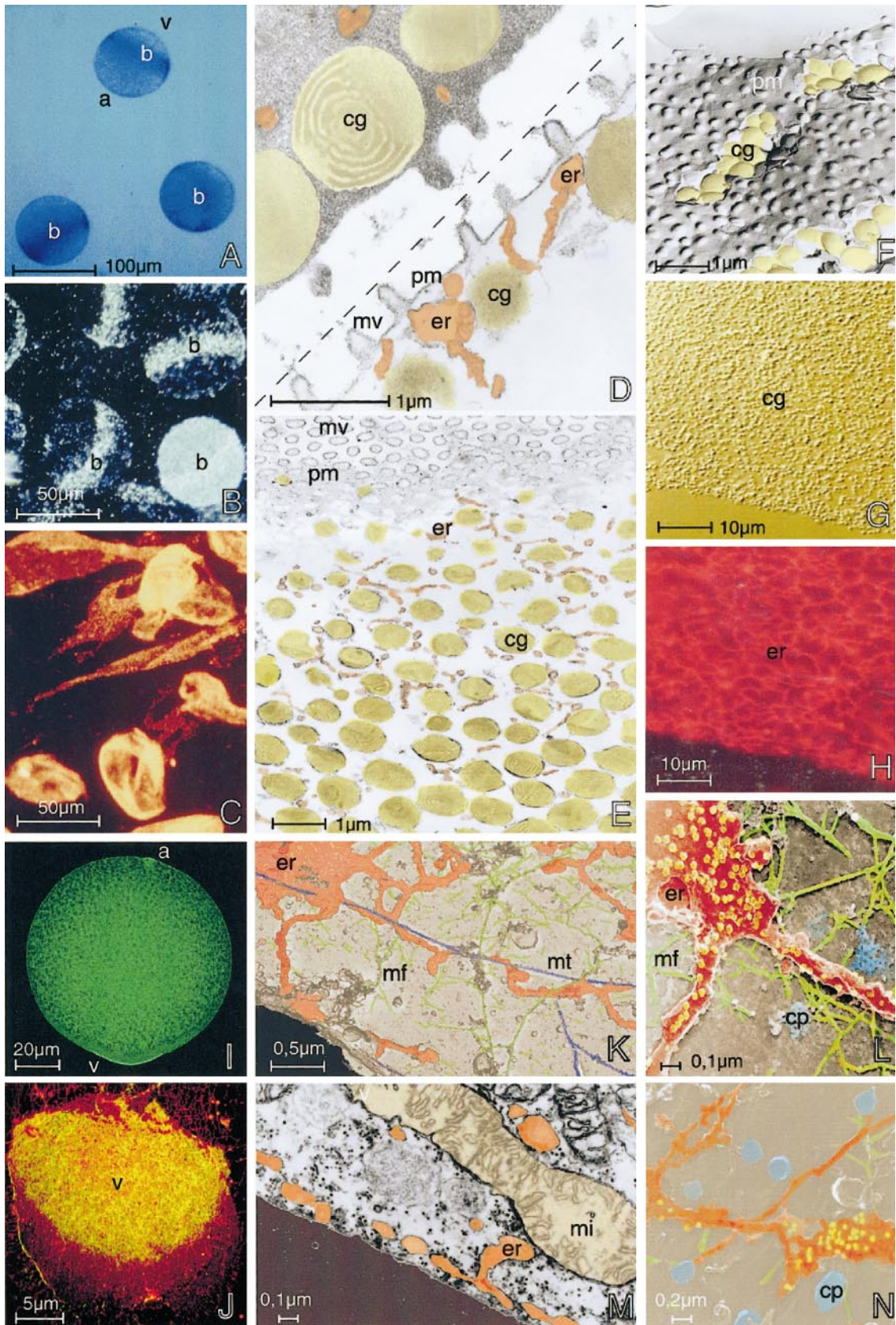
Key Words: egg; oocyte; fertilization; maturation; oogenesis; cortex; cytoskeleton; polarity; mRNA localization.

A. WHAT IS THE CORTEX?

Today, as in the early days of experimental cell biology, the word cortex is used rather imprecisely to designate the

¹ To whom correspondence should be addressed. Fax: 33-4-93-76-37-92. E-mail: Sardet@obs-vlfr.fr; Web site: www.obs-vlfr.fr/biomarcell.html.

periphery of the cell. The cortex functions as a boundary zone that gives the cell its shape, a region of selective exchanges and transductions, an active partner for the cytosolic cytoskeleton and organelles, and a preferred site for the localization of RNA and protein determinants. It has properties of a visco-elastic gel and functions as a unit during many cell behaviors. There is no consensus definition of the limits of the cell cortex. It generally refers to a



layer immediately beneath the cell surface, but depending on the researcher's point of view this layer may include only the plasma membrane (PM) and subsurface coating of actin microfilaments (MFs) or continue into the deeper cytoplasm. Therefore the thickness of the cell cortex may be anywhere between 0.1 and 20 μm , and may vary with the cell type, its functional state, the biological process under study, and the resolution of the observations.

The Egg Cortex

The egg cortex is a large assemblage of the plasma membrane, extracellular coat, and subsurface skeleton to which a characteristic set of cytoskeletal elements, organelles and macromolecules adhere. It behaves as a functional unit during the reorganizations that precede and follow fertilization. The egg cortex is also the repository for spatiotemporal developmental information since it contains determinants of axis and tissue formation. The existence of a dynamic egg cortex was recognized in the first half of the 20th century when histological observations and experimental manipulations with rods, micropipettes, magnetic particles, and using centrifugation showed that certain properties of the cortex (morphology, stiffness, thickness) changed during maturation, fertilization and the first cell cycles (reviewed in Harvey, 1956; Chambers and Chambers, 1961; Raven, 1970; Hiramoto, 1970; Sardet and Chang, 1987). Then in the 1970s several groups achieved preparations of "isolated cortices", "cortical lawns", "cell surface complexes," or "cortical hulls" (Figs. 1B and 1C) that retained the ability to contract and undergo exocytosis *in*

vitro following addition of ATP or calcium (Sakai, 1960; Kane and Stephens, 1969; Guerrier, 1972; Mabuchi and Sakai, 1972; Vacquier, 1975; Schatten and Mazia, 1976; Begg and Rebhun, 1979). It is now possible to isolate the egg cortex from echinoderms, molluscs, ascidians, mouse, the amphibian *Xenopus laevis*, and the annelid *Tubifex hattai*. Since the multiple connections with the deeper cytoplasm are severed during the procedure, isolated *in vitro* cortices only retain part of the interconnected networks of organelles and cytoskeletal elements which adhere to the PM in *in vivo* cortices (Figs. 1D, 1E, 1G, 1H, and 1K). This situation resembles that of isolated nuclei, which can be obtained only at the expense of severing the endoplasmic reticulum (ER) and cytoskeletal elements that connect it with the rest of the cell. Despite this limitation, isolated cortices, like isolated nuclei, retain functional properties. They have been shown to pump and release calcium (Terasaki and Sardet, 1991), to retain localized mRNAs (Elinson *et al.*, 1993; Alarcon and Elinson, 2001), and to sustain organelle and cytoskeleton movements as well as furrow progression (Shimizu, 1985; Walker *et al.*, 1994).

Our present knowledge is relatively limited when one considers published work devoted exclusively to the structure, function, or polarity of the egg cortex. At the same time, the amount of information concerning the cortex is enormous if one takes into account papers in which the egg cortex is discussed with respect to PM permeability, calcium release, exocytosis, endocytosis, the dynamics and positioning of microtubules (MTs) and MFs, or the localization of mRNAs, to name just a few topics. Reviews treating the egg or oocyte cortex proper are few and far apart. Those

FIG. 1. Views of the egg cortex. Additional material can be found on our Web site: <http://www.obs-vlfr.fr/biomarcell.html>. Eggs and isolated cortices of sea urchin (A–H), ascidian (I–M), and amphibian (N). The ER is colored orange/red in D, E, K, L, M, and N; ribosomes on the ER are colored yellow in K, L, and N; the CGs are colored ocre in D, E, and F; the MFs are colored green in K, L, and N; the MTs are colored dark blue in K; the cytoplasmic face of the PM is light brown in K, L, and N; and coated plaques and vesicles are colored light blue in L and N. (A) Living eggs of the sea urchin *Paracentrotus lividus* display a band of pigmented vesicles (b), one of the earliest described cortical markers of animal (a)–vegetal (v) polarity (bright field optics, blue filter). (B) Isolated cortices attached to a polylysine coated surface. The band of pigmented vesicles (b) is retained in cortices. The cortex in the lower right corner has also retained cortical granules (dark field optics, blue filter). (C) Isolated cortices (cortical hulls) suspended in homogenization milieu (dark field optics). (D) An egg (top left) and an isolated cortex (bottom right) side by side. In the isolated cortex, the cER network (er) and cortical granules (cg) remain attached to the plasma membrane (pm) which is covered by microvilli (mv) (electron micrograph (EM) thin section). (E) Oblique tangential section through an isolated cortex showing the cER network (er) surrounding cortical granules (cg). Microvilli (mv) and plasma membrane (pm) are on top (EM, thin section). (F) External face of the egg plasma membrane (pm) studded with short micropapillae. A lawn of cortical granules is situated beneath (EM, freeze fracture replica). (G and H) The same isolated egg cortex. The cortical granules (cg) visualized in G are surrounded by cER (er) labeled with the lipophilic dye DiI(C16)3 in H (DIC optics in G and fluorescence microscopy in H). (I) A fertilized egg of the ascidian *Phallusia mammillata*. The animal (a)–vegetal (v) polarity is evident by the concentration of actin microfilaments (labeled with fluorescent Phalloidin) which forms near the vegetal pole (v) 5 min after fertilization (confocal microscopy). (J) Cortex isolated from the vegetal (v) region of an egg 5 min after fertilization (such as the egg in I). The cER network labeled with a lipophilic dye (red) and MF network labeled with fluorescent phalloidin (green) are concentrated in the vegetal (v) pole (confocal microscopy). (K) Fragment of isolated cortex from an unfertilized egg showing the networks of cER (er), microfilaments (mf), and microtubules (mt) (EM, fast freeze/deep etch replica). (L) Detail of an isolated cortex like that shown in K. The cER (er) is studded with ribosomes and particles. Microfilaments (mf) and coated plaques (cp) are visible as well as the cytoplasmic face of the plasma membrane (EM, fast freeze/deep etch replica). (M) Vegetal cortical region of an egg showing the cER (er) and one mitochondrion (mi) (EM, thin section). (N) Fragment of a *Xenopus* oocyte cortex isolated during vitellogenesis. A large number of coated plaques and vesicles (cp) are present on the underside of the plasma membrane (EM, fast freeze/deep etch replica).

of Elinson (1980), Vacquier (1981), Longo (1985), and Sardet and Chang (1987) provide an access to the earlier literature on cortical changes in starfish, sea urchins, amphibians, mammals and a half dozen other organisms. More recent publications by Sardet *et al.*, (1992), Larabell (1993), Shimizu (1995), and Chang *et al.*, (1999b) concern cortices from oocytes and eggs of the ascidian *Phallusia mammillata*, the annelid *Tubifex* and the amphibian *Xenopus*. Attention is also given to cortical changes in a few general reviews dealing with cytoplasmic reorganizations in leeches, annelids, the nematode *Caenorhabditis elegans*, ascidians and *Xenopus* (Elinson and Houlston, 1990; Sardet *et al.*; 1994; Shimizu, 1995; Fernandez *et al.*, 1998; Gard, 1999). We are also in a somewhat paradoxical situation with regard to the contributions of different species to the cortex field. A large part of what we know regarding the structure and the transformations of the cortex is the result of studies using eggs of genetically intractable echinoderms, molluscs, and annelids, while very little is known about the structure or evolution of the cortex in *Caenorhabditis elegans* and *Drosophila melanogaster*, the two species in which rapid progress is being made in defining cortical determinants of axis and tissue formation. An intermediate situation prevails in eggs of *Xenopus* and ascidians, where identified cortical determinants can be studied both *in vivo* and in the isolated cortex.

The Scope of This Review

We will first examine the structural and functional properties of the egg cortex during oogenesis as information is stored in the form of localized determinants, as it endocytoses and recycles surface components during vitellogenesis, and as it matures after hormone stimulation and egg laying. We then detail how the cortex is profoundly reorganized during fertilization or artificial activation and briefly introduce the establishment and amplification of cortical polarity, a subject which will be covered in detail in a second review. What emerges from the synthesis of a large and diverse body of literature is that the basic cortical scaffold, comprised of the PM, a 3-dimensional matrix of submembranous skeleton based on MFs, and an attached network of cortical ER, appears conserved in many oocytes and eggs of different species (see Figs. 1 and 2). The presence or absence in the cortex of many other components (MTs, intermediate filaments (IFs), organelles, granules, particles) depends on what type of oocyte, egg, zygote, or embryo is considered, and if the cortex is isolated, what isolation procedures and what milieu are used (reviewed in Vacquier, 1981; Schroeder, 1981; Sardet and Chang, 1987; Chang *et al.*, 1999b).

We have emphasized recent references and reviews, only mentioning the older literature where it remains essential or represents an interesting historical source. We have also attempted to point out areas where research on somatic cells contributes concepts and models that could be applied to the egg cortex. In addition, we introduce notions of

cytoplasmic reorganizations and polarity when necessary to understand causes and consequences of cortical events. It is our hope that this review constitutes a useful access to sources and concepts for the many biologists who like to use the word cortex freely while describing their favorite process, cytoskeletal element, organelle or molecule.

B. STORING INFORMATION AND BUILDING THE CORTEX DURING OOGENESIS

The oocyte cortex evolves and enlarges with the rest of the cell as the small germ cell precursors pass through successive stages of migration, proliferation and then differentiation and growth within the gonads, a process that can take days, months, or even years (Matova and Cooley, 2001). The largest part of the oocyte's content generally comes from surrounding follicle cells and/or neighboring nurse cells via endocytosis and/or by bulk cytoplasmic transfer through intercellular bridges.

Embryonic polarity in *Xenopus* and *Drosophila* and muscle differentiation in ascidians are dependent on the localization of mRNA determinants in the oocyte cortex (Van Eeden and St Johnston, 1999; Houston and King, 2000b; Mowry and Cote, 1999; Nishida and Sawada, 2001). A cortical scaffold of cytoskeletal elements and organelles beneath the PM must be assembled in preparation for the localization of determinant mRNAs during oogenesis, maturation, and fertilization, and for the expression of these determinants during development. The early stages of oogenesis are best understood in *Drosophila* and *Xenopus* while the later steps of maturation and cortex reorganization in preparation for fertilization have been best described in *Xenopus*, echinoderms, and mammals.

Localizing Maternal Determinants in the *Drosophila* Cortex

The key event in the establishment of embryonic axes in *Drosophila* is the localization of maternal mRNA determinants and proteins to the cortex during oogenesis. These cortical macromolecules essential for axis formation and germ plasm formation are a set of mRNA determinants (*bicoid*, *nanos*, *oskar*) and an increasing number of proteins (Vasa, Tudor, Stauf, Egl, Exu, Bic-D, Lis-1, Par-1, . . .) which are synthesized in the 15 nurse cells, translocated into the oocyte, and anchored in its anterior or posterior cortex or poles (these terms are used interchangeably in the field to designate the 1–20 micron thick region beneath the oocyte surface). As a result of these precocious cortical localizations of determinants, and in contrast to most other species, the future antero-posterior and dorso-ventral polarities of the *Drosophila* embryo are established in the gonad well before maturation and fertilization of the egg (reviewed in Van Eeden and St Johnston, 1999; Lasko, 1999; Reichmann and Ephrussi, 2001). The localization of deter-

minant mRNAs and proteins produced in the nurse cells requires first the formation of large particles containing messages and proteins and their passage from the nurse cells into the oocyte, and then the translocation of these particles in the oocyte to reach their cortical destination. These events depend on several phases of reorganizations during early, mid, and late oogenesis, involving principally the MT network, the cortex and the GV of the oocyte (Theurkauf, 1997; Megraw and Kaufman, 2000; Cha *et al.*, 2001).

During early *Drosophila* oogenesis (previtellogenic stages 1–6), the microtubular network is established by transferring centrosomal foci from the nurse cells into the oocyte via a distinctive intracellular structure (the fusome) which interconnects all 16 cells of the cyst. The fusome, an asymmetric structure made of proteins generally associated with the cortex (spectrins, annexins, ankyrins, adducins, Par-1), apparently plays a major role in determining which of the 16 cells in the cyst will be the oocyte (DeCuevas and Spradling, 1998). By using the fusome as a polarized guide, all the centrosomal foci (harboring the minus ends of MTs) and the centrioles from the 15 other cyst cells are transported through cytoplasmic bridges called ring canals towards the differentiating oocyte (Theurkauf, 1997; Gonzalez-Reyes and St Johnston, 1998; Grieder *et al.*, 2000; Navarro *et al.*, 2001). Most centrioles and microtubule organizing centers (MTOCs) are later found between the posterior cortex and the large oocyte nucleus (the germinal vesicle: GV). Although some γ -tubulin foci still remain in the anterior cortex (Mahowald and Strassheim, 1970), the posterior centrosomal cluster sandwiched between the cortex and the GV radiates the bulk of the oocyte's MTs. This posterior centrosomal cluster accumulates important proteins such as Bicaudal-D (Bic-D), a protein essential for oocyte specification and polarization of the oocyte (Paré and Suter, 2000). The positioning of the complex containing Bic-D and other proteins such as Egalitarian (Egl) apparently requires the previous localization in the oocyte cortex of DLis-1, a regulator of cytoplasmic dynein implicated in nuclear migration in *Drosophila* and also in fungi and mammals (Swan *et al.*, 1999). How the translocation and posterior positioning of the MT network is accomplished is not entirely clear but it has been recently discovered that the *Drosophila* homolog of the Par-1 protein is essential for this process and for specifying which of the 16 cells of the cyst will become the oocyte (Cox *et al.*, 2001; Huynh *et al.*, 2001). Par-1 protein is a serine/threonine kinase whose polarized cortical distribution is necessary for axis specification of the *Caenorhabditis* zygote and for polarity of mammalian epithelial cells (Böhm *et al.*, 1997; Kempfues, 2000). *Drosophila* oocyte Par-1 is involved like its mammalian homologues (called MARKs) in the establishment and stabilization of the MT network (Drewes *et al.*, 1997; Navarro *et al.*, 2001).

The next major reorganization of the MT network with respect to the cortex occurs during midoogenesis (stages 7–8, when vitellogenesis starts). It is linked to the MT-

dependent repositioning of the GV from the posterior to anterior cortex which is the essential step in the establishment of oocyte polarity (Cooley and Theurkauf, 1994; Van Eeden and St Johnston, 1999; Reichmann and Ephrussi, 2001). The relocation of the bulk of the MT network from the posterior to the antero-dorsal cortex is triggered by extracellular signals from neighboring posterior follicle cells in response to the transcription, synthesis and secretion of the growth factor-like protein Gurken in the posterior region of the oocyte where the GV is first localized (Gonzalez-Reyes and St Johnston, 1998; Micklem *et al.*, 1997). After relocation of the GV, the MTOC and radiating MTs are nested in a crater of the GV in the antero-dorsal corner of the oocyte and many MT foci are located in the anterior and lateral cortex (Paré and Suter, 2000; Cha *et al.*, 2001). This reorganization of the MT network is again dependent on the cortical protein Par-1 which localizes first transiently in the anterior cortex and then more stably in the posterior cortex by stage 9 (Tomanek *et al.*, 2000; Shulman *et al.*, 2000). The understanding of the interaction between the MTs and the cortex is far from complete. The MT reorganization could result from a disassembly of the MTs at the posterior pole and their selective growth from foci located in the anterior cortex, or possibly from a rotation of the centrosomal aster and attached GV with respect to the cortex. The ER and the secretory pathway, which is known to carry important constituents for MT-cortex interactions in yeast, may be involved (Roth *et al.*, 1995; Heil-Chapelaine *et al.*, 1999).

The changes in the position of the GV and the reorganization of the MT network in the anterior cortex during midoogenesis have two consequences. First, a second round of *gurken* transcription, localized synthesis, secretion and interaction with neighboring follicle cells takes place, fixing the antero-dorsal axis of the embryo. Second, the presence of numerous MTs in the anterior cortex mediates the arrival of particles containing the anterior determinant *bicoid* mRNA which is produced in the nurse cells. Although it is generally believed that an asymmetric MT network accounts for the anterior localization of *bicoid*, recent observations show that the MT network lacks sufficient antero-posterior polarity to explain the vectorial translocation of the large irregular particles containing *bicoid* to the anterior cortex (Theurkauf and Hazelrigg, 1998; Cha *et al.*, 2001). The specificity of anterior *bicoid* localization probably lies in the specialized properties of the MT network in the anterior cortex to which a translocating machinery attaches. Some of the translocating machinery is in the process of being uncovered, since *bicoid* mRNAs apparently attach to MTs coursing along the anterior cortex via the linker protein Swallow and the minus end-directed motor Dynein I (Schnorrer *et al.*, 2000). The nature of the large *bicoid* and Exuperantia (Exu)-containing particles remains mysterious; they could be the so-called "sponge bodies," which are microdomains identified by electron microscopy containing ER and Vasa-containing nuage material (Wilsch-Brauninger *et al.*, 1997). By stage 8–9, *bicoid*

has been translocated and anchored by a process which requires Bic-D and DLis, (Swan *et al.*, 1999; Vallee *et al.*, 2001). These molecules are themselves localized and anchored in the cortex during earlier stages of oogenesis by unknown mechanisms apparently independent of MTs (Liu *et al.*, 1999; Paré and Suter, 2000).

A somewhat similar scenario directs the posterior cortical localization of *oskar* mRNA, a determinant important for the formation of polar granules (*Drosophila* germ plasm), and for the subsequent localization and expression of the posterior determinant *nanos* (Ephrussi and Lehman, 1992). After transient anterior localization, around stage 8–9 *oskar* mRNA migrates to the posterior pole in close association with the cortex. *Oskar* is thought to travel in large RNP particles containing the protein Staufen and plus-end-directed MT motors of the Kinesin I family (Micklem *et al.*, 1997; Brendza *et al.*, 2000; Reichmann and Ephrussi, 2001). It is not known what the exact composition of these RNP particles is, or if they travel freely along MTs or in association with a “sponge body”-like structure containing ER. A role for internal membranes in *Oskar* localization has also been suggested (Ruden *et al.*, 2000; Jankovics *et al.*, 2001). A possible involvement of ER in mRNA localization in *Drosophila* is further implied by the fact that Staufen, a double stranded RNA binding protein necessary for *oskar* mRNA and protein localization, is strongly associated with rough ER in fibroblasts and nerve cells. In these cells, Staufen is also involved in the translocation of particulate mRNAs (Wickham *et al.*, 1999; Rogiers and Jan, 2000).

In late oogenesis (stages 10b–14), large quantities of nurse cell cytoplasm are transferred to the oocyte through ring canals by cytoplasmic streaming motions (Cooley and Theurkauf, 1994). The process apparently involves non-muscle Myosin II and the establishment of sarcomere-like actin MF bundles in the nurse cells (Riparbelli and Callaini, 1995; Wheatly *et al.*, 1995). The cytoplasm mixing movements are thought to depend in part on acto-myosin and to participate in the anchoring of many cytoplasmic components to the polarized cortical scaffold. This bulk cytoplasmic transport completes the recruitment of macromolecules initiated in the cortex at earlier stages of oogenesis. Such is the case for *nanos* which accumulates in the posterior pole at the end of oogenesis (Mahajan-Miklos and Cooley, 1994; Lasko, 1999).

How mRNA determinants are anchored to the cortex after being targeted to the proper location is still a matter of debate. MFs and proteins that bind RNA or are involved in the control of polyadenylation, such as Staufen or Orb, have been implicated (Chang *et al.*, 1999a). A precise role for a specific organelle or cytoskeletal component is difficult to pinpoint because the close interactions of the cortical constituents make it likely that perturbing one partner, whether MT, MF, or ER, will destabilize the others (Lantz *et al.*, 1999; Sider *et al.*, 1999; Waterman-Storer and Salmon, 1999).

Structuring and Positioning of Information in the *Xenopus* Cortex

Oocyte growth, cortical transformations and acquisition of cortical RNAs have been reviewed extensively in *Xenopus* (Etkin, 1997; Mowry and Cote, 1999; Gard, 1999; King *et al.*, 1999; Chang *et al.*, 1999b; Houston and King, 2000b). As in *Drosophila*, a cyst of 16 pear-shaped cells give rise to germ cell precursors, but in *Xenopus* all cells in the cluster become oocytes. Germ cell precursors (stage 0 oocytes) are polarized with acetylated MTs, mitochondria and golgi situated at one pole towards the center of the cluster, but it is not known if that asymmetry bears a relationship to the future animal-vegetal axis of the oocyte because this polarity is lost by stage I (reviewed in Gard, 1995, 1999).

Early stage I oocytes have no identifiable centrosomes or obvious polarity in the structure of their MF, MT or intermediate filament (IF) networks. The IFs are principally type I and type II cytokeratins (keratin filaments: KFs) which appear at mid stage I (Gard and Klymkowsky, 1998). Cortical polarity first arises at stage II with the arrival at the cortex of the “mitochondrial cloud” with its associated MTs and KFs. The site at which it attaches to the cortex defines the vegetal pole. The mitochondrial cloud appears to develop from one of several fibrous aggregates of mitochondria, ER, and electron dense nuage material found around the GV in early stage I oocytes (Heasman *et al.*, 1984; Kloc *et al.*, 1996; Wylie, 1999; Houston and King, 2000b). By stage II, cortical MTs with their minus ends in the cortex are present, along with cortical ER (cER), cortical granules (CGs), and numerous coated pits which populate the underside of the PM (Chang *et al.*, 1999b). Vitellogenesis begins at this stage, with yolk uptake occurring uniformly over the surface (Danilchik and Gerhart, 1987). During stages III and IV, dark pigment granules concentrate in the animal hemisphere cortex, and the vegetal accumulation of large yolk platelets displaces the GV animally. At stage III the MT and KF networks are organized radially and symmetrically throughout the oocyte. The MTs emanate from γ -tubulin foci in the cortex with many plus ends pointing inwards towards the GV (Gard, 1994, 1995). MT and KF networks become polarized along the AV axis at stage IV. Cortical KFs bundle into thicker arrays detected principally in the vegetal hemisphere, whereas radial arrays of MTs are more obvious in the animal hemisphere where yolk platelets are smaller. γ -Tubulin foci accumulate in the vegetal cortex during this period (reviewed in Gard, 1999).

By stage VI the oocyte, still surrounded by follicle cells to which it is connected by numerous gap junctions, has grown and accumulated yolk platelets through a cortical endocytic process (see vitellogenesis below). It has acquired a submembranous layer of CGs and a cortical network of ER tubes and cisternae continuous with stacks of subcortical annulate lamellae (Campanella and Andreucetti, 1977; Campanella *et al.*, 1984; Terasaki *et al.*, 2001). The organization and localization of KFs has been modified to form anastomosing networks 3–4 μ m thick in the vegetal cortex

and around the GV. A finer and deeper array of KFs can be discerned in the animal hemisphere 10 to 15 μm beneath the PM (Gard *et al.*, 1995a; Pfeiffer and Gard, 1999). The MTs are organized radially with minus ends predominantly close to the vegetal cortex where γ -tubulin foci are present (reviewed in Gard, 1999). In the animal hemisphere MTs are more abundant and more acetylated, suggesting that they are less dynamic (Gard, 1991). The network of MFs in the animal cortex is thicker and more contractile than that in the vegetal cortex, however there is no detailed description of the organization or evolution of MFs during oogenesis. The use of inhibitors suggests that the cortical actin layer is necessary for the anchoring of the MT and IF networks, and that γ -tubulin foci in the cortex are enmeshed in the cortical MF network (Gard *et al.*, 1997).

Many specific mRNAs associated with germ cell determination such as *Xcat2*, *Xdazl* (related to *Drosophila* *nanos* and *boule*, respectively), and *DEADSouth* (an RNA helicase related to the eukaryotic initiation factor eIF4A), are found in the germinal granules (derived from nuage material) of the mitochondrial cloud (Mosquera *et al.*, 1993; Houston *et al.*, 1998; MacArthur *et al.*, 1999, 2000). When the mitochondrial cloud arrives at the vegetal cortex, it fragments into islands of germ plasm measuring about 5 μm in diameter. These microdomains disperse across the vegetal cortex and form characteristic patches rich in mitochondria and germ plasm that contain a number of mRNAs (*Xlsirts*, *Xwnt11*, *Xcat2*, *Xdazl*, *DEADSouth*, *Xpat*, and *Xotx1*), individual mRNAs taking up different positions in the patches (Ku and Melton, 1993; Kloc *et al.*, 1993; Kloc *et al.*, 1998; Hudson and Woodland, 1998; King *et al.*, 1999; Mowry and Cote, 1999; Houston and King 2000a,b; Pannese *et al.*, 2000). This early transport pathway of RNA localization (the so-called METRO pathway, Kloc and Etkin, 1995) is likely due to the bulk translocation of germ plasm material towards the vegetal cortex. Its reliance on the MT and MF interdependent networks is still unclear. The ER-rich particles transported by the METRO pathway may be analogous to the large mRNA particles or the nuage- and ER-rich "sponge bodies" described in *Drosophila* oocytes which are translocated in a MT-dependent fashion (Cha *et al.*, 2001; Wilsh-Brauninger *et al.*, 1997; Saffman and Lasko, 1999).

There is also a late pathway of RNA localization in *Xenopus* (stage II-IV oocytes), during which a second set of mRNAs arrives at the cortex via another route which may require the early METRO pathway (Kloc and Etkin 1998; Kloc *et al.*, 1998). Among these mRNAs are *Vg1* and *VegT* which function in patterning the mesodermal and endodermal layers (Joseph and Melton, 1998; Zhang and King, 1996; Zhang *et al.*, 1998). *Vg1* message binds to the RNA binding protein Vera/Vg1RBP, a homologue of the β -actin RNA localizing protein ZBP-1, and to homologues of the human hnRNP1 protein (Havin *et al.*, 1998; Zhang *et al.*, 1999; reviewed in Mowry and Cote, 1999). The *Vg1* RNA/protein complex is displaced in a MT-dependent process to an ER-rich region that extends between the GV and the vegetal

cortex (Deshler *et al.*, 1997, 1998; reviewed in Mowry and Cote, 1999). Other mRNAs such as *fatvg* seem to use both early and late pathways to localize to the vegetal cortex (Kloc *et al.*, 1998; Chan *et al.*, 1999).

Although limited in their interpretation because of the interdependency of cytoskeletal and organelle networks, experiments using drugs which depolymerize MTs and MFs and injection of antibodies which interfere with KFs at first suggested that MFs and KFs as well as noncoding RNAs (*Xlsirts*) are involved in anchoring the 2 populations of mRNAs in the vegetal cortex (Klymkowsky *et al.*, 1991; Kloc and Etkin, 1994). Studies based on the isolation of cortices from stage VI oocytes show that at least three mRNAs localized through the early and late pathways (*Xcat2*, *Xwnt11*, and *Vg1*) are retained in the cortices (Elinson *et al.*, 1993; Alarcon and Elinson, 2001). More recent selective treatment of eggs or isolated cortices with drugs, antibodies, or detergents which perturb or extract the major components of the isolated cortex (the MF, KF, and ER networks) indicate that *Vg1* and *Xwnt11* interact with the cortical KF network and possibly the cER, while *Xcat2* is apparently not tethered to any of these major constituents (Alarcon and Elinson, 2001).

Most of the cortically anchored mRNAs have not been assigned clear functions for the moment except for the endoderm determinant *VegT* and *Xdazl* which is involved in primordial germ cell differentiation (Zhang *et al.*, 1996, 1998; Houston and King, 2000a,b). Experiments using UV treatment (which acts only within a few microns below the surface), oocyte inversion, and transfer of cortical material from full-grown but not matured oocytes suggest that mRNAs localized close to the surface are important for axis establishment and germ cell determination (Holowacz and Elinson, 1993, 1995; Kageura, 1997; Marikawa *et al.*, 1997; discussed and reviewed in Chang *et al.*, 1999b)

Cortical Polarity and Storage of Information in Other Oocytes

Compared with *Drosophila* and *Xenopus* our knowledge of cortical polarity and mRNA localization in other organisms is very poor. There are indications that mRNAs are localized in the animal and vegetal poles of zebrafish oocytes (Howley and Ho, 2000; Suzuki *et al.*, 2000). In this organism the arrival of some of these RNAs precedes morphological manifestations of polarity, so whether their localization causes or results from the establishment of the animal-vegetal axis remains an open question. In eggs of the sea urchin *Paracentrotus lividus* in which the gradient of cortical pigmented granules represents one of the earliest reports of oocyte polarity (Boveri, 1901; Sardet and Chang 1985; see Figs. 1A and 1B), there is recent evidence that maternal mRNAs and proteins (called BEP) localized in the animal cortex are involved in patterning the embryo along the animal-vegetal axis (Romancino *et al.*, 1998, 2001; Romancino and Di Carlo, 1999). A striking example of cortical polarity occurs in ascidian oocytes, in which a large

class of cortically located mRNAs (the *PEMs* for Posterior End Mark) are distributed along an animal-vegetal gradient (Nishida and Makabe, 1999; Sasakura *et al.*, 2000). Some of these *PEM* mRNAs and particularly *macho*, the likely tadpole muscle determinant, are retained with the isolated cortices and appear to localize on the cER which is itself polarized in unfertilized eggs (Sardet *et al.*, 1992; Nishida and Sawada, 2001; Sardet and Nishida, unpublished).

The Cortex during Vitellogenesis

Because of the intense endocytic activity of the cortex associated with accumulation of yolk vesicles, vitellogenesis has the potential to remodel the PM and the attached cortical cytoskeleton. Recent observations of actin-dependent propulsion of endosomes via comet tail formation in activated *Xenopus* eggs raise the possibility that endosomes generated during vitellogenesis may contribute to the establishment of a cortical MF scaffold (Taunton *et al.*, 2000).

Morphological observations in eggs of insects and amphibians have provided most of our knowledge of vitellogenesis. In *Drosophila*, yolk vesicles filled with vitellogenins start accumulating in the oocyte around stage 8 when polarity is being established (Schonbaum *et al.*, 2000). Vitellogenin receptor (VTGR) mRNA is synthesized in the nurse cells, translocated into the oocyte through stage 7, and translated. By stage 8–9 the VTGR proteins, which are LDL receptor subtypes, have moved to the cortex. VTGRs dock at the PM and vitellogenin proteins secreted by surrounding follicle cells are endocytosed, forming vesicles and endosomes that recycle the receptors back to the surface. VTGRs are inserted into the oocyte PM by a process resembling hormone-stimulated exocytic insertion of glucose transporters (such as Glut4) in adipocytes or muscle cells (Holman and Sandoval, 2001). Coated vesicle-mediated endocytosis of vitellogenin, triggered by a juvenile hormone, is followed by formation in the cortex of tubulovesicular early endosomal components and multivesicular bodies considered to be late endosomes. Yolk vesicles form from the maturing endosomes while receptors are recycled back to the oocyte surface until vitellogenesis is completed (Schonbaum *et al.*, 2000). This vitellogenic phase of oogenesis bears common themes with late oogenesis in other insects, fish, birds, and frogs which also have LDL Receptor type VTGRs, multivesicular bodies that are precursors of yolk vesicles, and amplified coated vesicle endocytosis, a process originally discovered in mosquito oocytes (Roth and Porter, 1964). Recent investigations of oogenesis in *Caenorhabditis* show that the basic components of VTGR mediated trafficking are also conserved in nematodes (Grant and Hirsh, 1999; Greener *et al.*, 2001). We can expect rapid progress in this domain from an experimental model where forward and reverse genetic analysis can be used extensively.

C. MATURATION PREPARES THE CORTEX FOR FERTILIZATION

Maturation is the process which takes the oocyte from the GV stage (4n chromosomes) to a stage of meiosis at which the oocyte can be fertilized. Depending on the species, the mature oocyte arrests in meiotic metaphase I (4n chromosomes: ascidians, some molluscs. . .), metaphase II (2n chromosomes: amphibians, mammals. . .) or proceeds all the way to interphase (n chromosomes: echinoderms, cnidarians. . .). In many species the mature oocyte possesses an animal-vegetal polarity with the small nucleus or meiotic spindle under the animal pole surface and/or opposed to the polar bodies, the smallest of cells generated by unequal cleavages (Shimizu, 1990; Verlhac *et al.*, 2000). During maturation, secretory vesicles or CGs localize and anchor to the cortex, awaiting the activating calcium signal which will induce a massive exocytosis. Reorganizations of the cortex during maturation affect all the major networks in the oocyte (cER, MT, MF, and IF) and lead to relocation of some cortical mRNAs. Among the cortical changes that occur during oocyte maturation, it is difficult to distinguish those directly related to cell cycle changes (completion of meiosis) from those which prepare the egg to respond to sperm. A full understanding of the cell cycle-related functional and structural changes in the cortex during meiotic completion awaits a description of the effects of changing activities of important factors such as MPF and MAPKs on the dynamics of the cortical ER, MF, MT, and IF networks (Whitaker, 1996; Nebreda and Ferby, 2000).

Restructuring Cortical Endoplasmic Reticulum and Organelles during Maturation

Response to the fertilizing sperm requires that the egg cortex is able to undergo a full calcium response, massive exocytosis and endocytosis, as well as propagated contractility (Suzuki *et al.*, 1995). These properties are made possible by the development just beneath the PM of an extensive network of tubes and sheets of cER which stores and releases calcium (Gardiner and Grey, 1983; Campanella *et al.*, 1984; Sardet 1984; Larabell and Chandler, 1988; Henson *et al.*, 1990; Terasaki *et al.*, 2001; see Figs. 1H, 1K, 1L, 1M and 1N). The positioning of a large population of exocytic vesicles (often called CGs) and coated plaques beneath the PM is also necessary (Chandler and Heuser, 1981; Sardet, 1984; Sardet *et al.*, 1992; Chang *et al.*, 1996; 1999b; see Figs. 1L and 1N), as well as the stabilization of a meshwork of cortical MFs responsive to the secondary messengers generated by sperm fusion and entry (Ducibella *et al.*, 1990; Johnson and Capco, 1997). The most extensive information in this field comes from starfish and *Xenopus* oocytes which can be stimulated to mature by the hormones 1 MeAdenine and progesterone, respectively, and to proceed either to completion of meiosis II (starfish) or to arrest in metaphase of meiosis II (*Xenopus*). Additional

observations come from spontaneously maturing eggs of ascidians and sea urchins (Swalla *et al.*, 1991; Jeffery, 1995; Berg and Wessel, 1997).

During maturation oocytes acquire responsiveness to IP₃, the main calcium-releasing messenger used at fertilization. This explains why in many species, including mammals, the sperm has the ability to bind and fuse to an immature GV stage oocyte but fails to activate it (Evans *et al.*, 2000). The ability to respond to IP₃ is likely due to the establishment of the extensive cER network underneath the PM (Campanella *et al.*, 1984; Larabell and Chandler, 1988; Callamaras and Parker, 1999; Terasaki *et al.*, 2001; reviewed in Sardet and Chang, 1987; Stricker *et al.*, 1998; Kline, 2000). This cER network reorganizes from stacks of perinuclear and subcortical ER, as well as from stacked ER which forms the annulate lamellae. Recent observations of GFP-labeled ER networks in *Xenopus* show that small clusters of ER develop in the vegetal cortex at the time of GVBD, then disappear and reappear at the time of the second meiotic metaphase block (Terasaki *et al.*, 2001). In mammals, clusters of cER are small and sparse in the immature oocyte and they grow in size and number until meiotic arrest is established (Shiraishi *et al.*, 1995; Kline, 2000). These data suggest that changes in cER organization are coupled to MPF activity and that cER clustering confers the ability to release calcium in response to IP₃ (Mehlman *et al.*, 1995, 1996; Shiraishi *et al.*, 1995; Terasaki *et al.*, 2001). In cytoplasmic extracts of *Xenopus* eggs and in somatic cells, ER tubules have been observed to attach to MTs and MFs, leading to the elaboration of a 3 dimensional ER network (Klopfenstein *et al.*, 1998; Lane and Allan, 1998; Tabb *et al.*, 1998), but it is not known whether the same mechanisms preside over the establishment of the cortical ER network in live eggs. The cER which progressively surrounds CGs is strongly tethered to the PM by the time the oocyte becomes ready to be fertilized. It is not known however whether these attachment sites play a role in rendering the cER network competent to release calcium in response to the factors introduced by sperm (Stricker, 1999).

During maturation in *Xenopus*, starfish, sea urchins and mammals, thousands of golgi-derived cortical vesicles line up beneath the PM, ready for exocytosis in response to the rise in cytoplasmic calcium concentration triggered by sperm entry. The CGs arrive there either progressively (in vertebrates and starfish) or synchronously (in sea urchins) during the period between GV migration and GVBD (Ducibella *et al.*, 1994; Berg and Wessel, 1997). In sea urchins, these vesicles, filled with secretory proteins and reticulating enzymes, are attached to the PM by specialized sites (Vacquier, 1975; Zimmerberg *et al.*, 1985). By the time GVBD has occurred, membrane microvilli and associated actin bundles have retracted and the oocyte membrane has decreased its surface area by half. In both *Xenopus* and starfish the PM has also acquired new ion channels and electrical properties (reviewed in Sardet and Chang, 1987; Nuccitelli and Ferguson, 1994). Studies of insertion-

retrieval of channels in *Xenopus* PM shows that in quiescent oocytes, the PM proteins turn over and that the membrane is constantly renewed via endocytotic-exocytotic trafficking, possibly regulated by protein kinase C (PKC) (Forster *et al.*, 1999). Interestingly, some PM components can be replaced in 24 h, as demonstrated by the injection of connexin50 mRNA, and the study of the expression of the protein and of its insertion and retrieval in the PM of the *Xenopus* oocyte (Zampighi *et al.*, 1999). As this experimental model is used by a growing number of physiologists inserting their favorite channels in the PM by expression of injected mRNA, it is expected that soon we will be able to visualize what types of microdomains exist in the PM of *Xenopus* oocytes and understand what physiological functions they may perform (Singer-Lahat *et al.*, 2000).

Restructuring the Cytoskeleton during Maturation

The cytoskeletal networks of MFs, MTs, and IFs are restructured and further polarized during maturation. A spectacular example of polarization is found in oocytes of the annelid *Chaetopterus*, where a cortical attractor at the animal pole tethers the meiotic spindle, even when the spindle is displaced by violent needle manipulations (Lutz *et al.*, 1988). Similarly in starfish oocytes a distinct astral MT scaffold sandwiched between the PM and the GV, called the premeiotic aster, connects the GV to the animal pole cortex (Miyazaki *et al.*, 2000). This polar spot is devoid of the reticulated network of KFs resembling a hair net (called a "snood," Schroeder and Otto, 1991) situated 1 μm beneath the surface in sea urchin and starfish oocytes. This anastomosing KF network disappears before the end of maturation, and at the same time the length and number of cortical MTs diminish (Boyle and Ernst, 1989). In *Xenopus*, cortical KFs also disassemble during maturation, probably as a result of MPF activation, and disappear completely in the vegetal hemisphere at the time the egg is fertilized (reviewed in Gard, 1999). In eggs blocked in meiosis I or II (ascidians, mammals), the meiotic spindle also seems to interact with the cortex and there is evidence that chromosomes themselves can provoke the accumulation of MFs at the cell surface independently of spindle MTs (Maro *et al.*, 1986). The presence of an actin-rich plaque covering the meiotic spindle appears to be a common feature of the animal cortex region (Sardet *et al.*, 1992; Evans *et al.*, 2000; Verlhac *et al.*, 2000).

Changes in the MF network associated with the morphology of surface microvilli have also been reported during maturation (reviewed in Sardet and Chang, 1987; Johnson and Capco, 1997). The most spectacular structures are the giant actin spikes that appear in starfish oocytes in places where the oocyte had established junctions with surrounding follicle cells (Otto and Schroeder, 1984). These spikes which contain fascin and other Actin Binding Proteins (ABPs) disappear 30 min before GVBD and are replaced by flacid projections which shorten further as maturation progresses. Remodeling of the actin cortex also occurs

during maturation in *Xenopus* and is manifest as the development of the ability to contract when calcium levels are elevated by natural or artificial means (Merriam et al., 1983; Benink et al., 2000).

Relocalization of Cortical mRNAs and Domains during Maturation

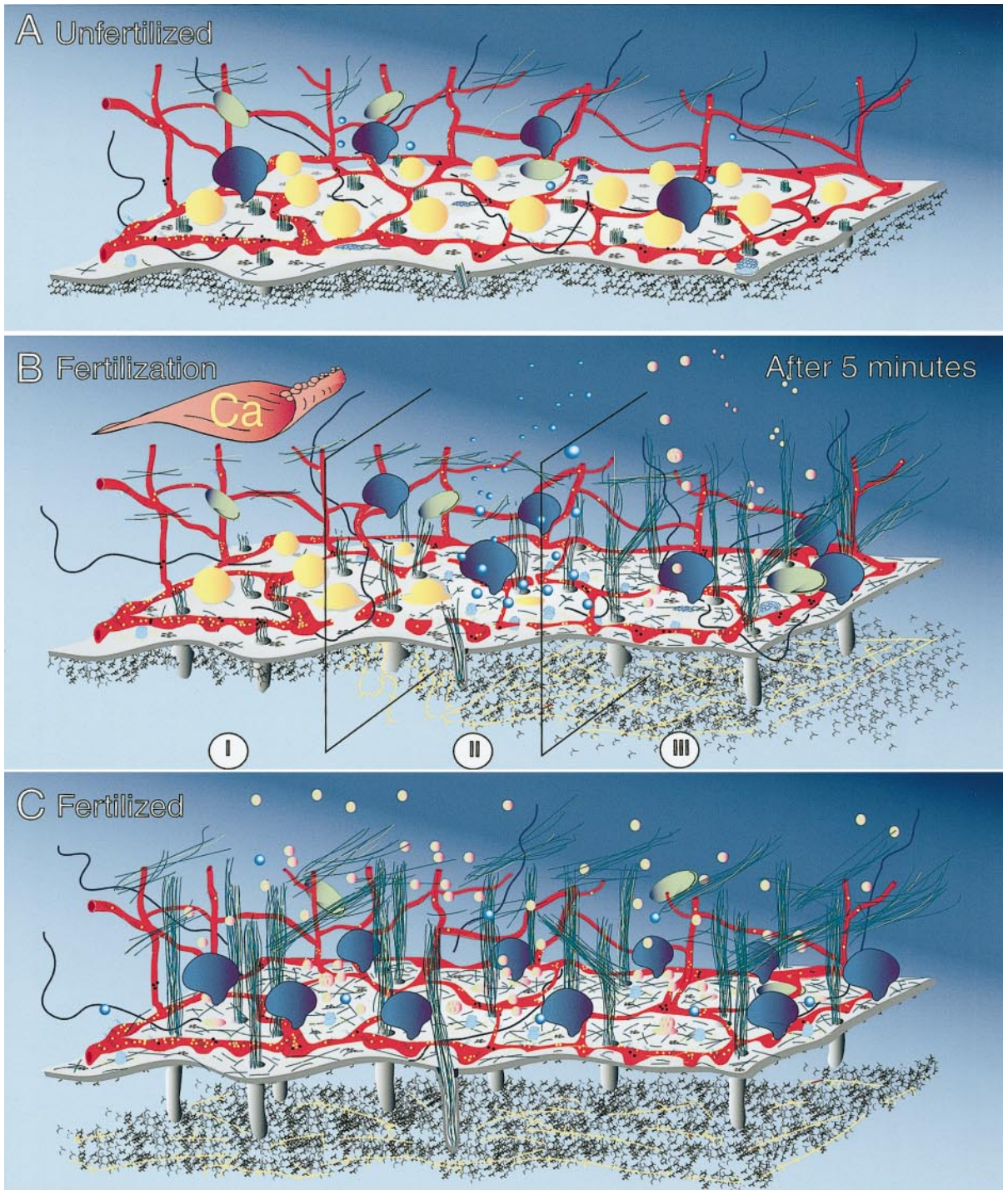
Some of the cortical changes which take place during meiotic maturation have significant consequences on cortical determinant and mRNA localizations. In *Xenopus*, the sensitivity of dorso-anterior determinants to UV irradiation of the vegetal pole decreases up to GVBD, indicating that the UV target undergoes a change in nature or position during maturation (Holowacz and Elinson, 1993; 1995; Marikawa et al., 1997). Messages for *Vg1* (but not *Xcat2*) are released from their tight cortical association, independently of the disappearance of the KF network, and spread out over the vegetal hemisphere from the time of GVBD until meiotic metaphase arrest (Forristal et al., 1995; Klymkowsky et al., 1991). Patches of germinal granules and mitochondria containing mRNAs such as *Xcat2* congregate to form larger islands of germ plasm that remain close to the vegetal surface (Savage and Danilchik, 1993). It is interesting to note that mRNAs encoding Par-6, which associates with the important cell polarity protein Par-3 (ASIP/Bazooka), is restricted to the animal hemisphere of *Xenopus* oocytes (Choi et al., 2000), while *Xenopus* homologues of the Par-3 protein and aPKC are progressively localized in the animal cortex (Izumi et al., 1998; Nakaya et al., 2000). These recent observations on the Par-3/Par-6/aPKC complex, and other studies concerning conserved germ plasm components (*Xcat2* and *Nos*), emphasize that similarities may exist between the establishment of the animal-vegetal axis in the amphibian oocyte and the acquisition of anterior-posterior polarity in the *Caenorhabditis* zygote (Subramaniam and Seydoux, 1999; Kemphues, 2000; Houston and King, 2000b; Ohno, 2001). It remains to correlate these changes in Par proteins and mRNA localization with the maturation of the cortical ER, MF, MT and KF networks and also to analyze the changing locations of mRNAs in cortices isolated at different times during maturation (Aларcon and Elinson, 2001).














Not much is known about cortical mRNA relocalizations during maturation in species other than *Xenopus*. As mentioned earlier, ascidian eggs contain a large population of cortically localized PEM mRNAs (Nishida and Makabe, 1999). The positioning of these cortical mRNAs has not been examined in detail, but it is probable that they reach their cortical location at the same time the mitochondria-rich myoplasm layer is positioned in the subcortical vegetal hemisphere of the maturing oocyte (Jeffery and Capco, 1978; Swalla et al., 1991; Sasakura et al., 2000).

The Cortex as the Site of Attachment and Fusion of Sperm and Egg

In the sea urchin and starfish eggs, sperm fusion occurs randomly on the egg surface. In eggs of the amphibian (*Xenopus*) and ascidian (*Phallusia*), preferred sperm entry is in the animal hemisphere. The underlying mechanisms cannot yet be associated with any clear differentiation in the cortex of these species (Elinson, 1975; Speksnijder et al., 1989), although in *Xenopus* oocytes surface microvilli are more sparse in the animal hemisphere (Monroy and Baccetti, 1975). On mouse eggs, sperm binding and fusion is restricted to the vegetal 2/3 of the egg which is characterized by microvilli covered by specific molecules associated with sperm binding (Evans et al., 2000). The smooth patch lacking microvilli around the animal pole arises during maturation as precocious exocytosis of CGs and reorganizations of the MF network take place (Ducibella et al., 1990; Simerly et al., 1998). Many eggs have special extracellular structures (micropyles) which form conduits to guide the sperm towards the animal or, in rare cases, the vegetal pole. Animal and vegetal differentiations in the egg cortex related to a specific sperm entry site clearly exist in some cnidarians (Carré and Sardet, 1981) and annelids (Focarelli and Rosati, 1995).

Although sperm-egg binding proteins seem to vary widely among different phyla (Vacquier, 1998), surface macromolecules of the integrin family are apparently main actors in sperm-egg interaction in mammals, amphibians and invertebrates. In mice, different surface proteins of the Integrin family are specifically located in the microvillar (vegetal hemisphere and equatorial region) or the amicrovillar (animal pole) regions and are implicated in the binding of sperm proteins and possibly in sperm-egg fusion through association with receptor partners (LeNaour et al., 2000; Evans et al., 2000; Wassarman et al., 2001). In sea urchins, β -integrins combine at the tip of microvilli where they may act as posts anchoring the vitelline membrane. These integrins are severed from the surface by proteases during CG exocytosis, leaving their cleaved transmembrane and cytoplasmic fragments attached to the PM where they may anchor the cortical MF cytoskeleton and participate in its transformation during fertilization (Murray et al., 2000). In many species, fusion between the gametes is thought to take place at the tip of microvilli, a recurring theme in cell-cell adhesion and fusion (Wilson and Snell, 1998). The MFs abutting the tip of the microvilli may participate in the maintenance of membrane microdomains involved in sperm-egg binding, signal transduction and/or MF polymerization. In sea urchins, a candidate sperm receptor apparently localizes to the tip of microvilli (Ohlendieck and Lennarz, 1995; Giusti et al., 1997). The mechanism of sperm-egg fusion is not well understood. Recently CD9 proteins, members of the tetraspan family of integral membrane proteins associating with $\beta 1$ integrins, have been proposed to participate in fusion based on the phenotype of mutant mice (LeNaour et al., 2000; Wassarman et al., 2001).



- | | | | |
|--|--|--|---|
|  Plasma membrane |  Acidic/pigmented vesicle |  Exocytotic vesicles/ Endosomes |  Microtubule |
|  Endoplasmic Reticulum/ Ribosomes |  Cortical Granule |  Extracellular Coat |  Microfilament |
|  Microvillus |  Mitochondria |  Coated Plaques |  Microdomains |
| | |  Calcium wave | |

D. FERTILIZATION TRANSFORMS THE EGG CORTEX

Fusion with sperm brings enormous changes to the egg cortex (Fig. 2). These changes have been best described in sea urchins, amphibians, ascidians and mammals. They are immediate consequences of the massive release of intracellular calcium that floods the egg, propagating through it from the cortical site of sperm entry (Stricker, 1999). It is interesting to note that in *Medaka* fish eggs, where the first calcium waves were discovered more than 20 years ago, the propagation of the waves is restricted to the cortex (Gilkey *et al.*, 1978).

Sperm-Induced Calcium Waves and Membranes

The fusion of egg and sperm PMs brings about large changes in membrane permeability and electrical potential as well as the calcium-mediated activation of the egg (reviewed in Nuccitelli and Ferguson, 1994; Stricker, 1999; Swann and Parrington, 1999; Jaffe *et al.*, 2001). The initial depolarization of the PM causes a brief and nonactivating calcium influx all around the cortex in sea urchins and a more substantial calcium influx in mollusc eggs where it is the signal for activation. In annelids, sea urchins, ascidians, amphibians, and mammals, it has been hypothesized that the signal for activation is a factor locally introduced by the sperm that acts on the cortex to trigger a single or multiple calcium waves (Oda *et al.*, 1999; reviewed in Sardet *et al.*, 1998; Stricker, 1999; Jaffe *et al.*, 2001). In sea urchins a single wave of propagated calcium release from the ER sweeps through the egg cortex and cytoplasm at an approximate speed of 5–10 $\mu\text{m/s}$ (Suzuki *et al.*, 1995; McDougall *et al.*, 2000). In annelids, ascidians, and mammals, multiple waves are produced during the completion of the meiotic cell cycles (Speksnijder *et al.*, 1990a,b; Stricker *et al.*, 1998; Kline *et al.*, 1999; Deguchi *et al.*, 2000). The cER and the deeper interconnected network of rough ER tubes and sheets are reorganized at the time free cytoplasmic calcium concentration reaches activating levels (5–20 μM). In sea urchins, starfish, and *Xenopus* the single calcium wave sweeps through the egg from the sperm entry site and causes a wave of vesiculation of the ER network which persists as long as the intracellular calcium concentration

remains elevated (Terasaki and Jaffe, 1991; Jaffe and Terasaki, 1994; Terasaki *et al.*, 2001). The continuous 3-dimensional network of ER tubes and sheet reforms after the intracellular calcium level decreases.

In ascidians and the annelid *Cerebratulus* where fertilization triggers repetitive calcium waves, the ER network does not fragment. Instead, sheets and tubes of the ER network aggregate into clusters (microdomains) after fertilization (Speksnijder *et al.*, 1993; Stricker *et al.*, 1998). In mouse eggs cER clusters are also visible as sites of IP₃ receptor accumulation which line the PM in the vegetal hemisphere (Kline *et al.*, 1999; Kline, 2000). In the ascidian egg cortex, the aggregation of cER in the vegetal contraction pole is amplified by a spectacular actomyosin-driven cortical contraction triggered by calcium release (Sardet *et al.*, 1989; Roegiers *et al.*, 1995; see Fig. 11, J). A domain of cER accumulation 20 μm long and 2–5 μm thick, which can be isolated as a cell surface complex and is not dislodged by centrifugation, acts as a calcium wave pacemaker emitting 6–12 cortical waves which originate in the vegetal cortex (Speksnijder *et al.*, 1990a,b; McDougall and Sardet, 1995; Dumollard and Sardet, 2001). It is probable that in mouse and annelids, like in ascidians, the cortical spots of cER accumulation act as calcium wave pacemakers during the completion of the meiotic cell cycle (Stricker *et al.*, 1998; Kline *et al.*, 1999; Deguchi *et al.*, 2000). It is intriguing that calcium wave pacemakers are all located in the vegetal cortex in the eggs of widely different organisms (annelids, ascidians, mammals). Whether such directional waves play a role in later development remains an open question (Sardet *et al.*, 1998; Jones, 1998).

It is not known what happens to the sites of attachment of the cER to the PM after fertilization. It has been hypothesized that these cER-PM junctions might be like PM-sarcoplasmic reticulum junctions characteristic of muscle cells (Gardiner and Grey, 1983; Sardet, 1984; Sardet *et al.*, 1992). There may also exist a link between these ER-PM junctions and the presence of store-operated calcium channels described in ascidian and *Xenopus* eggs (Arnoult *et al.*, 1996; Jaconi *et al.*, 1997; Yao *et al.*, 1999; Machaca and Haun, 2000). Considering the recently-described association between store-operated calcium channels and IP₃ or Ryanodine Receptors (Csutora *et al.*,

FIG. 2. Transformation of the cortex during activation. This schematic representation is inspired from the work done on sea urchins and *Xenopus* eggs where major exocytotic and endocytic events take place after the egg is fertilized. (A) Unfertilized egg cortex phenotype. The plasma membrane (grey) has a cell surface coat (black) and its cytoplasmic face comprises coated plaques (blue), short microfilaments (green), cortical vesicles (cortical granules: ocre, acidic/pigmented vesicles: blue) and an attached network of cortical endoplasmic reticulum (red) studded with ribosomes (yellow). (B) At fertilization, the calcium wave triggers waves of cortical reorganizations. Cortical granules fuse with the plasma membrane (I left), exocytosing structural proteins and enzymes which form the fertilization membrane. After the passage of the calcium wave, endocytic activity is stimulated, the endoplasmic reticulum is fragmented (II), and microfilaments polymerize and bundle in elongating microvilli and underneath the plasma membrane (II middle, and III right). (C) Fertilized egg cortex phenotype. Microvilli have elongated and bundles of microfilaments are abundant underneath the plasma membrane. Acidic/pigmented vesicles have moved closer to the plasma membrane.

1999; Kaznacheyeva *et al.*, 2000; Kiselyov *et al.*, 2000), it is tempting to speculate that the ER network in eggs is tethered to the PM via specialized regions of the cER containing these calcium channels. Such junctional plaques could represent one type of PM microdomain important for signal transduction, like the many other raft-like structures present in the PM of somatic cells (Simons and Toomre, 2000). The presence of microdomains or raft-like structures in the egg PM and their possible reorganization at fertilization have not yet received much attention, although older observations pointed out a mosaic composition of the sea urchin egg PM after fertilization (Carron and Longo, 1983). There are interesting recent reports on the presence of lipid rafts containing signaling complexes important for fertilization in the PM of sea urchins eggs (Ohta *et al.*, 2000; Belton *et al.*, 2001), and it is clear that membrane proteins and ion channels inserted in the *Xenopus* oocyte PM form small patch-like domains (Singer-Lahat *et al.*, 2000). We do know that, at least in the sea urchin egg, there are no large differences in lipid or protein mobilities at the animal and vegetal poles and that fluidity tends to decrease after the doubling in surface area created by the exocytosis of CGs (Peters *et al.*, 1984; Hirano, 1991).

Waves of Exocytosis and Endocytosis at Fertilization

In sea urchins successive waves of CG fusion, microvilli elongation, and microvilli stiffening propagate with the same directionality and speed as the initial calcium wave (Suzuki *et al.*, 1995). These waves of reorganization transform the egg cortex in 15 min from a fairly flat, soft, layer studded with short micropapillae to a highly microvillated and stiffened surface with a new set of cortical vesicles (acidic/pigmented vesicles) replacing the exocytosed CGs (see Figs. 2A, 2B, and 2C).

In many eggs, the most visible consequence of the rise in intracellular calcium is the massive secretion due to exocytosis and subsequent transformations of the extracellular layer. In many species this exocytotic event erects a physical barrier (the fertilization envelope), which participates in the block to polyspermy and more generally protects the developing embryo. In sea urchin eggs a wave of exocytosis of some 15,000 CG (about 5000 in mouse eggs) attached to the PM at specialized sites follows the wave of calcium release in the cortex (Figs. 1D, 1E, 1F, 1G, and 1H and Fig. 2B I and II). This exocytic burst results in increases in PM surface area on the order of 50% in sea urchin, and 10% in mouse (Schroeder, 1981; Ducibella *et al.*, 1994). In sea urchins several classes of exocytic vesicles can be distinguished (Matese *et al.*, 1997). In recent years, proteins universally involved in the exocytotic-endocytic pathway (SNAREs, GTPases, synaptotagmin, Rab3, Rabphilin. . .) have been found to be located in the CG membranes or their vicinity (Conner *et al.*, 1997; Masumoto *et al.*, 1998). It is expected that identification and localization of these

molecules combined with manipulations of isolated cortices of sea urchins in which CG-PM fusions can be triggered *in vitro* by raising calcium will bring rapid progress to this field (Zimmerberg *et al.*, 1999).

Following the wave of exocytosis, endocytosis takes place, in part to limit and reverse the PM surface area increase caused by addition of exocytotic vesicle membrane. Endocytic coated plaques observed in sea urchins, ascidians, and *Xenopus* eggs are another class of membrane microdomain, which participate in the endocytic burst after fertilization (Figs. 1L and 1N). In the 1980s several investigations suggested that these coated vesicles retrieved much of the membrane inserted during CG exocytosis (reviewed in Sardet and Chang, 1987). Recent observations point to a more complex situation (Vogel *et al.*, 1999). In sea urchins, the endocytotic period that follows the wave of exocytosis is apparently limited to sites of exocytosis and not simply the result of the endocytosis of small-coated vesicles (Smith *et al.*, 2000). In fact it seems that this compensatory endocytosis is linked to the transitory insertion and retrieval of P-type calcium channels in the egg PM. Interestingly, the calcium influx through these newly inserted P-type channels drives the endocytic process and can specify that only CG-derived membrane is retrieved (Vogel *et al.*, 1999; Smith *et al.*, 2000). This points to at least 2 types of endocytic processes which profoundly modify the PM after exocytosis: one would be removing the CG membrane inserted during CG exocytosis, and the other would be based on the endocytosis of thousands of small coated vesicles coalescing into endosome-like structures. The endosomes may add to the populations of acidic vesicles/pigment granules which are present in the egg cortex before fertilization in *Paracentrotus* (forming the pigment band, see Fig. 1A, B) or which migrate to the cortex soon after fertilization in other sea urchin species (Lee and Epel 1983; Sardet, 1984; Allen *et al.*, 1992). The saltatory translocation of these large acidic, calcium containing vesicles in the cortex could be linked to their interactions with MFs or MTs via spectrin which coats the vesicles (Fishkind *et al.*, 1990a,b). An interesting idea recently put forward is that in *Xenopus* after fertilization (as during vitellogenesis) endosome-like vesicles which result from the intense endocytotic burst may contribute to restructuring of the cortical actin cytoskeleton (Taunton *et al.*, 2000). These endosomes apparently bind PKC and NWASP on their surface and are able to nucleate actin MF comet tails that resemble those produced by bacteria such as *Listeria*.

Fertilization-Induced Microfilament Reorganizations

Just after fertilization, the cortical MF cytoskeleton is reorganized and contracts in a wave-like manner starting from the site of sperm entry. In echinoderms a massive polymerization of actin filaments takes place, forming a giant fertilization cone that engulfs the sperm (Tilney and Jaffe, 1980). The elevations in calcium and in intracellular

pH cause an increase in the number of cortical MFs and their restructuring into bundles within 10 min of fertilization (Wong *et al.*, 1997; Heil-Chapdelaine and Otto, 1996). It is clear that the reorganizations of the MF network can be disconnected from the massive CG exocytosis. Observations of live sea urchin eggs suggest that MFs may be recruited from deeper layers in the egg by translocating over a scaffold (possibly the ER network). After fertilization, MF bundles shoot away from the membrane (Terasaki, 1996), a process that may bear some resemblance to the generation of endosome-nucleated MF comets mentioned above.

The results of all these transformations is that 10 min after fertilization, a thicker cortical MF cytoskeleton has been assembled which is intimately connected to the PM via abundant microvilli and which bathes a collection of cortical organelles (cER, vesicles, and mitochondria in some species like *Tubifex*). Cortical MFs within elongated rigid microvilli are bundled and form characteristic rootlets extending 5–8 μm into the egg (see Figs. 2B III and 2C). The transformation of the microvilli from flacid to rigid apparently involves the rise in pH (Begg and Rebhun, 1979). Fascin is probably involved in the bundling, and a dozen other ABPs have been detected and participate in cortical actin polymerization and MF reorganization (Terasaki *et al.*, 1997). At this stage what is needed is a careful analysis of the evolution of the cortical MF network using high resolution live imaging of MFs and of various ABPs in the presence or absence of the other major cortical constituents (ER, MTs, IFs). High resolution analysis of the cortical structures and macromolecules by a combination of EM techniques combined with immunochemistry is possible in eggs or isolated cortices of sea urchins, ascidians, and amphibians (Chandler and Heuser 1981; Sardet *et al.*, 1992; Chang *et al.*, 1996; 1999b; Heuser, 2000).

An important functional capability acquired by the egg cortex after fertilization or artificial activation is the ability to undergo calcium-dependent contraction-relaxation (Sardet *et al.*, 1989; Roegiers *et al.*, 1995; 1999; Benink *et al.*, 2000). The properties of the cortical MF network are harnessed in producing cortical flows (translocation of cortical and subcortical material parallel to the plane of the PM) which are essential for cortical reorganizations and relocalizations between fertilization and first cleavage (Canman and Bement, 1997; Benink *et al.*, 2000). What these cortical flows might be and how they are regulated has been addressed recently in fertilized *Ceanorhabditis* eggs and also in *Xenopus* oocytes artificially stimulated by Phorbol esters (O'Connel *et al.*, 2000; Benink *et al.*, 2000). Phorbol esters such as PMA activate PKC and induce a contraction or cortical flow similar to or more violent than that naturally triggered at fertilization. Such cortical flows would principally depend on the control of myosin II activity via the action of the small GTPases Rac and Rho. MT polymerization and the presence of large MT arrays, in particular asters, are seemingly able to control the speed and direction of such cortical flows (Canman and Bement, 1997). Concepts mostly developed in somatic cells concerning the

interplay of the MF and MT networks (Sider *et al.*, 1999; Waterman-Storer and Salmon, 1999; Mandato *et al.*, 2000) are expected to provide interesting models for understanding what drives and controls cortical flows during development, a subject that will be dealt with in another review.

The Fertilized Egg Cortex

The fertilized cortex has been transformed by the flux of calcium out of the ER as well as by changes in secondary messengers and pH which have pleiotropic effects at the levels of enzymes and other molecules which regulate the cytoskeleton, signaling pathways, exocytosis, and endocytosis. Exocytosis and changes in the MF network from the unfertilized to the fertilized state can be controlled to a certain extent in isolated cortices by manipulating calcium and pH (Begg and Rebhun, 1979; Zimmerberg *et al.*, 1999). The fertilized egg cortex is generally thicker than the unfertilized cortex, principally due to the growth of microvilli with their cores of MF bundles intermingling with the MF meshwork underlying the membrane (Wong *et al.*, 1997). We are now starting to suspect that cytoskeletal transformations of the cortex are intimately dependent on membrane traffic and the presence of cortical organelles such as endosomes and acidic vesicles. These organelles often display saltatory motions near the cortex, especially just after fertilization when pH, calcium, secondary messengers, MPF activity, and phosphorylation status are rapidly changing (Allen *et al.*, 1992). These excursions of organelles may contribute both to their positioning in the cortex and also to the elaboration of the cortical MF network (Taunton *et al.*, 2000). After sperm entry and the introduction of the paternally derived centrosome, giant astral or bundled MTs may firmly attach to the cortex such that they can be isolated with it (Schatten and Mazia, 1976). At appropriate times during the meiotic or mitotic cell cycles, elongated cortical MTs may slide with respect to major components of the cortex (cER, cMFs, PM), as is the case during cortical rotation in *Xenopus* and ascidians or during spindle positioning of the first mitosis (Chang *et al.*, 1999b; Roegiers *et al.*, 1999). The cortical MF network will also be activated to contract and relax during specific phases of the meiotic and mitotic cell cycles, a process which seems to be regulated by the presence of MTs (Mandato *et al.*, 2000). The interplay between the cMF network and the microtubular structures mediates in turn the formation of particular protrusions such as polar lobes in molluscs and annelids, the initiation and propagation of surface contraction waves best described in amphibians, and subsequent equal or unequal cleavages (Speksnijder *et al.*, 1986; Sardet *et al.*, 1994; Chang *et al.*, 1999b). These later transformations of the cortex will be dealt with in a forthcoming review.

E. WHAT THE CORTEX IS

In summary, we can consider that what comprises the egg cortical scaffold and its continuity is the assemblage of the basic three: (1) the plasma membrane (PM) and extracellular layer, (2) the cortical endoplasmic reticulum (cER) network tethered to it, and (3) the 3 dimensional matrix of microfilaments (MFs) bathing the underside of the PM. Within the basic cortical scaffold, microtubules (MTs) and intermediate filaments (IFs) seem to come and go depending principally on the calcium signals, cell cycle factors, and binding proteins that regulate their dynamic behavior (see Fig. 2). The layer of cortical MTs, MFs, and IFs should be seen as forming a highly interconnected meshwork in which cER and organelles are embedded (Sider *et al.*, 1999; Chou *et al.*, 2001). It is this interconnected assemblage that can be isolated by gently lysing or homogenizing eggs, by shearing eggs attached to a surface, or by microdissecting eggs and oocytes (Sardet *et al.*, 1992; Elinson *et al.*, 1993; Kageura, 1997; Kline *et al.*, 1999; Alarcon and Elinson, 2001). One consequence of the interconnectivity is that the PM/cER/MF scaffold remains unperturbed when organelles and cytoskeletal elements are stratified by low centrifugal forces (Shimizu, 1985, 1988; Sardet *et al.*, 1992). Depending on the abundance and degree of interconnectivity of the MF, cER, MT, and KF networks, the cortex will be several microns thick as in the case of the *Xenopus* stage VI oocyte or about a micron thick as in mature ascidian, mollusc, or sea urchin eggs. Although they remain to be characterized, a large cohort of linker molecules and motors attach to the cortical scaffold and mediate the interactions between the MTs, MFs, PM, cER and other cortical organelles (Fishkind *et al.*, 1990a,b). Because the cER is strongly anchored to the PM we hypothesize that in the cortex, MTs may slide onto the tubes and sheets of cER rather than pull and stretch them into a reticulated network as they do in homogenates or in the deeper cytoplasm (Lane and Allan, 1998). The layer of cER may provide the scaffold on which motors anchor to move the microtubular structures (asters and MT bundles).

The Cortex as a Repository of Spatiotemporal Information

In *Xenopus*, *Drosophila*, and the ascidian *Halocynthia* all mRNA determinants identified to date are positioned and anchored in the cortex during oogenesis and maturation for use after fertilization and during development. There is evidence for strong physical tethering of these mRNAs to one or several components of the cortical scaffold, since at least in *Xenopus* and ascidians many cortical mRNAs remain attached to the cortex once it is isolated (Alarcon and Elinson, 2001; Sardet and Nishida, unpublished). In addition cortical mRNAs can be transplanted with the microdissected cortex, and their attachment to the cortex resists centrifugal forces that stratify cytoplasmic egg components (Kageura, 1997; Sasakura *et al.*, 2000). In *Xenopus* oocytes, the cortical KF network has been proposed to be a

main binding site for some *Vg1* type mRNAs and the cER may also participate (Alarcon and Elinson, 2001). It is reasonable to think that mRNAs would associate with the cER, considering the presence of ribosomes on the cER (see Figs. 1L and 1N) and the involvement of Staufin, a rough ER-associated molecule, in mRNA localization (reviewed in Roegiers and Jan, 2000). Finally the abundant cortical MF network may also provide RNA binding sites as is believed to be the case in cells as different as yeast, fibroblasts, or eggs (reviewed in Bashirullah *et al.*, 1998; Lasko, 1999; Kwon and Schnapp, 2001).

The localization of mRNAs to specific regions of the cortex implies an inherent polarity in the egg cortical scaffold. In the ascidian egg cortex, cER and MFs are polarized along the animal-vegetal axis in the mature oocyte and move after fertilization, potentially providing the structural support for the initial animal-vegetal gradient distribution of cortical PEM mRNAs and their relocation after fertilization (Sardet *et al.*, 1992; Speksnijder *et al.*, 1993; Roegiers *et al.*, 1999; Sasakura *et al.*, 2000; Sardet and Nishida, unpublished). In *Xenopus* oocytes at stage II, when the first localizing mRNAs (*Xcat2* type) are brought by the mitochondrial cloud to the vegetal cortical region, there are no apparent differences between the animal and vegetal cortices (Gard, 1999). In the oocyte of *Drosophila*, some proteins such as the MT stabilizer Bic-D arrive in the anterior cortex well before *bicoid* mRNAs localize there (Pare and Suter, 2000). We still do not know much about the structures (particles, granules, networks of ER, MTs, MFs, KFs) that mediate the translocation and binding of these cortically localized proteins and mRNAs, one problem being that the structure and evolution of the *Drosophila* oocyte cortex have not yet been studied in detail. Many cortical proteins or complexes (such as the γ tubulin foci in *Xenopus* oocytes, Pfeiffer and Gard, 1999) influence the MT network and its positioning with respect to the cortex, allowing macromolecular complexes, cytoskeletal elements, organelles and particles to be motored to specific regions of the cortex. We can expect that a large collection of motor molecules and proteins such as the formins or the Par-1/MARKs will mediate these polarized interaction of MTs with the cortex in the oocyte and egg cortex as they do in yeast or mammalian epithelial cells (Drewes *et al.*, 1997; Korinek *et al.*, 2000; Lee *et al.*, 2000; reviewed in Heil-Chapdelaine *et al.*, 1999; Segal and Bloom, 2001).

We may wonder why spatiotemporal information is stored in the egg cortex. This may be because the cortex is a relatively stable point of reference, especially during cell division when partitioning of informational material to different blastomeres takes place. Spatial information acquired during oogenesis (related to sites of previous cell divisions, site of attachment to ovary, . . .), maturation (related to the expression of animal-vegetal polarity, . . .), and fertilization (related to the point of sperm entry, . . .) could thus be stored in the cortex for later use during development. In this sense, the cortex provides a memory of these events and of the stored information to be communi-

cated to the cytoplasm and nuclei and to be used for axis and tissue formation. However, we still know very little about the underlying mechanisms that dictate how this spatial information is stored and when it is released.

The Questions That Remain

As usual, we are left with more questions than answers. Looking at the cortex and its components (schematically drawn in Fig. 2), we can address a series of interesting problems:

- What physical and functional links exist between the PM, the cER, and the MF networks that constitute the basic cortical scaffold of the egg? When and how is the cortical scaffold first established during oogenesis and maturation?
- When does polarity first arise in the cortical scaffold? By what mechanisms is it established? How are other cortical polarities set up after fertilization? How is this cortical polarity preserved and expressed following cell divisions?
- How are important proteins, mRNAs and their complexes brought to the cortex and anchored to it? What information do they provide locally or to the rest of the egg or embryo? How is translation of the cortical mRNAs controlled?
- How does the cortical scaffold collect and anchor organelles, or cytoplasmic domains like germ plasm and asters? What factors (cell cycle factors, factors introduced by sperm. . .) control their translocation.
- What structure tethers the cER to the PM of eggs? How and when are these cER-PM junctions established? How are they reorganized after fertilization? What roles do they play in signaling and during development and differentiation?
- What other types of microdomains like caveolae and coated plaques exist in the PM and subsurface skeleton? Do they have a polarized distribution? What roles do they play in sperm binding, signaling, remodeling of the cytoskeleton, or the endocytic-exocytic cycles?
- How do MTs attach to the basic cortical scaffold and move with respect to it during important events (meiotic spindle attachment, cortical rotations, equal and unequal cleavages)?
- How do the components of the cortical scaffold cooperate to undergo MF-based contractions-relaxations? What are the relationships between these MF based events and signaling and trafficking? How are cortical flows and cleavages controlled in time and space and what role do MTs play?

We now have many good molecular and cellular tools to tackle some of these questions. First, mRNA determinants (*Bicoid*, *VegT*, *Macho*) located in the cortex have been identified at the poles of eggs of widely different species. Second, we know important proteins localized within the basic cortical scaffold (such as the Par proteins) are involved in polarization of different eggs and many somatic cell types. Third, we know of many motors, regulators, and

linkers interacting with MFs, MTs, cER, and KFs. Many of these structures, proteins, and mRNAs can be manipulated by genetic means, selectively labeled, and studied using high resolution live imaging of the cortex. We should also be able to dissect isolated cortices and locate important RNAs or proteins by selectively detaching or extracting MFs, cER, MTs, IFs, organelles, ribosomes, RNP particles, and other macromolecular complexes. Because they are thin and flat and can be rapidly frozen, cortex preparation are ideally suited for high-resolution localization of important structures and macromolecules. Furthermore, the isolated cortex (an "open cell preparation" obtained without permeabilization) can be combined with cytoplasmic extracts of different cell cycle stages to induce movements of cytoskeletal components. Finally, many functional properties of the cortex (exocytosis of CGs, calcium pumping and release, contractibility, organelle translocations. . .) can be studied and micromanipulated at the same time *in vitro* and *in vivo*. Like asters or the nucleus, the cortex is a main actor of the egg cell and of its transformations during development. Understanding the cortex and the multiple roles it plays remains a worthwhile challenge.

ACKNOWLEDGMENTS

We are very grateful to Anne Marie Gomez for helping to sort through the massive cortical literature, to Mohamed Khamla, Christian Rouvière, and Philippe Dru for their help with the imaging, and to Richard Elinson, Anne Ephrussi, Evelyn Houlston, Mary Lou King, Gérard Prulière, and Mark Terasaki, for discussions and cortical reading of the manuscript.

REFERENCES

- Alarcon, V. B., and Elinson, R. P. (2001). RNA anchoring in the vegetal cortex of the *Xenopus* oocyte. *J. Cell Sci.* **114**, 1731–1741.
- Allen, P. G., Baltz, J. M., and Begg, D. A. (1992). Fertilization alters the orientation of pigment granule saltations in *Arbacia* eggs. *Cell Motil. Cytoskeleton* **21**, 223–234.
- Arnoult, C., Grunwald, D., and Villaz, M. (1996). Novel postfertilization inward Ca^{2+} current in ascidian eggs ensuring a calcium entry throughout meiosis. *Dev. Biol.* **174**, 322–334.
- Bashirullah, A., Cooperstock, R. L., and Lipshitz, H. D. (1998). RNA localization in development. *Annu. Rev. Biochem.* **67**, 335–394.
- Begg, D. A., and Rebhun, L. I. (1979). pH regulates the polymerization of actin in the sea urchin egg cortex. *J. Cell Biol.* **83**, 241–248.
- Belton, R. J., Jr., Adams, N. L., and Foltz, K. R. (2001). Isolation and characterization of sea urchin egg lipid rafts and their possible function during fertilization. *Mol. Reprod. Dev.* **59**, 294–305.
- Benink, H. A., Mandato, C. A., and Bement, W. M. (2000). Analysis of cortical flow models *in vivo*. *Mol. Biol. Cell* **11**, 2553–2563.
- Berg, L. K., and Wessel, G. M. (1997). Cortical granules of the sea urchin translocate early in oocyte maturation. *Development* **124**, 1845–1850.
- Böhm, H., Brinkmann, V., Drab, M., Henske, A., and Kurzchalia, V. (1997). Mammalian homologues of *C. elegans* PAR-1 are asymmetrically focalized in epithelial cells and may influence their polarity. *Curr. Biol.* **7**, 603–606.

- Boveri, T. (1901). Ueber die polarität des seeigeleies. *Ver. der phys. med. Ges. zu Würzburg* **34**, 145–175.
- Boyle, J. A., and Ernest, S. G. (1989). Sea urchin oocytes possess elaborate cortical arrays of microfilaments, microtubules and intermediate filaments. *Dev. Biol.* **134**, 72–84.
- Brendza, R. P., Serbus, L. R., Duffy, J. B., and Saxton, W. M. (2000). A function for kinesin I in the posterior transport of oskar mRNA and stau protein. *Science* **289**, 2120–2122.
- Callamaras, N., and Parker, I. (1999). Radial localization of inositol 1,4,5-trisphosphate-sensitive Ca²⁺ release sites in *Xenopus* oocytes resolved by axial confocal linescan imaging. *J. Gen. Physiol.* **113**, 199–213.
- Campanella, C., and Andreucetti, P. (1977). Ultrastructural observations on cortical endoplasmic reticulum and on residual cortical granules in the egg of *Xenopus laevis*. *Dev. Biol.* **56**, 1–10.
- Campanella, C., Andreucetti, P., Taddei, C., and Talevi, R. (1984). The modifications of cortical endoplasmic reticulum during in vitro maturation of *Xenopus laevis* oocytes and its involvement in cortical granule exocytosis. *J. Exp. Zool.* **229**, 283–293.
- Canman, J. C., and Bement, W. M. (1997). Microtubules suppress actomyosin-based cortical flow in *Xenopus* oocytes. *J. Cell Sci.* **110**, 1907–1917.
- Carré, D., and Sardet, C. (1981). Sperm chemotaxis in siphonophores. *Biol. Cell* **40**, 119–128.
- Carron, C. P., and Longo, F. J. (1983). Filipin/sterol complexes in fertilized and unfertilized sea urchin egg membranes. *Dev. Biol.* **99**, 482–488.
- Cha, B., Koppetsch, B. S., and Theurkauf, W. E. (2001). *In Vivo* Analysis of *Drosophila* bicoid mRNA Localization Reveals a Novel Microtubule-Dependent Axis Specification Pathway. *Cell* **106**, 35–46.
- Chambers, R., and Chambers, E. L. (1961). "Exploration into the nature of the living cell." Harvard Univ. Press, Cambridge, MA.
- Chan, A. P., Kloc, M., and Etkin, L. D. (1999). fatvg encodes a new localized RNA that uses a 25-nucleotide element (FVLE1) to localize to the vegetal cortex of *Xenopus* oocytes. *Development* **126**, 4943–4953.
- Chandler, D. E., and Heuser, J. (1981). Postfertilization growth of microvilli in the sea urchin egg: New views from eggs that have been quick-frozen, freeze-fractured, and deeply etched. *Dev. Biol.* **82**, 393–400.
- Chang, J. S., Tan, L., and Schedl, P. (1999a). The *Drosophila* CPEB homolog, Orb, is required for Oskar protein expression in oocytes. *Dev. Biol.* **215**, 91–106.
- Chang, P., LeGuellec, K., and Houlston, E. (1996). Immunodetection of cytoskeletal structures and the Eg5 motor protein on deep-etch replicas of *Xenopus* egg cortices isolated during the cortical rotation. *Biol. Cell* **88**, 89–98.
- Chang, P., Perez-Mongiovi, D., and Houlston, E. (1999b). Organization of *Xenopus* oocyte and egg cortices. *Micro. Res. Techn.* **44**, 415–429.
- Choi, S. C., Kim, J., and Han, J. K. (2000). Identification and developmental expression of par-6 gene in *Xenopus laevis*. *Mech. Dev.* **91**, 347–350.
- Chou, Y. H., Helfand, B. T., and Goldman, R. D. (2001). New horizons in cytoskeletal dynamics: Transport of intermediate filaments along microtubule tracks. *Curr. Opin. Cell Biol.* **13**, 106–109.
- Conner, S., Leaf, D., and Wessel, G. (1997). Members of the SNARE hypothesis are associated with cortical granule exocytosis in the sea urchin egg. *Mol. Reprod. Dev.* **48**, 106–118.
- Cooley, L., and Theurkauf, W. E. (1994). Cytoskeletal functions during *Drosophila* oogenesis. *Science* **266**, 590–596.
- Cox, D. N., Lu, B., Sun, T. Q., Williams, L. T., and Jan, Y. N. (2001). *Drosophila* par-1 is required for oocyte differentiation and microtubule organization. *Curr. Biol.* **11**, 75–87.
- Csutora, P., Su, Z., Kim, H. Y., Bugrim, A., Cunningham, K. W., Nuccitelli, R., Keizer, J. E., Hanley, M. R., Blalock, J. E., and Marchase, R. B. (1999). Calcium influx factor is synthesized by yeast and mammalian cells depleted of organellar calcium stores. *Proc. Natl. Acad. Sci. USA* **96**, 121–126.
- Danilchik, M. V., and Gerhart, J. C. (1987). Differentiation of the animal-vegetal axis in *Xenopus laevis* oocytes. I. Polarized intracellular translocation of platelets establishes the yolk gradient. *Dev. Biol.* **122**, 101–112.
- De Cuevas, M., and Spradling, A. C. (1998). Morphogenesis of the *Drosophila* fusome and its implications for oocyte specification. *Development* **125**, 2781–2789.
- Deguchi, R., Shirakawa, H., Oda, S., Mohri, T., and Miyazaki, S. (2000). Spatiotemporal analysis of Ca²⁺ waves in relation to the sperm entry site and animal-vegetal axis during Ca²⁺ oscillations in fertilized mouse eggs. *Dev. Biol.* **218**, 299–313.
- Deshler, J. O., Highett, M. I., Abramson, T., and Schnapp, B. J. (1998). A highly conserved RNA-binding protein for cytoplasmic mRNA localization in vertebrates. *Curr. Biol.* **8**, 489–496.
- Deshler, J. O., Highett, M. I., and Schnapp, B. J. (1997). Localization of *Xenopus* Vg1 mRNA by Vera protein and the endoplasmic reticulum. *Science* **276**, 1128–1131.
- Drewes, G., Ebneith, A., Preuss, U., Mandelkow, E. M., and Mandelkow, E. (1997). MARK, a novel family of protein kinases that phosphorylate microtubule-associated proteins and trigger microtubule disruption. *Cell* **89**, 297–308.
- Ducibella, T., Duffy, P., and Buetow, J. (1994). Quantification and localization of cortical granules during oogenesis in the mouse. *Biol. Reprod.* **50**, 467–473.
- Ducibella, T., Duffy, P., Reindollar, R., and Su, B. (1990). Changes in the distribution of mouse oocyte cortical granules and ability to undergo the cortical reaction during gonadotropin-stimulated meiotic maturation and aging in vivo. *Biol. Reprod.* **43**, 870–876.
- Dumollard, R., and Sardet, C. (2001). 3 different calcium wave pacemakers in ascidian eggs. *J. Cell Sci.* **114**, 2471–2481.
- Elinson, R. P. (1975). Site of sperm entry and a cortical contraction associated with egg activation in the frog *Rana pipiens*. *Dev. Biol.* **47**, 257–268.
- Elinson, R. P. (1980). The amphibian egg cortex in fertilization and early development. In "The Cell Surface: Mediator in developmental processes." (S. a. W. Subtelny, N.K., Ed.). Academic Press, New York.
- Elinson, R. P., and Houlston, E. (1990). Cytoskeleton in *Xenopus* oocytes and eggs. *Sem. Cell Biol.* **1**, 349–357.
- Elinson, R. P., King, M. L., and Forristall, C. (1993). Isolated vegetal cortex from *Xenopus* oocytes selectively retains localized mRNAs. *Dev. Biol.* **160**, 554–562.
- Ephrussi, A., and Lehman, R. (1992). Induction of germ cell formation by oskar. *Nature* **358**, 387–392.
- Etkin, D. L. (1997). A new face for the endoplasmic reticulum: RNA localization. *Dev. Biol.* **276**, 1092–1093.
- Evans, J. P., Foster, J. A., McAvey, B. A., Gerton, G. L., Kopf, G. S., and Schultz, R. M. (2000). Effects of perturbation of cell polarity on molecular markers of sperm-egg binding sites on mouse eggs. *Biol. Reprod.* **62**, 76–84.

- Fernandez, J., Roegiers, F., Cantillana, V., and Sardet, C. (1998). Formation and localization of cytoplasmic domains in leech and ascidian zygotes. *Int. J. Dev. Biol.* **42**, 1075–1084.
- Fishkind, D. J., Bonder, E. M., and Begg, D. A. (1990a). Subcellular localization of sea urchin egg spectrin: Evidence for assembly of the membrane-skeleton on unique classes of vesicles in eggs and embryos. *Dev. Biol.* **142**, 439–452.
- Fishkind, D. J., Bonder, E. M., and Begg, D. A. (1990b). Sea urchin spectrin in oogenesis and embryogenesis: A multifunctional integrator of membrane-cytoskeletal interactions. *Dev. Biol.* **142**, 453–464.
- Focarelli, R., and Rosati, F. (1995). The 220-kDa vitelline coat glycoprotein mediates sperm binding in the polarized egg of unio elongatulus through O-linked oligosaccharides. *Dev. Biol.* **171**, 606–614.
- Forristall, C., Pondel, M., Chen, L., and King, M. L. (1995). Patterns of localization and cytoskeletal association of two vegetally localized RNAs, Vg1 and Xcat-2. *Development* **121**, 201–8.
- Forster, I. C., Traebert, M., Jankowski, M., Stange, G., Biber, J., and Murer, H. (1999). Protein kinase C activators induce membrane retrieval of type II Na⁺-phosphate cotransporters expressed in *Xenopus* oocytes. *J. Physiol.* **517**, 327–340.
- Gard, D. L. (1991). Organization, nucleation and acetylation of microtubules in *Xenopus laevis* oocytes: A study by confocal immunofluorescence microscopy. *Dev. Biol.* **143**, 346–362.
- Gard, D. L. (1994). γ -tubulin is asymmetrically distributed in the cortex of *Xenopus* oocytes. *Dev. Biol.* **161**, 131–140.
- Gard, D. L. (1995). Axis formation during amphibian oogenesis: Re-evaluating the role of the cytoskeleton. *Curr. Top. Dev. Biol.* **30**, 215–252.
- Gard, D. L. (1999). Confocal microscopy and 3-D reconstruction of the cytoskeleton of *Xenopus* oocytes. *Micros. Res. Techn.* **44**, 388–414.
- Gard, D. L., Affleck, D., and Error, B. M. (1995a). Microtubule organization, acetylation, and nucleation in *Xenopus laevis* oocytes: II. A developmental transition in microtubule organization during early diplotene. *Dev. Biol.* **168**, 189–201.
- Gard, D. L., Cha, B. J., and King, E. (1997). The organization and animal-vegetal asymmetry of cytokeratin filaments in stage VI *Xenopus* oocytes is dependent upon F-actin and microtubules. *Dev. Biol.* **184**, 95–114.
- Gard, D. L., Cha, B. J., and Schroeder, M. M. (1995b). Confocal immunofluorescence microscopy of microtubules, microtubule-associated proteins, and microtubule-organizing centers during amphibian oogenesis and early development. *Curr. Top. Dev. Biol.* **31**, 383–431.
- Gard, D. L., and Klymkowsky, M. W. (1998). Intermediate filament organization during oogenesis and early development in the clawed frog. *Xenopus laevis*. *Subcellular biochemistry: Intermediate filaments* **31**, 35–69.
- Gardiner, D. M., and Grey, R. D. (1983). Membrane junctions in *Xenopus* eggs. Their distribution suggests a role in calcium regulation. *J. Cell Biol.* **96**, 1159–1163.
- Gilkey, J. C., Jaffe, L. F., Ridgway, E. B., and Reynolds, G. T. (1978). A free calcium wave traverses the activating egg of the medaka. *Oryzias latipes*. *J. Cell Biol.* **76**, 448–466.
- Giusti, A. F., Hoang, K. M., and Foltz, K. R. (1997). Surface localization of the sea urchin egg receptor for sperm. *Dev. Biol.* **184**, 10–24.
- Gonzalez-Reyes, A., and St Johnston, D. (1998). The *Drosophila* AP axis is polarised by the cadherin-mediated positioning of the oocyte. *Development* **125**, 3635–3644.
- Grant, B., and Hirsh, D. (1999). Receptor-mediated endocytosis in the *Caenorhabditis elegans* oocyte. *Mol. Biol. Cell* **10**, 4311–4326.
- Greener, T., Grant, B., Zhang, Y., Wu, X., Greene, L. E., Hirsh, D., and Eisenberg, E. (2001). *Caenorhabditis elegans* auxilin: A J-domain protein essential for clathrin-mediated endocytosis in vivo. *Nat. Cell Biol.* **3**, 215–219.
- Grieder, N. C., De Cuevas, M., and Spradling, A. C. (2000). The fusome organizes the microtubule network during oocyte differentiation in *Drosophila*. *Development* **127**, 4253–4264.
- Guerrier, P. (1972). Technique générale pour l'extraction du système périphérique total de l'ovule d'oursin. In "Cahiers de Biologie Marine", **XIII**, 475–477.
- Harvey, E. B. (1956). "The American Arbacia and Other Sea Urchins." Princeton University.
- Havin, L., Git, A., Elisha, Z., Oberman, F., Yaniv, K., Schwartz, S., Standart, N., and Yisraeli, J. (1998). RNA-binding protein conserved in both microtubule- and microfilament-based RNA localization. *Genes Dev.* **12**, 1593–1598.
- Heasman, J., Quarumby, J., and Wylie, C. C. (1984). The mitochondrial cloud of *Xenopus* oocytes: The source of the germinal granule material. *Dev. Biol.* **105**, 458–469.
- Heil-Chapdelaine, R., and Otto, J. J. (1996). Relative changes in F-actin during the first cell cycle: Evidence for two distinct pools of F-actin in the sea urchin egg. *Cell Motil. Cytoskeleton* **34**, 26–35.
- Heil-Chapdelaine, R. A., Adames, N. R., and Cooper, J. A. (1999). Formin the connection between microtubules and the cell cortex. *J. Cell Biol.* **144**, 809–811.
- Henson, J. H., Beaulieu, S. M., Kaminer, B., and Begg, D. A. (1990). Differentiation of a calsequestrin-containing endoplasmic reticulum during sea urchin oogenesis. *Dev. Biol.* **142**, 255–269.
- Heuser, J. (2000). The production of 'cell cortices' for light and electron microscopy. *Traffic* **1**, 545–52.
- Hiramoto, Y. (1970). Rheological properties of sea urchin eggs. *Biorheology* **6**, 201–234.
- Hirano, K. I. (1991). Change in membrane fluidity of sand dollar egg cortices caused by Ca²⁺ induced exocytosis: Microscopic analysis with fluorescence anisotropy. *Dev. Growth Diff.* **33**, 451–458.
- Holman, G. D., and Sandoval, I. V. (2001). Moving the insulin-regulated glucose transporter GLUT4 into and out of storage. *Trends Cell Biol.* **11**, 173–179.
- Holowacz, T., and Elinson, R. P. (1993). Cortical cytoplasm, which induces dorsal axis formation in *Xenopus*, is inactivated by UV irradiation of the oocyte. *Development* **119**, 277–285.
- Holowacz, T., and Elinson, R. P. (1995). Properties of the dorsal activity found in the vegetal cortical cytoplasm of *Xenopus* eggs. *Development* **121**, 2789–2798.
- Houston, D. W., and King, M. L. (2000a). A critical role for Xdzal, a germ plasm-localized RNA, in the differentiation of primordial germ cells in *Xenopus*. *Development* **127**, 447–456.
- Houston, D. W., and King, M. L. (2000b). Germ plasm and molecular determinants of germ cell fate. *Curr. Top. Dev. Biol.* **50**, 155–182.
- Houston, D. W., Zhang, J., Maines, J. Z., Wasserman, S. A., and King, M. L. (1998). A *Xenopus* DAZ-like genes encodes an RNA component of germ plasm and is a functional homologue of *Drosophila* boule. *Development* **125**, 171–180.
- Howley, C., and Ho, R. K. (2000). mRNA localization patterns in zebrafish oocytes. *Mech. Dev.* **92**, 305–309.
- Hudson, C., and Woodland, H. (1998). Xpat, a gene expressed specifically in germ plasm and primordial germ cells of *Xenopus laevis*. *Mech. Dev.* **73**, 159–168.

- Huynh, J. R., Shulman, J. M., Benton, R., and St Johnston, D. (2001). PAR-1 is required for the maintenance of oocyte fate in *Drosophila*. *Development* **128**, 1201–1209.
- Izumi, Y., Hirose, T., Tamai, Y., Hirai, S. I., Nagashima, Y., Fujimoto, T., Tabuse, Y., Kempfues, K. J., and Ohno, S. (1998). An atypical PKC directly associates and colocalizes at the epithelial tight junction with ASIP, a mammalian homologue of *Caenorhabditis elegans* polarity protein PAR-3. *J. Cell Biol.* **143**, 95–106.
- Jaconi, M., Pyle, J., Bortolon, R., Ou, J., and Clapham, D. (1997). Calcium release and influx colocalize to the endoplasmic reticulum. *Curr. Biol.* **7**, 599–602.
- Jaffe, L. A., Giusti, A. F., Carroll, D. J., and Foltz, K. R. (2001). Ca²⁺ signalling during fertilization of echinoderm eggs. *Sem. in Cell & Dev. Biol.* **12**, 45–51.
- Jaffe, L. A., and Terasaki, M. (1994). Structural changes in the endoplasmic reticulum of starfish oocytes during meiotic maturation and fertilization. *Dev. Biol.* **164**, 579–587.
- Jankovics, F., Sinka, R., and Erdelyi, M. (2001). An Interaction Type of Genetic Screen Reveals a Role of the Rab11 Gene in oskar mRNA Localization in the Developing *Drosophila melanogaster* Oocyte. *Genetics* **158**, 1177–1188.
- Jeffery, W. R. (1995). Development and evolution of an egg cytoskeletal domain in ascidians. *Curr. Top. Dev. Biol.* **31**, 243–276.
- Jeffery, W. R., and Capco, D. G. (1978). Differential accumulation and localization of maternal poly(A)-containing RNA during early development of the ascidian *Styela*. *Dev. Biol.* **67**, 152–166.
- Johnson, J., and Capco, D. G. (1997). Progesterone acts through protein kinase C to remodel the cytoplasm as the amphibian oocyte becomes the fertilization-competent egg. *Mech. Dev.* **67**, 215–226.
- Jones, K. T. (1998). Ca²⁺ oscillations in the activation of the egg and development of the embryo in mammals. *Int. J. Dev. Biol.* **42**, 1–10.
- Joseph, E. M., and Melton, D. A. (1998). Mutant Vg1 ligands disrupt endoderm and mesoderm formation in *Xenopus* embryos. *Development* **125**, 2677–2685.
- Kageura, H. (1997). Activation of dorsal development by contact between the cortical dorsal determinant and the equatorial core cytoplasm in eggs of *Xenopus laevis*. *Development* **124**, 1543–1551.
- Kane, R. E., and Stephens, R. E. (1969). A comparative study of the isolation of the cortex and the role of the calcium-insoluble protein in several species of sea urchin egg. *J. Cell. Biol.* **41**, 133–144.
- Kaznacheeva, E., Zubov, A., Nikolaev, A., Alexeenko, V., Bezprozvanny, I., and Mozhayeva, G. N. (2000). Plasma membrane calcium channels in human carcinoma A431 cells are functionally coupled to inositol 1,4,5-trisphosphate receptor-phosphatidylinositol 4,5-bisphosphate complexes. *J. Biol. Chem.* **275**, 4561–4564.
- Kempfues, K. (2000). PARsing embryonic polarity. *Cell* **101**, 345–348.
- King, M. L., Zhou, Y., and Bubunenko, M. (1999). Polarizing genetic information in the egg: RNA localization in the frog oocyte. *BioEssays* **21**, 546–557.
- Kiselyov, K. I., Min Shin, D., Wang, Y., Pessah, I. N., Allen, P. D., and Muallem, S. (2000). Gating of store-operated channels by conformational coupling to ryanodine receptors. *Mol. Cell* **6**, 421–431.
- Kline, D. (2000). Attributes and dynamics of the endoplasmic reticulum in mammalian eggs. *Curr. Top. Dev. Biol.* **50**, 125–153.
- Kline, D., Mehlmann, L., Fox, C., and Terasaki, M. (1999). The cortical endoplasmic reticulum (ER) of the mouse egg: Localization of ER clusters in relation to the generation of repetitive calcium waves. *Dev. Biol.* **215**, 431–442.
- Kloc, M., and Etkin, L. D. (1994). Delocalization of Vg1 mRNA from the vegetal cortex in *Xenopus* oocytes after destruction of Xlsirt RNA. *Science* **265**, 1101–1103.
- Kloc, M., and Etkin, L. D. (1995). Two distinct pathways for the localization of RNAs at the vegetal cortex in *Xenopus* oocytes. *Development* **121**, 287–297.
- Kloc, M., and Etkin, L. D. (1998). Apparent continuity between the messenger transport organizer and late RNA localization pathways during oogenesis in *Xenopus*. *Mech. Dev.* **73**, 95–106.
- Kloc, M., Larabell, C., Chan, A.P.Y., and Etkin, L. D. (1998). Contribution of METRO pathway localized molecules to the organization of the germ cell lineage. *Mech. Dev.* **75**, 81–93.
- Kloc, M., Larabell, C., and Etkin, L. D. (1996). Elaboration of the messenger transport organizer pathway for localization of RNA to the vegetal cortex of *Xenopus* oocytes. *Dev. Biol.* **180**, 119–130.
- Kloc, M., Spohr, G., and Etkin, L. D. (1993). Translocation of repetitive RNA sequences with the germ plasm in *Xenopus* oocytes. *Science* **262**, 1712–1714.
- Klopfenstein, D.R.C., Kappeler, F., and Hauri, H. P. (1998). A novel direct interaction of endoplasmic reticulum with microtubules. *EMBO J.* **17**, 6168–6177.
- Klymkowsky, M. W., Maynell, L. A., and Nislow, C. (1991). Cytokeratin phosphorylation, cytoskeleton filament severing and the solubilization of the maternal mRNA Vg1. *J. Cell Biol.* **114**, 787–797.
- Korinek, W. S., Copeland, M. J., Chaudhuri, A., and Chant, J. (2000). Molecular linkage underlying microtubule orientation toward cortical sites in yeast. *Science* **287**, 2257–2259.
- Ku, M., and Melton, D. A. (1993). *Xwnt-11*: A maternally expressed *Xenopus wnt* gene. *Development* **119**, 1161–1173.
- Kwon, S., and Schnapp, B. J. (2001). RNA localization: SHEDding light on the RNA-motor linkage. *Curr. Biol.* **11**, R166–R168.
- Lane, J., and Allan, V. (1998). Microtubule-based membrane movement. *Biochim. et Biophys. Acta* **1376**, 27–55.
- Lantz, V. A., Clemens, S. E., and Miller, K. G. (1999). The actin cytoskeleton is required for maintenance of posterior pole plasm components in the *Drosophila* embryo. *Mech. Dev.* **85**, 111–122.
- Larabell, C. A. (1993). A new technique for isolation and visualization of the *Xenopus* egg cortex reveals a complex cytoskeleton. *J. Struct. Biol.* **110**, 154–159.
- Larabell, C. A., and Chandler, D. E. (1988). Freeze-fracture analysis of structural reorganization during meiotic maturation in oocytes of *Xenopus laevis*. *Cell Tissue Res.* **251**, 129–136.
- Lasko, P. (1999). RNA sorting in *Drosophila* oocytes and embryos. *Faseb. J.* **13**, 421–433.
- Lee, H. C., and Epel, D. (1983). Changes in intracellular acidic compartments in sea urchin eggs after activation. *Dev. Biol.* **98**, 446–454.
- Lee, L., Tirnauer, J. S., Li, J., Schuyler, S. C., Liu, J. Y., and Pellman, D. (2000). Positioning of the mitotic spindle by a cortical-microtubule capture mechanism. *Science* **287**, 2260–2263.
- LeNaour, F., Rubinstein, E., Jasmin, C., Prenant, M., and Boucheix, C. (2000). Severely reduced female fertility in CD9-deficient mice. *Science* **287**, 319–321.
- Liu, Z., Xie, T., and Steward, R. (1999). Lis1, the *Drosophila* homolog of a human lissencephaly disease gene, is required for germline cell division and oocyte differentiation. *Development* **126**, 4477–4488.

- Longo, F. J. (1985). Fine structure of the mammalian egg cortex. *Am. J. Anatomy* **174**, 303–315.
- Lutz, D. A., Hamaguchi, Y., and Inoue, S. (1988). Micromanipulation studies of the asymmetric positioning of the maturation spindle in *Chaetopterus* sp. oocytes: I. Anchorage of the spindle to the cortex and migration of a displaced spindle. *Cell Motil. Cytoskeleton* **11**, 83–96.
- Mabuchi, I., and Sakai, H. (1972). Cortex protein of sea urchin eggs. I. Its purification and other protein components of the cortex. *Dev. Growth Differ.* **14**, 247–261.
- MacArthur, H., Bubunenko, M., Houston, D. W., and King, M. L. (1999). *Xcat-2* RNA is a translationally sequestered germ plasm component in *Xenopus*. *Mech. Dev.* **84**, 75–88.
- MacArthur, H., Houston, D. W., Bubunenko, M., Mosquera, L., and King, M. L. (2000). *DEADSouth* is a germ plasm specific DEAD-box RNA helicase in *Xenopus* related to eIF4A. *Mech. Dev.* **95**, 291–295.
- Machaca, K., and Haun, S. (2000). Store-operated calcium entry inactivates at the germinal vesicle breakdown stage of *Xenopus* meiosis. *J. Biol. Chem.* **275**, 38710–38715.
- Mahajan-Miklos, S., and Cooley, L. (1994). Intercellular cytoplasm transport during *Drosophila* oogenesis. *Dev. Biol.* **165**, 336–351.
- Mahowald, A. P., and Strassheim, J. M. (1970). Intercellular migration of centrioles in the germarium of *Drosophila melanogaster*. *J. Cell Biol.* **45**, 306–320.
- Mandato, C. A., Benink, H. A., and Bement, W. M. (2000). Microtubule-actomyosin interactions in cortical flow and cytokinesis. *Cell Motil. Cytoskeleton* **45**, 87–92.
- Marikawa, Y., Li, Y., and Elinson, R. P. (1997). Dorsal determinants in the *Xenopus* egg are firmly associated with the vegetal cortex and behave like activators of the Wnt pathway. *Dev. Biol.* **191**, 69–79.
- Maro, B., Johnson, M. H., Webb, M., and Flach, G. (1986). Mechanism of polar body formation in the mouse oocyte: An interaction between the chromosomes, the cytoskeleton and the plasma membrane. *J. Embryol. Exp. Morphol.* **92**, 11–32.
- Masumoto, N., Ikebuchi, Y., Tahara, M., Yokoi, T., Tasaka, K., Miyake, A., and Murata, Y. (1998). Expression of Rab3A in the cortical region in mouse metaphase II eggs. *J. Exp. Zool.* **280**, 91–96.
- Matese, J. C., Black, S., and McClay, D. R. (1997). Regulated exocytosis and sequential construction of the extracellular matrix surrounding the sea urchin zygote. *Dev. Biol.* **186**, 16–26.
- Matova, N., and Cooley, L. (2001). Comparative aspects of animal oogenesis. *Dev. Biol.* **231**, 291–320.
- McDougall, A., and Sardet, C. (1995). Function and characteristics of repetitive calcium waves associated with meiosis. *Curr. Biol.* **5**, 318–328.
- McDougall, A., Shearer, J., and Whitaker, M. (2000). The initiation and propagation of the fertilization wave in sea urchin eggs. *Biol. Cell* **92**, 205–214.
- Megraw, T., and Kaufman, T. C. (2000). The centrosome in *Drosophila* oocyte development. *Curr. Top. Dev. Biol.* **49**, 385–407.
- Mehlman, L. M., Mikoshiba, K., and Kline, D. (1996). Redistribution and increase in cortical inositol 1,4,5-trisphosphate receptors after meiotic maturation of the mouse oocyte. *Dev. Biol.* **180**, 489–498.
- Mehlman, L. M., Terasaki, M., Jaffe, L. A., and Kline, D. (1995). Reorganization of the endoplasmic reticulum during meiotic maturation of the mouse oocyte. *Dev. Biol.* **170**, 607–615.
- Merriam, R. W., Sauterer, R., and Christensen, K. (1983). A subcortical, pigment containing structure in *Xenopus* eggs with contractile properties. *Dev. Biol.* **95**, 437–446.
- Micklem, D. R., Dasgupta, R., Elliott, H., Gegerly, F., Davidson, C., Brand, A., Gonzalez-Reyes, A., and St Johnston, D. (1997). The mago nashi gene is required for the polarisation of the oocyte and the formation of perpendicular axes in *Drosophila*. *Curr. Biol.* **7**, 468–478.
- Miyazaki, A., Kamitsubo, E., and Nemoto, S. I. (2000). Premeiotic aster as a device to anchor the germinal vesicle to the cell surface of the presumptive animal pole in starfish oocytes. *Dev. Biol.* **218**, 161–171.
- Monroy, A., and Baccetti, B. (1975). Morphological changes of the surface of the egg of *Xenopus laevis* in the course of development. I. Fertilization and early cleavage. *J. Ultrastr. Res.* **50**, 131–142.
- Mosquera, L., Forristall, C., Zhou, Y., and King, M. L. (1993). An mRNA localized to the vegetal cortex of *Xenopus* oocytes encodes a protein with a nanos-like zinc finger domain. *Development* **117**, 377–386.
- Mowry, K. L., and Cote, C. A. (1999). RNA sorting in *Xenopus* oocytes and embryos. *Faseb J.* **13**, 435–445.
- Murray, G., Reed, C., Marsden, M., Rise, M., Wang, D., and Burke, R. D. (2000). The $\alpha\beta\beta\text{C}$ integrin is expressed on the surface of the sea urchin egg and removed at fertilization. *Dev. Biol.* **227**, 633–647.
- Nakaya, M., Fukui, A., Izumi, Y., Akimoto, K., Asashima, M., and Ohno, S. (2000). Meiotic maturation induces animal-vegetal asymmetric distribution of aPKC and ASIP/PAR-3 in *Xenopus* oocytes. *Development* **127**, 5021–5031.
- Navarro, C., Lehmann, R., and Morris, J. (2001). Oogenesis: Setting one sister above the rest. *Curr. Biol.* **11**, R162–R165.
- Nebreda, A. R., and Ferby, I. (2000). Regulation of the meiotic cell cycle in oocytes. *Curr. Opin. Cell Biol.* **12**, 666–675.
- Nishida, H., and Makabe, K. W. (1999). Maternal information and localized maternal mRNAs in eggs and early embryos of the ascidian *Halocynthia roretzi*. *Invert. Reprod. Dev.* **36**, 41–49.
- Nishida, H., and Sawada, K. (2001). macho-1 encodes a localized mRNA in ascidian eggs that specifies muscle fate during embryogenesis. *Nature* **409**, 724–729.
- Nuccitelli, R., and Ferguson, J. E. (1994). Fertilization and ion channels. *Science* **263**, 988.
- O'Connell, K. F., Maxwell, K. N., and White, J. G. (2000). The *spd-2* gene is required for polarization of the anteroposterior axis and formation of the sperm asters in the *Caenorhabditis elegans* zygote. *Dev. Biol.* **222**, 55–70.
- Oda, S., Deguchi, R., Mohiri, T., Shikano, T., Nakanishi, S., and Miyazaki, S. (1999). Spatiotemporal dynamics of the $(\text{Ca}^{2+})_i$ rise induced by microinjection of sperm extract into mouse eggs: Preferential induction of a Ca^{2+} wave from the cortex mediated by the inositol 1,4,5-trisphosphate receptor. *Dev. Biol.* **209**, 172–185.
- Ohlendieck, K., and Lennarz, W. J. (1995). Role of the sea urchin egg receptor for sperm in gamete interactions. *Trends Biochem. Sci.* **20**, 29–33.
- Ohno, Shigeo (2001). Intercellular junctions and cellular polarity: The PAR-aPKC complex, a conserved core cassette playing fundamental roles in cell polarity. *Curr. Opin. Cell Biol.* **13**, 641–648.
- Ohta, K., Sato, C., Matsuda, T., Toriyama, V., Vacquier, V. D., Hirohashi, N., Lennarz, W. J., and Kitajima, K. (2000). Lipid raft

- on gametic cells as a functional domain for sperm-egg interaction coupled with signal transduction. *Zygote* **8**, S63.
- Otto, J. J., and Schroeder, T. E. (1984). Assembly-disassembly of actin bundles in starfish oocytes: An analysis of actin-associated proteins in the isolated cortex. *Dev. Biol.* **101**, 263–273.
- Pannese, M., Cagliani, R., Pardini, C. L., and Bocinelli, E. (2000). Xotxl maternal transcripts are vegetally localized in *Xenopus laevis* oocytes. *Mech. Dev.* **90**, 111–114.
- Paré, C., and Suter, B. (2000). Subcellular localization of Bic-D:GFP is linked to an asymmetric oocyte nucleus. *J. Cell Sci.* **113**, 2119–2127.
- Peters, R., Sardet, C., and Richter, H. P. (1984). Mobility of membrane lipids and proteins at the animal and vegetal poles of the sea urchin egg. *Dev. Growth Differ.* **26**, 105–110.
- Pfeiffer, D. C., and Gard, D. L. (1999). Microtubules in *Xenopus* oocytes are oriented with their minus-ends towards the cortex. *Cell Motil. Cytoskeleton* **44**, 34–43.
- Raven, C. P. (1970). The cortical and subcortical cytoplasm of the *Lymnaea* egg. *Int. Rev. Cytol.* **28**, 1–44.
- Rebagliati, M. R., Weeks, D. L., Harvey, R. P., and Melton, D. A. (1985). Identification and cloning of localized maternal RNAs from *Xenopus* eggs. *Cell* **42**, 769–777.
- Riechmann, V., and Ephrussi, A. (2001). Axis formation during *Drosophila* oogenesis. *Curr. Opin. Genet. Dev.* **11**, 374–383.
- Riparbelli, M. G., and Callaini, G. (1995). Cytoskeleton of the *Drosophila* egg chamber: New observations of microfilament distribution during oocyte growth. *Cell Motil. Cytoskeleton* **31**, 298–306.
- Roegiers, F., Djediat, C., Dumollard, R., Rouviere, C., and Sardet, C. (1999). Phases of cytoplasmic and cortical reorganizations of the ascidian zygote between fertilization and first division. *Development* **126**, 3101–3117.
- Roegiers, F., and Jan, Y. N. (2000). Staufen: A common component of mRNA transport in oocytes and neurons? *Trends Cell Biol.* **10**, 220–224.
- Roegiers, F., McDougall, A., and Sardet, C. (1995). The sperm entry point defines the orientation of the calcium-induced contraction wave that directs the first phase of cytoplasmic reorganization in the ascidian egg. *Development* **121**, 3457–3466.
- Romancino, D. P., and Di Carlo, M. (1999). Asymmetrical localization and segregation of *Paracentrotus lividus* Bep4 maternal protein. *Mech. Dev.* **87**, 3–9.
- Romancino, D. P., Montana, G., Dalmazio, S., and Di Carlo, M. (2001). Bep4 protein is involved in patterning along the animal-vegetal axis in the *Paracentrotus lividus* embryo. *Dev. Biol.* **234**, 107–119.
- Romancino, D. P., Montana, G., and Di Carlo, M. (1998). Involvement of the cytoskeleton in localization of *Paracentrotus lividus* maternal BEP mRNAs and proteins. *Exp. Cell Res.* **238**, 101–109.
- Roth, S., Neuman-Silberberg, F. S., Barcelo, G., and Schüpbach, T. (1995). Cornichon and the EGF receptor signalling process are necessary for both anterior-posterior and dorsal-ventral pattern formation in *drosophila*. *Cell* **81**, 967–968.
- Roth, T. F., and Porter, K. R. (1964). Yolk protein uptake in the oocyte of the mosquito *Aedes aegypti* L. *J. Cell Biol.* **20**, 313–332.
- Ruden, D. M., Sollars, V., Wang, X., Mori, D., Alterman, M., and Lu, X. (2000). Membrane fusion proteins are required for oskar mRNA localization in the *Drosophila* egg chamber. *Dev. Biol.* **218**, 314–325.
- Saffman, E. E., and Lasko, P. (1999). Germline development in vertebrates and invertebrates. *Cell Mol. Life Sci.* **55**, 1141–1163.
- Sakai, H. (1960). Studies on sulfhydryl groups during cell division of sea urchin egg. *J. Biophys. Biochem. Cytol.* **8**, 603–607.
- Sardet, C. (1984). The ultrastructure of the sea urchin egg cortex isolated before and after fertilization. *Dev. Biol.* **105**, 196–210.
- Sardet, C., and Chang, P. (1985). A marker of animal-vegetal polarity in the egg of the sea urchin cortex isolated before and after fertilization. *Dev. Biol.* **105**, 196–210.
- Sardet, C., and Chang, P. (1987). The egg cortex: From maturation through fertilization. *Cell Differ.* **21**, 1–19.
- Sardet, C., McDougall, A., and Houlston, E. (1994). Cytoplasmic domains in egg. *Trends Cell Biol.* **4**, 166–171.
- Sardet, C., Roegiers, F., Dumollard, R., Rouviere, C., and McDougall, A. (1998). Calcium waves and oscillations in eggs. *Biophys. Chem.* **72**, 131–140.
- Sardet, C., Speksnijder, J. E., Inoue, S., and Jaffe, L. F. (1989). Fertilization and ooplasmic movements in the ascidian egg. *Development* **105**, 237–249.
- Sardet, C., Speksnijder, J. E., Terasaki, M., and Chang, P. (1992). Polarity of the ascidian egg cortex before fertilization. *Development* **115**, 221–237.
- Sasakura, Y., Ogasawara, M., and Makabe, K. W. (2000). Two pathways of maternal RNA localization at the posterior-vegetal cytoplasm in early ascidian embryo. *Dev. Biol.* **220**, 365–378.
- Savage, R., and Danilchik, M. (1993). Dynamics of germ plasm localization and its inhibition by ultraviolet irradiation in early cleavage *Xenopus* embryo. *Dev. Biol.* **157**, 371–382.
- Schatten, G., and Mazia, D. (1976). The penetration of the spermatozoon through the sea urchin egg surface at fertilization. *Exp. Cell Res.* **98**, 325–337.
- Schnorrer, F., Bohmann, K., and Nüsslein-Volhard, C. (2000). The molecular motor dynein is involved in targeting Swallow and bicoid RNA to the anterior pole of *Drosophila* oocytes. *Nature Cell Biol.* **2**, 185–190.
- Schonbaum, C. P., Perrino, J. J., and Mahowald, A. P. (2000). Regulation of the vitellogenin receptor during *Drosophila melanogaster* oogenesis. *Mol. Biol. Cell* **11**, 511–521.
- Schroeder, T. E. (1981). Interrelations between the cell surface and the cytoskeleton in cleaving sea urchin eggs. In “Cytoskeletal Elements and Plasma Membrane Organization.” (G. N. Poste, G.L., Ed.), Vol. 5, pp. 168–215. Elsevier, North-Holland Biomedical Press.
- Schroeder, T. E., and Otto, J. J. (1991). Snoods: A periodic network containing cytokeratin in the cortex of starfish oocytes. *Dev. Biol.* **144**, 240–247.
- Segal, M., and Bloom, K. (2001). Control of spindle polarity and orientation in *Saccharomyces cerevisiae*. *Trends Cell Biol.* **11**, 160–166.
- Shimizu, T. (1985). Movements of mitochondria associated with isolated egg cortex. *Dev. Growth Differ.* **27**, 149–154.
- Shimizu, T. (1988). Localization of actin networks during early development of *Tubifex* embryos. *Dev. Biol.* **125**, 321–331.
- Shimizu, T. (1990). Polar body formation in *Tubifex* eggs. *Ann. NY Acad. Sci.* **582**, 260–272.
- Shimizu, T. (1995). Role of the cytoskeleton in the generation of spatial patterns in *Tubifex* eggs. *Curr. Top. Dev. Biol.* **31**, 197–235.
- Shiraishi, K., Okada, A., Shirakawa, H., Nakanishi, S., Mikoshiba, K., and Miyazaki, S. (1995). Developmental changes in the distribution of the endoplasmic reticulum and inositol 1,4,5-trisphosphate receptors and the spatial pattern of Ca²⁺ release during maturation of hamster oocytes. *Dev. Biol.* **170**, 594–606.

- Shulman, J. M., Benton, R., and St Johnston, D. (2000). The *Drosophila* homolog of *C. elegans* PAR-1 organizes the oocyte cytoskeleton and directs oskar mRNA localization to the posterior pole. *Cell* **101**, 377–388.
- Sider, J. R., Mandato, C. A., Weber, K. L., Zandy, A. J., Beach, D., Finst, R. J., Skoble, J., and Bement, W. M. (1999). Direct observation of microtubule-f-actin interaction in cell free lysates. *J. Cell Sci.* **112**, 1947–1956.
- Simerly, C., Nowak, G., de Lanerolle, P., and Schatten, G. (1998). Differential expression and functions of cortical myosin IIA and IIB isotypes during meiotic maturation, fertilization, and mitosis in mouse oocytes and embryos. *Mol. Biol. Cell* **9**, 2509–2525.
- Simons, K., and Toomre, D. (2000). Lipid Rafts, and Signal Transduction. *Nat. Rev. Mol. Cell Biol.* **1**, 31–39.
- Singer-Lahat, D., Dascal, N., Mittelman, L., Peleg, S., and Lotan, I. (2000). Imaging plasma membrane proteins in large membrane patches of *Xenopus* oocytes. *Eur. J. Physiol.* **440**, 627–633.
- Smith, R. M., Baibakov, B., Ikebuchi, Y., White, B. H., Lambert, N. A., Kaczmarek, L. K., and Vogel, S. S. (2000). Exocytosis insertion of calcium channels constrains compensatory endocytosis to sites of exocytosis. *J. Cell Biol.* **148**, 755–767.
- Speksnijder, J. E., de Jong, K., Linnemans, W. A., and Dohmen, M. R. (1986). The ultrastructural organization of the isolated cortex of a molluscan egg. *Prog. Clin. Biol. Res.* **217B**, 353–356.
- Speksnijder, J. E., Jaffe, L. F., and Sardet, C. (1989). Polarity of sperm entry in the ascidian egg. *Dev. Biol.* **133**, 180–184.
- Speksnijder, J. E., Sardet, C., and Jaffe, L. F. (1990a). Periodic calcium waves cross ascidian eggs after fertilization. *Dev. Biol.* **142**, 246–2469.
- Speksnijder, J. E., Sardet, C., and Jaffe, L. F. (1990b). The activation wave of calcium in the ascidian egg and its role in ooplasmic segregation. *J. Cell Biol.* **110**, 1589–1598.
- Speksnijder, J. E., Terasaki, M., Hage, W. J., Jaffe, L. F., and Sardet, C. (1993). Polarity and reorganization of the endoplasmic reticulum during fertilization and ooplasmic segregation in the ascidian egg. *J. Cell Biol.* **120**, 1337–1346.
- Stricker, S. A. (1999). Comparative biology of calcium signaling during fertilization and egg activation in animals. *Dev. Biol.* **211**, 157–176.
- Stricker, S. A., Silva, R., and Smythe, T. (1998). Calcium and endoplasmic reticulum dynamics during oocyte maturation and fertilization in the marine worm *Cerebratulus lacteus*. *Dev. Biol.* **203**, 305–322.
- Subramaniam, K., and Seydoux, G. (1999). nos-1 and nos-2, two genes related to *Drosophila* nanos, regulate primordial germ cell development and survival in *Caenorhabditis elegans*. *Development* **126**, 4861–4871.
- Suzuki, H., Maegawa, S., Nishibu, T., Sugiyama, T., Yasuda, K., and Inoue, K. (2000). Vegetal localization of the maternal mRNA encoding an EDEN-BP/Bruno-like protein in zebrafish. *Mech. Dev.* **93**, 205–209.
- Suzuki, K., Tanaka, Y., Nakajima, Y., Hirano, K., Itoh, H., Miyata, H., Hayakawa, T., and Kinoshita, K. J. (1995). Spatiotemporal relationships among early events of fertilization in sea urchin eggs revealed by multiview microscopy. *Biophys. J.* **68**, 739–748.
- Swalla, B. J., Badgett, M. R., and Jeffery, W. R. (1991). Identification of a cytoskeletal protein localized in the myoplasm of ascidian eggs: Localization is modified during anural development. *Development* **111**, 425–436.
- Swan, A., Nguyen, T., and Suter, B. (1999). *Drosophila* Lissencephaly-1 functions with Bic-D and dynein in oocyte determination and nuclear positioning. *Nature Cell Biol.* **1**, 444–449.
- Swann, K., and Parrington, J. (1999). Mechanism of Ca²⁺ release at fertilization in mammals. *J. Exp. Zool.* **285**, 267–275.
- Tabb, J. S., Molyneaux, B. J., Cohen, D. L., Kuznetsov, S. A., and Langford, G. M. (1998). Transport of ER vesicles on actin filaments in neurons by myosin V. *J. Cell Sci.* **111**, 3221–3234.
- Taunton, J., Rowning, B. A., Coughlin, M. L., Wu, M., Moon, R. T., Mitchison, T. J., and Larabell, C. A. (2000). Actin-dependent propulsion of endosomes and lysosomes by recruitment of N-WASP. *J. Cell Biol.* **148**, 519–530.
- Terasaki, A. G., Ohnuma, M., and Mabuchi, I. (1997). Identification of actin-binding proteins from sea urchin eggs by F-actin affinity column chromatography. *J. Biochem.* **122**, 226–236.
- Terasaki, M. (1996). Actin filament translocations in sea urchin eggs. *Cell Motil. Cytoskeleton* **34**, 48–56.
- Terasaki, M., and Jaffe, L. A. (1991). Organization of the sea urchin egg endoplasmic reticulum and its reorganization at fertilization. *J. Cell Biol.* **114**, 929–940.
- Terasaki, M., Runft, L. L., and Hand, A. R. (2001). Changes in organization of the endoplasmic reticulum during *Xenopus* oocyte maturation and activation. *Mol. Biol. Cell* **12**, 1103–1116.
- Terasaki, M., and Sardet, C. (1991). Demonstration of calcium uptake and release by sea urchin egg cortical endoplasmic reticulum. *J. Cell Biol.* **115**, 1031–1037.
- Theurkauf, W. E. (1997). Oocyte differentiation: A motor makes a difference. *Curr. Biol.* **7**, R548–R551.
- Theurkauf, W. E., and Hazelrigg, T. I. (1998). *In vivo* analysis of cytoplasmic transport and cytoskeletal organization during *Drosophila* oogenesis: Characterization of a multi-step anterior localization pathway. *Development* **125**, 3655–3666.
- Tilney, L. G., and Jaffe, L. A. (1980). Actin, microvilli, and the fertilization cone of sea urchin eggs. *J. Cell Biol.* **87**, 771–82.
- Tomancak, P., Piano, F., Reichmann, V., Gunsalus, K. C., Kempthues, K. J., and Ephrussi, A. (2000). A *Drosophila melanogaster* homologue of *Caenorhabditis elegans* par-1 acts at an early step in embryonic-axis formation. *Nature Cell Biol.* **2**, 458–460.
- Vacquier, V. D. (1975). The isolation of intact cortical granules from sea urchin eggs: Calcium ions trigger granule discharge. *Dev. Biol.* **43**, 62–74.
- Vacquier, V. D. (1981). Dynamic changes of the egg cortex. *Dev. Biol.* **84**, 1–26.
- Vacquier, V. D. (1998). Evolution of gamete recognition proteins. *Science* **281**, 1995–1998.
- Vallee, R. B., Tai, C. Y., and Faulker, N. E. (2001). LIS1: Cellular function of a disease-causing gene. *Trends Cell Biol.* **11**, 155–160.
- Van Eeden, F., and St Johnston, D. (1999). The polarisation of the anterior-posterior and dorsal-ventral axes during *Drosophila* oogenesis. *Curr. Opin. Genet. Dev.* **9**, 396–404.
- Verlhac, M. H., Lefebvre, C., Guillaud, P., Rassiner, P., and Maro, B. (2000). Asymmetric division in mouse oocytes: With or without Mos. *Curr. Biol.* **10**, 1303–1306.
- Vogel, S. S., Smith, R. M., Baibakov, B., Ikebuchi, Y., and Lambert, N. A. (1999). Calcium influx is required for endocytotic membrane retrieval. *Proc. Natl. Acad. Sci.* **96**, 5019–5024.
- Walker, G. R., Kane, R., and Burgess, D. R. (1994). Isolation and characterization of a sea urchin zygote cortex that supports *in vitro* contraction and reactivation of furrowing. *J. Cell Sci.* **107**, 2239–2248.
- Wassarman, P. M., Jovine, L., and Litscher, E. S. (2001). A profile of fertilization in mammals. *Nature Cell Biol.* **3**, E59–E64.

- Waterman-Storer, C. M., and Salmon, E. D. (1999). Positive feedback interactions between microtubule and actin dynamics during cell motility. *Curr. Opin. Cell Biol.* **11**, 61–67.
- Wheatley, S., Kulkarni, S., and Karsenti, R. (1995). *Drosophila* nonmuscle myosin II is required for rapid cytoplasmic transport during oogenesis and for axial nuclear migration in early embryos. *Development* **121**, 1937–1946.
- Whitaker, M. (1996). Control of meiotic arrest. *J. Reprod. Fert.* **1**, 127–135.
- Wickham, L., Duchaine, T., Luo, M., Nabi, I. R., and DesGroseillers, L. (1999). Mammalian stau68 is a double-stranded-RNA-and tubulin-binding protein which localizes to the rough endoplasmic reticulum. *Mol. Cell Biol.* **19**, 2220–2230.
- Wilsch-Brauninger, M., Schwarz, H., and Nüsslein-Volhard, C. (1997). A sponge-like structure involved in the association and transport of maternal products during *Drosophila* oogenesis. *J. Cell Biol.* **139**, 817–829.
- Wilson, N. F., and Snell, W. J. (1998). Microvilli and cell-cell fusion during fertilization. *Trends Cell Biol.* **8**, 93–96.
- Wong, G. K., Allen, P. G., and Begg, D. A. (1997). Dynamics of filamentous actin organization in the sea urchin egg cortex during early cleavage divisions: Implications for the mechanism of cytokinesis. *Cell Motil. Cytoskeleton* **36**, 30–42.
- Wylie, C. (1999). Germ cells. *Cell* **96**, 165–174.
- Yao, Y., Ferrer-Montiel, A. V., Montal, M., and Tsien, R. Y. (1999). Activation of store-operated Ca²⁺ current in *Xenopus* oocytes requires SNAP-25 but not a diffusible messenger. *Cell* **98**, 475–485.
- Zampighi, G. A., Loo, D.D.F., Kreman, M., Eskandari, S., and Wright, E. M. (1999). Functional and morphological correlates of connexin50 expressed in *Xenopus laevis* oocytes. *J. Gen. Physiol.* **113**, 507–523.
- Zhang, J., Houston, D. W., King, M. L., Payne, C., Wylie, C., and Heasman, J. (1998). The role of maternal VegT in establishing the primary germ layers in *Xenopus* embryos. *Cell* **94**, 515–524.
- Zhang, J., and King, M. L. (1996). *Xenopus* VegT RNA is localized to the vegetal cortex during oogenesis and encodes a novel T-box transcription factor involved in mesoderm patterning. *Development* **122**, 4119–4129.
- Zhang, Q., Yaniv, K., Oberman, F., Wolke, U., Git, A., Fromer, M., Taylor, W. L., Meyer, D., Standart, N., Raz, E., and Yisraeli, J. K. (1999). Vg1 RBP intracellular distribution and evolutionarily conserved expression at multiple stages during development. *Mech. Dev.* **88**, 101–106.
- Zimmerberg, J., Coorsen, J. R., Vogel, S. S., and Blank, P. S. (1999). Sea urchin egg preparations as systems for the study of calcium-triggered exocytosis. *J. Physiol.* **520 Pt 1**, 15–21.
- Zimmerberg, J., Sardet, C., and Epel, D. (1985). Exocytosis of sea urchin egg cortical vesicles *in vitro* is retarded by hyperosmotic sucrose: Kinetics of fusion monitored by quantitative light-scattering microscopy. *J. Cell Biol.* **101**, 2398–2410.

Received for publication May 18, 2001

Revised September 8, 2001

Accepted September 8, 2001

Published online November 28, 2001