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Organization of Cytokeratin Cytoskeleton and Germ Plasm in the Vegetal Cortex of *Xenopus Laevis* Oocytes Depends on Coding and Non-Coding RNAs: Three-Dimensional and Ultrastructural Analysis

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Abstract

Recent studies discovered a novel structural role of RNA in maintaining the integrity of the mitotic spindle and cellular cytoskeleton. In *Xenopus laevis*, noncoding Xlsirts and coding VegT RNAs play a structural role in anchoring localized RNAs, maintaining the organization of the cytoskeleton and germinal granules in the oocyte vegetal cortex, and subsequent development of the germline in the embryo. We studied the ultrastructural effects of antisense oligonucleotide driven ablation of Xlsirts and VegT RNAs on the organization of the cytoskeleton, germ plasm and other components of the vegetal cortex. We developed a novel method to immunolabel and visualize cytoskeleton at the electron microscopy level, which allowed us to reconstruct the ultrastructural organization of the cytoskeleton network relative to the components of the vegetal cortex in *Xenopus* oocytes. The removal of Xlsirts and VegT RNAs not only disrupts the cytoskeleton but also has a profound transcript-specific effect on the anchoring and distribution of germ plasm islands and their germinal granules and the arrangement of yolk platelets within the vegetal cortex. We suggest that the cytoskeleton plays a role in anchoring of germ plasm islands within the vegetal cortex and germinal granules within the germ plasm islands.

Keywords

structural RNA; *Xenopus*; cytoskeleton; germ plasm; localized RNA

Introduction

Localized RNAs play an important role in the establishment of molecular asymmetry in somatic cells, germ cells and developing embryos in various systems (1-6). In many organisms the formation of oocyte polarity, early stages of embryogenesis and patterning of the embryo rely

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on the proper spatial and temporal distribution of maternal localized RNAs produced during oogenesis (1-4,7-9). In addition, various RNAs localized to the germ/pole plasm are components of germinal or polar granules and serve as germ cell determinants involved in the specification of the germ cell fate (1,3,10-14). Recently, RNAs were also shown to play a novel, structural role in the organization of mitotic spindle in HeLa cells and *Xenopus* egg extracts (15) and in the organization of cytoskeleton in *Xenopus* oocytes (16). In *Xenopus* oocytes, which are polarized along an animal/vegetal axis (3,17), the localized, noncoding RNA *Xlsirts* and coding mRNA *VegT* play a structural role in the organization of the cytokeratin cytoskeleton in the oocyte vegetal cortex. Destruction of these RNAs causes the disruption of cytokeratin in a transcript-specific manner, which results in the release of several different RNAs from the vegetal cortex (16,18). In addition, destruction of *VegT* mRNA interferes with the proper organization of germinal granules and the development of the germline in the embryo (16). It was observed that in *VegT* mRNA-depleted oocytes and embryos the germinal granules fuse and coalesce into large aggregates (16) but mechanism for this transformation remains, thus far, unknown.

The vegetal cortex of *Xenopus* oocytes and eggs is an extremely complex spatial and temporal arrangement of molecules, cytoskeleton and subcellular structures that is built on and shaped during the long process of oogenesis, and is the repository for spatiotemporal developmental cues indispensable for the proper development of the embryo (19,20). The vegetal cortex contains elements of cytoskeleton such as microtubules, actin and cytokeratin filaments (which anchor various localized RNAs), cortical granules, yolk platelets and germ plasm islands containing germ line mitochondria and germinal granules (17,19-29). Although there is a substantial volume of data on the ultrastructure of the vegetal cortex in *Xenopus* oocytes, and on the changes occurring in the cortex during oocyte maturation and egg activation (19,30, 31; reviewed in 20), the information on the organization of the cytokeratin cytoskeleton in *Xenopus* oocytes and eggs comes mainly from light and confocal microscopy (17,19,24,32; reviewed in 20) and so far, there is no detailed information on the ultrastructural organization of the cytokeratin in relation to other components of the cortex such as the yolk, cortical granules and germ plasm. We developed an electron microscopy method to immunostain cytokeratin filaments that allowed us to visualize the ultrastructural distribution of cytokeratin and its relation to various components of cytoskeleton in stage VI oocytes, mature oocytes and activated eggs. We also studied the effect of ablation of *Xlsirts* and *VegT* RNAs on the ultrastructure and organization of the cytokeratin, germ plasm and other components of the vegetal cortex. We found that the presence of these RNAs is necessary for the proper organization and spatial distribution of the components of the vegetal cortex, and the removal of these RNAs not only disrupts the cytokeratin cytoskeleton but also has a profound effect on the arrangement of yolk and the anchoring and distribution of germ plasm islands within the vegetal cortex and the distribution of germinal granules within the germ plasm islands, which are probable cause of abnormalities observed in the germline development of resulting embryos.

Material and Methods

Antisense oligonucleotide injection and cytokeratin staining for light and confocal microscopy

Xenopus laevis stage VI oocytes, mature oocytes and activated eggs were fixed in 100% methanol overnight at -20°C and stained with anti-cytokeratin C11-FITC conjugated antibody (Sigma-Aldrich, St. Louis, MO) as described previously (16). Experimental oocytes were injected with antisense deoxynucleotides against *Xlsirts* and *VegT* RNA exactly as described previously (16)

Whole-mount immunostaining of cytokeratin for electron microscopy

Stage VI oocytes, mature oocytes and activated eggs were fixed in 1 or 4% formaldehyde in 100% methanol overnight at -20°C . We found that the cytokeratin epitope and ultrastructure preservation of components of the oocyte cortex were identical in samples fixed formaldehyde which concentration ranged from 1 to 4%. The next day, samples were rehydrated with the decreased concentration of ethanol, washed 2×5 min with phosphate-buffered saline (PBS) containing 0.05% Tween 20 (Bio-Rad Laboratories, Hercules, CA), incubated in PBS-0.25% Triton X100 for 10 min, and washed 2×5 min with PBS-0.05% Tween 20. Next, they were blocked for 6 hr in caseine blocking buffer (BioRad) with 0.05% Tween 20 at room temperature. Subsequently samples were incubated with anti-cytokeratin C11 monoclonal antibody (Sigma-Aldrich) at 1:50 dilution, overnight at 4°C . The next day, samples were washed for several hours with PBS- 0.05% Tween 20, and incubated overnight at 4°C with anti-mouse antibody conjugated with 1.4nm gold (Nanoprobes, Yaphank, NY) diluted at 1:50 with blocking buffer. The next day, samples were washed 3×15 min with PBS-0.05% Tween 20, post-fixed for 20 min in 1% glutaraldehyde in PBS-0.05% Tween 20, washed 3×15 min in water, silver enhanced and processed for electron microscopy without the osmium tetroxide treatment, as described previously (13).

Embedding and sectioning

Whole mount immunostained oocytes and eggs were contrasted with 0.5% uranyl acetate only (without osmium tetroxide) and embedded in Epon. Semi-thin ($0.7\mu\text{m}$) serial sections were stained lightly with 1% toluidine blue diluted in 1% borax and examined and photographed under a Leica DMR microscope (Leica Microsystems, Wetzlar, Germany) equipped with Nomarski optics (DIC). Thin (70-100nm) serial sections were examined and photographed in JEOL1200 transmission electron microscope. The images of semi-thin and thin serial sections were used for three- dimensional (3-D) reconstruction and analysis.

Three-dimensional reconstruction and analysis

Two groups of three unaligned TIFF image sets (3 sets containing ten semi-thin sections each, and 3 sets containing eight thin sections each) were processed using the scientific visualization software Amira (tgs.com). Typical image datasets were $1000 \times 1000 \times 10$, with a density range of 0-255 represented as unsigned bytes. The first step of processing was to align the images manually using the AlignSlices module; this resulted in three volumes. The second step of processing was to manually identify the cytokeratin, yolk, germ plasm, and cortical granules, using the segmentation editor. A Wacom tablet and a ShuttlePro control surface facilitated dissection. The third step of processing was the rescaling of the Z-axis to approximate spherical shapes of the yolk platelets. The fourth step of processing was the re-sampling of the datasets into volumes of $256 \times 256 \times 100$. The fifth step of processing was to apply 2D/3D image filters to the individual dissected volumes. The dissected features were sequentially processed using a float conversion, XY unsharp, 3D unsharp, 3D median, and XY histogram filters. The final step of processing was to generate, integrate and display the isosurfaces in Amira. Original aligned 2D images were used to select the appropriate isosurface threshold.

Results

In *Xenopus*, the vegetal cortex of fully-grown stage VI oocyte contains a geodesic network of cytokeratin filaments (16,32). The cytokeratin network disassembles into small cytokeratin foci during, natural or artificial, progesterone- induced oocyte maturation, and reconstitutes upon natural or artificial activation by sperm or pricking (17,32). We showed previously that the organization and maintenance of the cytokeratin network in oocytes, and its reconstitution upon egg activation, depend on the presence of vegetally localized, noncoding Xlirts and coding VegT RNAs (16).

Ultrastructural analysis of cytokeratin filament distribution in relation to the components of the vegetal cortex in stage VI oocytes

We developed an electron microscopy method to immunostain cytokeratin filaments with nanogold and silver enhancement that allowed us to visualize the ultrastructural distribution of cytokeratin and its relation to various components of the vegetal cortex. We fixed oocytes in methanol that, because it preserves cytokeratin immunogenicity extremely well, is commonly used for immunostaining of cytokeratin for light and confocal microscopy (21), but is not a fixative of choice for electron microscopy and ultrastructural analysis. Although the preservation of the ultrastructure in methanol fixed oocytes was compromised to a certain degree, the addition of formaldehyde to the fixative resulted in the ultrastructure preservation that was still good enough to recognize various components of the oocyte cortex (judging from the proper preservation of layering of the cortical components, ultrastructure of mitochondria, germ plasm islands and germinal granules) and was excellent for the visualization of the cytokeratin organization and its relation to various organelles. Because, in our method, the use of silver enhancement (necessary to visualize nanogold) precluded the use of osmium tetroxide (applied in conventional electron microscopy to properly contrast the subcellular structures), the electron microscopy images presented in this study have much lower contrast than usual electron microscopy images.

Three-dimensional reconstruction from semi-thin sections of control and AS Xlirts and VegT ODN-ablated oocytes showed that these RNAs were not only needed for the maintenance of cytokeratin network at the oocyte cortex but also for the maintenance of cytokeratin organization away from the cortex, within the yolk-filled vegetal cytoplasm (Fig. 1). In control oocytes long cytokeratin filaments were not only present in the vegetal cortex but also dispersed between the yolk of vegetal cytoplasm (Fig. 1A). In oocytes injected with As VegT ODN these filaments were not visible (Fig. 1B), and in AS Xlirts ODN-injected oocytes they were much shorter than in control (Fig. 1C).

Analysis of the general ultrastructure and distribution of the components of the vegetal cortex of stage VI oocytes showed that they were distributed in very orderly fashion with a layer of cortical granules followed by a layer of small germ plasm islands (separated from each other) and a layer of yolk-filled cytoplasm (Fig. 2A). A network of immunogold-stained long filaments of cytokeratin was visible more internal to the cortical granules (Fig. 2B). Cytokeratin filaments were visible in close contact with, and also penetrating, the germ plasm islands (Fig. 2C-E). In addition, the small foci of cytokeratin were occasionally visible in the vicinity of cortical granules (Fig. 2B).

Ultrastructural analysis of the effect of Xlirts and VegT RNAs ablation on the cytokeratin filament distribution in relation to the components of vegetal cortex in stage VI oocytes

In oocytes injected with AS VegT ODN, and incubated overnight, cytokeratin filaments fragmented, and the short cytokeratin filaments were visible in contact with the surface and the interior of germ plasm islands (Fig. 3A, B). In addition there was a large subpopulation of very short fragments and granular foci, which surrounded the cortical granules (Fig. 3A, B and Fig. 7H). The analysis of the distribution of the germ plasm islands in the cortex of VegT-ablated oocytes showed very conspicuous aggregation of individual islands into larger aggregates (Fig. 3A). In oocytes injected with AS Xlirts ODN, long cytokeratin filaments formed a well-organized network, which was situated mainly below the germ plasm islands (Fig. 3C). The germ plasm islands formed large aggregates, and cytokeratin filaments only occasionally contacted the islands (Fig. 3C). The interiors of the germ plasm islands contained granular aggregates of cytokeratin (Fig. 3C, D) and occasionally, granular aggregates were present in the vicinity of cortical granules (Fig. 3C, D).

Ultrastructural analysis of cytokeratin distribution in relation to the germ plasm islands and germinal granules

In the vegetal cortex of stage VI oocytes the long cytokeratin filaments were visible in contact with the germ plasm islands (Fig. 4A) and also inside the islands in contact with germinal granules, which are situated between mitochondria (Fig. 4B, C). Forty sections from four different oocytes were analyzed and in all sections whenever the germ plasm was visible in contact with cytokeratin (about 60 germ plasm islands) these were long filaments of cytokeratin. In contrast, in AS VegT ODN injected oocytes (forty sections from four different oocytes were analysed) only short cytokeratin filaments were present in the vicinity and within the germ plasm island (Fig. 4D), and small foci of cytokeratin were occasionally visible in contact with abnormally large germinal granules (Fig. 4E, F), which probably formed by the fusion of several smaller granules (16). In AS Xlirts ODN-injected oocytes, very short fragments of cytokeratin filaments or foci were visible in the germ plasm interior (Fig. 4G) and in contact with germinal granules (Fig. 4G, H).

Ultrastructural analysis of cytokeratin distribution in mature oocytes and activated eggs

During progesterone-induced natural or artificial maturation, oocyte cortical granules move, in preparation for post-activation exocytosis, toward the oocyte surface, where they form a distinct band (Fig. 5A; 20) and the cytokeratin network dissipates into numerous, well defined foci (Fig. 5B;16). In the vegetal cortex of eggs activated by pricking (that mimics activation by sperm), we observed the layer of exocytosed cortical granules (Fig. 5C) and the reconstitution of the cytokeratin network in the form of long filaments (Fig. 5D;16). In mature oocytes originating from the oocytes injected with AS VegT and AS Xlirts ODNs, the cytokeratin network also dissipated into small foci but they were much less regularly spaced than the foci in control mature oocytes (Fig. 6A, C compare with Fig. 5B). In activated AS VegT-depleted eggs cytokeratin reconstituted into very short fragments or remained as dispersed cytokeratin foci (Fig. 6B), and in AS Xlirts depleted oocytes, the reconstitution of long cytokeratin filaments was observed (Fig. 6D).

Ultrastructural effect of anti-cytokeratin antibody injection on the cytokeratin distribution in the vegetal cortex of oocytes and eggs

The analysis of fluorescent and electron microscopy immunostaining showed that the injection of anti-cytokeratin antibody into stage VI oocytes had an effect similar to that of VegT RNA ablation, i.e., the disruption of the cytokeratin network and its fragmentation into cytokeratin foci (Fig. 7; 16). Electron microscopy analysis also showed that the subpopulation of cytokeratin foci became concentrated around the cortical granules, which was very similar to the effect of VegT ablation (Fig. 7 G, H). Upon oocyte maturation these cytokeratin foci became more dispersed (Fig. 7C, I) and interestingly, there was a partial reconstitution of short and thick cytokeratin filaments upon egg activation (Fig. 7D, J; 16).

Three-dimensional ultrastructural analysis of cytokeratin distribution in oocyte vegetal cortex

To overcome the limitation imposed by the analysis of single, ultra-thin electron microscopy sections on the acquisition of information pertaining to the spatial distribution of subcellular structures we performed 3-D reconstructions using serial thin sections of the vegetal cortex of control stage VI oocytes and oocytes injected with AS VegT and Xlirts ODNs (Fig. 8). These reconstructions showed very vividly that intact VegT mRNA and Xlirts RNA are necessary not only for the maintenance of the integrity of the cytokeratin network in the oocyte cortex but also for the proper distribution of germ plasm islands and yolk within the vegetal cortex. In oocytes injected with AS VegT ODN, the cytokeratin network fragmented, and in oocytes injected with AS Xlirts ODN, cytokeratin formed a distinct layer on the top of cortical

granules; in both cases the germ plasm islands coalesced into larger aggregates and yolk platelets become displaced toward the oocyte interior.

Discussion

The conventional role of RNA in the cell pertains to protein translation and regulatory functions. Recently, however, two independent laboratories showed a novel and unexpected, structural role of RNA in the organization and maintenance of the integrity of cellular cytoskeleton. They showed that the formation and maintenance of mitotic spindle in HeLa cells and extracts of *Xenopus* eggs (15), and the cytokeratin cytoskeleton in the vegetal cortex of *Xenopus* oocytes and eggs (16) depends on the presence of intact RNAs. In addition Heasman et al. (18) and Kloc and Etkin (33) showed that intact VegT mRNA and Xlirts RNA are required for the anchoring of other localized RNAs to the vegetal cortex of *Xenopus* oocytes. We showed previously using light and confocal microscopy analysis that the ablation of VegT mRNA in *Xenopus* oocytes causes the disruption of the cytokeratin network, which has profound consequences on germinal granules organization, causing them to coalesce into large aggregates, and germ line development in the embryo. We hypothesized that intact and properly spatially organized cytokeratin network is needed for the correct anchoring and separation of germinal granules (16). In the present study we developed a method for the immunostaining of cytokeratin for electron microscopy that allowed us to analyze the ultrastructural distribution of cytokeratin in relation to other components of the oocyte vegetal cortex. We showed, for the first time, that cytokeratin filaments not only remain in intimate contact with the surface of individual germ plasm islands but also penetrate into germ plasm island interior and contact individual germinal granules. We believe that when this contact between cytokeratin filaments and germ plasm islands becomes disrupted (either by filament fragmentation in VegT-depleted oocytes or by filament collapse toward the cortical granules in Xlirts-depleted oocytes) the germ plasm islands lose their individuality and coalesce into large aggregates. This results in the displacement of the yolk inward and away from the cortex. Thus, we suggest that cytokeratin filaments are necessary for the anchoring of germ plasm islands in the proper spatial arrangement within the oocyte vegetal cortex (see model in Fig. 9). We also suggest that the cytokeratin filaments are needed for the anchoring of the individual germinal granules and for the maintenance of their individuality and proper spatial arrangement within the germ plasm island (Fig. 9). We believe that the profound changes in the ultrastructural organization of the germ plasm islands and germinal granules in the oocyte vegetal cortex after VegT RNAs ablation explain the abnormalities observed in the germline development of resulting embryos (16).

The fact that in Xlirts-depleted oocytes the germinal granules do not fuse although they are devoid of contact with long filaments of cytokeratin strongly suggests that cytokeratin is not the only factor involved in the proper distribution of germinal granules within the germ plasm island. It is very possible that the separation and spatial distribution of germinal granules within the germ plasm island depends not only on the presence of intact cytokeratin filaments but also on other unknown factor(s) such as other cytoskeletal elements or structural proteins that are nonfunctional in AS VegT-depleted oocytes but still properly functioning in AS Xlirts-depleted oocytes. In our previous studies we indicated that the depletion of VegT and Xlirts RNAs did not have any visible effect on the structure of cortical actin (16). However we cannot exclude possibility that there are some subtle changes in the ultrastructure or function of actin filaments, which directly or indirectly affect the germ plasm distribution in the cortex. However this has to await further ultrastructural study.

In *Xenopus*, during egg activation and first four cleavages in the embryo, the germ plasm islands undergo fusion into large aggregates and ingress along the plasma membrane of vegetal blastomeres. It was shown that in early *Xenopus* embryo the germ plasm islands are embedded

in the cortical network of microtubules and microtubule motor kinase-like protein Xklp1 and surface contraction waves are required for germ plasm aggregation in the embryo (34,35).

Considering these facts, it is possible that in *Xenopus* oocytes, the changes in the organization of the cytoskeleton caused by the ablation of *Xlsirts* and *VegT* RNAs result, directly or indirectly, in precocious activation of molecular motor(s) and premature aggregation of germinal granules. However, the answer to this question requires further ultrastructural and functional studies.

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Figure 1.

Changes in the organization of the cytokeleton network in the vegetal cortex of stage VI oocytes, after XlSirts and VegT RNAs ablation. (A-C) Three-dimensional reconstruction of 3 sets of 10 semithin (0.7 μ m) sections of the fragments of the vegetal cortex of oocytes stained with anti-cytokeleton antibody, nanogold-conjugated secondary antibody, silver enhanced and stained lightly with 1% toluidine blue diluted in 1% borax. (A) In control oocytes, long filaments of the cytokeleton network are visible at the cortex (long arrow) and also away from the cortex between the yolk platelets (short arrow). (B) Oocytes injected with AS VegT ODN show fragmentation of filaments at the cortex (long arrow) and lack of long filaments between the yolk and only granular foci of cytokeleton (short arrows) visible between the yolk. (C)

Oocytes injected with AS Xisirts ODNs show a prominent narrow layer of cytokeratin at the cortex (long arrow) and also the presence of long but granular in appearance filaments between the yolk (short arrow). Yolk platelets, Y.

Figure 2.

Ultrastructural analysis of cyto keratin distribution in the vegetal cortex of stage VI

oocytes. Oocytes were immunolabeled with anti-cyto keratin antibody and nanogold conjugated secondary antibody and silver enhanced. (A) Low magnification view of the fragment of the oocyte showing the arrangement of the components of the vegetal cortex. The vitelline envelope is followed by the layer of cortical granules and yolk. The islands of germ plasma (arrows) are distributed at the boundary between the germinal granules and yolk. Cyto keratin is not visible at this magnification. (B) Fragment of the vegetal cortex showing the germ plasma islands (short arrows) and the long filaments of cyto keratin network (long arrows) situated between the layer of cortical granules and yolk and in contact with the

periphery of germ plasm islands. (C) Fragment of the vegetal cortex showing several (visibly separated from each other) germ plasm islands (short arrows) surrounded and penetrated by the long cytokeratin filaments (long arrows). (D, E) Two examples of germ plasm islands (short arrows) penetrated by the cytokeratin filaments (long arrows). Vitelline envelope, VE; Cortical granules, CG; yolk platelets, Y.

Figure 3.

The effect of Xlirts and VegT RNAs ablation on the organization of cytokeleton filaments and germ plasm islands distribution in the vegetal cortex of stage VI oocytes. Oocytes were injected with AS VegT (A, B) or AS Xlirts (C, D) ODNs and immunolabeled with anti-cytokeleton antibody and nanogold conjugated secondary antibody, and silver enhanced. (A) Fragment of the vegetal cortex of AS VegT-injected oocyte showing short and dispersed filaments of cytokeleton (long arrows) distributed between yolk and around the cortical granules and a thick band of interconnected germ plasm islands (short arrows). (B) Fragment of the vegetal cortex of AS VegT-injected oocyte showing fragmented cytokeleton filaments (long arrows) around and within the germ plasm islands (short arrows) and surrounding cortical granules. (C, D) Fragments of the vegetal cortex of AS Xlirts injected oocyte showing interconnected germ plasm islands (short arrows) and a compact layer of long cytokeleton filaments apposing cortical granules and yolk. Note the scarcity of cytokeleton filaments around the germ plasm islands. Vitelline envelope, VE; Cortical granules, CG; yolk platelets, Y.

Figure 4.

Cytokeratin distribution in relation to germ plasm islands and germinal granules in stage VI oocytes. (A-H) Stage VI oocytes immunolabeled with nanogold conjugated anti-cytokeratin antibody and silver enhanced. (A-C) Fragment of germ plasm island from stage VI control oocyte. Long cytokeratin filaments (long arrows) contact and penetrate germ plasm island and are in contact with the germinal granules (short arrows). Area of germ plasm island marked with square in panel A is shown at higher magnification in panel B. (D-F) Fragments showing germ plasm islands in oocytes injected with AS VegT ODN. (D) Short fragments of cytokeratin (long arrows) are visible in the vicinity of germ plasm islands. (E-F) Granular foci of disintegrated cytokeratin filaments (long arrows) are visible in the vicinity of huge germinal granules (short arrows). (G, H) Fragments showing the germ plasm island in oocytes injected with AS Xlirt ODN. Area of germ plasm island marked with square in panel G is shown at higher magnification in panel H. Granular foci of disintegrated cytokeratin filaments (long arrows) are visible in the vicinity of germinal granules (short arrows). Yolk platelets, Y; mitochondria, m.

Figure 5.

Ultrastructure of cytokeratin distribution in the vegetal cortex of mature oocyte and

activated egg. Oocytes and eggs were immunolabeled with anti-cytokeratin antibody and nanogold conjugated secondary antibody and silver enhanced. (A) Low magnification view of the fragment of the oocyte matured by the addition of progesterone showing the arrangement of the components of the vegetal cortex. The vitelline envelope is followed by the layer of cortical granules and yolk. (B) Fragment of oocyte cortex showing small foci of cytokeratin (long arrows) and small germ plasm islands (short arrows). (C) Low magnification view of the fragment of the egg, 1-3 hr after activation by pricking showing the arrangement of the components of the vegetal cortex with the layer of exocytosed cortical granules. (D) Fragment of activated egg cortex showing the reconstitution of long filaments of cytokeratin (long arrows) in contact with germ plasm islands (short arrows). Vitelline envelope, VE; Cortical granules, CG; Exocytosed cortical granules, EGC; yolk platelets, Y.

Figure 6.

The effect of VegT and Xlirts RNAs ablation on the ultrastructure of cytokeratin distribution in the vegetal cortex of mature oocytes and activated eggs. Oocytes were injected with AS VegT (A, B) or AS Xlirts (C, D) ODNs, matured by addition of progesterone, activated by pricking, and immunolabeled with anti-cytokeratin antibody and nanogold-conjugated secondary antibody and silver enhanced. (A) Fragment of the vegetal cortex of AS VegT ODN injected mature oocyte showing tiny foci of cytokeratin (long arrows) and large, interconnected germ plasm islands (short arrows). (B) In AS VegT ODN-injected and activated egg, short cytokeratin filaments (long arrows) reconstitute at the periphery of the cortex apposing vitelline envelope. Large germ plasm islands (short arrows) are also visible. (C) Fragment of the vegetal cortex of AS Xlirts ODN-injected mature oocyte showing foci of cytokeratin (long arrows) and large germ plasm islands (short arrows). (D) In AS Xlirts ODN injected and activated egg short and long cytokeratin filaments (long arrows) reconstitute at the periphery of the cortex apposing microvilli and also between the yolk and in vicinity of germ plasm islands (short arrows). Vitelline envelope, VE; Cortical granules, CG; Exocytosed cortical granules, EGC; yolk platelets, Y.

Figure 7.

The effect of injection of anti-cytokeratin antibody on the ultrastructure of cytokeratin filaments in the vegetal cortex of stage VI oocytes, mature oocytes, and activated eggs mimics the effect of VegT mRNA ablation.

(A) Cytokeratin network in the vegetal cortex of control stage VI oocyte stained with FITC conjugated anti-cytokeratin antibody. (B-D) Cytokeratin in vegetal cortex of stage VI oocytes and egg injected with FITC conjugated anti-cytokeratin antibody. (B) 3hr after the injection of anti-cytokeratin antibody, the cytokeratin network is disrupted and its remnants form thick foci. (C) After progesterone-induced maturation of injected oocytes, the cytokeratin is visible as very small and dispersed foci. (D) In eggs activated by pricking, cytokeratin reconstitutes into thick short fibrils. (E, F) Two areas of vegetal cortex of stage VI oocyte injected with anti-cytokeratin antibody and subsequently incubated with nanogold-conjugated secondary antibody and silver enhanced. A few hours after the injection of antibody the filaments of cytokeratin become shorter and visibly granular in appearance (long arrows, E) and also fragment into smaller foci, which surround cortical granules (long arrows, F). Germ plasm islands are also visible in the vicinity of fragmenting cytokeratin filaments (short arrow, E). The distribution of fragmented cytokeratin around cortical granules in anti-cytokeratin antibody-injected oocytes (G) resembles that in oocytes injected with AS VegT ODN (H). (I) In anti-cytokeratin antibody-injected mature oocytes (as in AS VegT ODN-injected mature oocytes) germ plasm islands are large and interconnected (short arrows), and cytokeratin forms tiny granular foci (long arrows). (J) In anti-cytokeratin-injected activated eggs (unlike in AS

VegT ODN-injected activated eggs) cytokeratin reforms as compact granular aggregates (long arrows). Cortical granules, CG; yolk platelets, Y. Scale bar in A-D: 12 μ m.

Figure 8.

Three dimensional reconstruction of distribution of cytokeratin and germ plasm islands in the vegetal cortex of stage VI oocytes after VegT and Xlirts RNAs ablation . (A) In

control stage VI oocytes small germ plasm islands are separated from each other and are distributed more internal to the layer of cortical granules and between the yolk. The majority of long cytokeratin filaments form the network between and within the layer of cortical granules and yolk and penetrate and surround the germ plasm islands. Shorter and granular in appearance filaments are also present at the surface of cortical granules. (B) In oocytes injected with AS VegT ODN the majority of the cytokeratin network disintegrates into short fragments, which aggregate around the cortical granules and some remain in contact with the germ plasm islands. The germ plasm islands fuse into larger interconnected aggregates, and yolk platelets are displaced inward. (C) In AS Xlirts ODN-injected oocytes the majority of long cytokeratin filaments form a distinct layer on the top of cortical granules. Filaments contact the surface of the aggregates of germ plasm islands (but do not penetrate them). Shorter and granular cytokeratin filaments are also present on the surface of cortical granules. Yolk platelets are displaced inward.

Figure 9.
Model of the effect of AS VegT and Xlirts RNA depletion on the ultrastructure of cytokeratin network and components of the vegetal cortex in *Xenopus* stage VI oocytes.
 (A) In control stage VI oocytes, the long filaments of cytokeratin (black) form a complex, interconnected network spanning between cortical granules (pink spheres) and yolk platelets (yellow spheres). Germ plasm islands (grey areas) are anchored by cytokeratin filaments in proper position within the cortex. Cytokeratin filaments also penetrate the germ plasm islands and anchor the germinal granules (red spheres) located within the islands. Proper multidimensional organization of cytokeratin filaments ensures proper spatial distribution of cortical granules and yolk platelets and separation of individual germ plasm islands and individual germinal granules within the germ plasm islands. (B) In AS VegT ODN-depleted oocytes, long cytokeratin filaments fragment into short pieces and granular foci. The disintegration of the cytokeratin network results in the disruption of the proper spatial distribution of the components of the cortex. The germ plasm islands become released from the cytokeratin anchor and fuse into larger aggregates displacing yolk platelets inward. In addition, the disintegration of cytokeratin filaments anchoring the germinal granules within the germ plasm islands causes the individual granules to fuse into larger aggregates. (C) In AS Xlirts ODN-depleted oocytes long cytokeratin filaments collapse at the surface of the cortical granule layer and lose contact with germ plasm islands. Loss of the cytokeratin anchor results in the fusion of germ plasm islands into larger aggregates and displacement of the yolk inward. At present it is unclear why germinal granules in Xlirts-depleted oocytes (although devoid of long cytokeratin filament anchors) do not fuse as in AS VegT-depleted oocytes. It is very possible that the proper separation and spatial distribution of germinal granules within the germ plasm island depends not only on the presence of intact cytokeratin filaments but also on other unknown factor(s) absent or nonfunctional in AS VegT-depleted oocytes but still properly functioning in AS Xlirts-depleted oocytes.