DEVELOPMENTAL BIOLOGY 155, 315-323 (1993)

Actin Is a Major Structural and Functional Element of the Egg Cortex of Giant Silkmoths during Oogenesis

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Accepted November 6, 1992

The cortex and subcortical regions of the developing follicles and eggs of silkmoths are rich in cytoskeletal elements, particularly actin. *In situ* analysis using [^{3}H]-polyuridylic acid and biotinylated oligo d(T) reveals a pattern of changes in poly(A)$^+$ RNA distribution during oogenesis. The developing pattern of distribution of actin filaments in the ooplasm closely resembles that of poly(A)$^+$ RNA. RNA polymerase II is also associated with the cortical cytoskeleton. Destruction of the actin filaments in the developing oocytes by cytochalasin D randomizes the distribution of mRNA and causes the displacement of RNA polymerase II from the cortex. Rhodamine-conjugated phalloidin and a monoclonal antibody against cytoskeletal actin were used in combination with laser scanning confocal microscopy to examine the details of actin distribution in the oocytes. RNA polymerase II was located in developing oocytes using both anti-*Drosophila* RNA polymerase II antibody and fluorescein-conjugated amanitin. © 1993 Academic Press, Inc.

INTRODUCTION

Unfertilized eggs, particularly those of insects, appear deceptively chaotic. There are no obvious structural correlates of the embryo which will form, often not even many signposts to indicate the major axes of the larva. It is clear that some cryptic organization must underlie the apparent randomness of the ooplasm. Work on the fruitfly, *Drosophila*, has indicated that a latent image of the embryo exists in the information contained in maternal messenger RNA (mRNA). This information must be distributed in a stereotyped spatial array in order to generate a coherent embryo rather than an amorphous collection of cells. The anterior-posterior axis, for instance, is determined by the product of such maternal genes as *bicoid*, *oskar*, *nanos*, *torso* and a number of others (see DePomerai, 1990 for a review). In order to function properly, these gene products must be organized in a precise spatial sequence which will establish the anterior-posterior axis and must be held in position until expressed in terms of translation into protein. In addition to mRNAs, one might consider that a similar sequestering of specific maternal enzymes crucial for further development would serve a useful function during oogenesis and early embryogenesis.

The particular mode of cellularization of many insect embryos allows us to designate the cortical layer of the egg as the likely site of immobilization of mRNA and possibly key enzymes. This cortical layer is the site of the formation of most, and debatably all, of the cells of the embryo, and thus it represents the strategic area for localization. The cortex of oocytes of both vertebrates and invertebrates has been postulated to be a potential site for storage of "morphogenetic substances" at least since the beginning of the 20th century (Wilson, 1904). Low centrifugal force exerted on eggs and very early embryos leaves the cortex intact and has little effect on subsequent development even though structures deeper in the ooplasm are displaced (Morgan, 1935; Clement, 1968; Verdonk, 1968; Jeffery and Meyer, 1983). By contrast, agents which disrupt the cortex can lead to changes in pattern formation or total disruption of embryogenesis (Schmidt *et al.*, 1975; Kalthoff, 1976). Various types of cytoskeletal networks have been detected in the cortex of eggs of many different species, and in *Drosophila*, actin in particular, appears to be a prominent constituent (Planques *et al.*, 1991, Theurkauf *et al.*, 1992). The cortex of unfertilized oocytes has been shown to be particularly rich in poly(A)$^+$ RNA in a variety of species (ascidians—Capco and Jeffery, 1978; Jeffery and Wilson, 1983; amphibians—Capco and Jeffery, 1982; Jeffery, 1984, 1985; Rebagliati *et al.*, 1985; silkmoths—Jarnot *et al.*, 1988), and the mRNA for the *Drosophila bicoid* product (Berleth *et al.*, 1988; Stephenson and Pokrywka, 1992) and the *Hyalophora cecropia Ec4b* gene (Kastern *et al.*, 1990) have been found to be localized specifically in the cortex.

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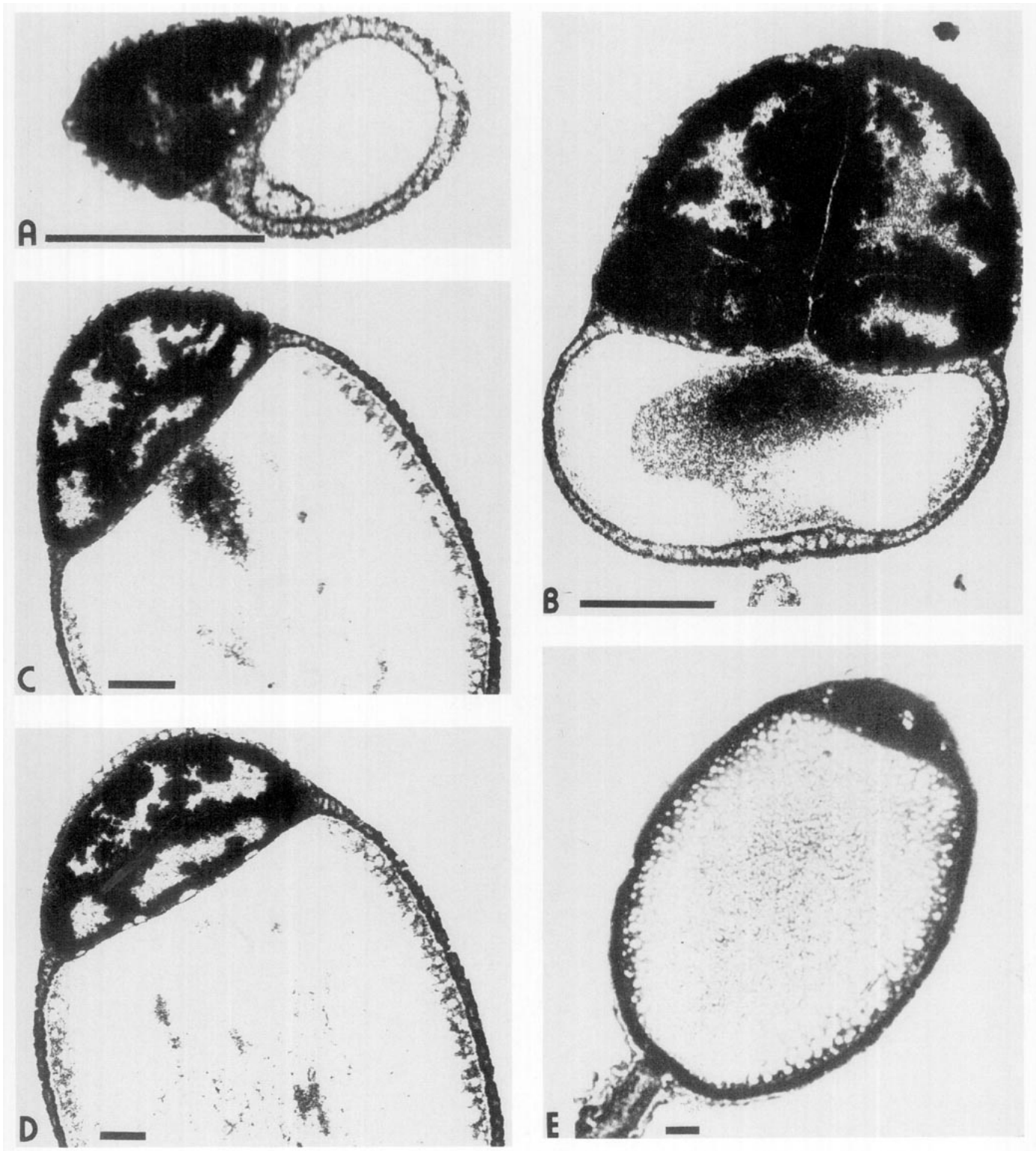


FIG. 1. Distribution of poly(A)$^+$ RNA detected by *in situ* hybridization to [^{3}H]polyuridylic acid during *H. cecropia* vitellogenesis. (A) Very early stage of follicle in vitellogenesis. Note the heavy distribution of silver grains over nurse cells and lighter concentration over follicle cells. Almost no staining was detectable in ooplasm except adjacent to germinal vesicle. (B) A stage somewhat later than A. Note that poly(U) hybridization is detectable over the central ooplasm (double arrow), and some silver grains are present in the developing cortex. (C and D) Successively later stages which show poly(A)$^+$ presence over the central ooplasm and increasing deposition in cortex. (E) A follicle shortly before the atrophy of the nurse cells. Sparse signal is detected over the general ooplasm, and heavier concentration of poly(A)$^+$ RNA in the cortical region of the oocyte. The scale bar represents 50 μm.

The experiments described in this report were designed to investigate the structure of the actin-containing cortical cytoskeleton of developing oocytes of the silkmoths *H. cecropia* and *Antheraea polyphemus*, to follow the process of mRNA localization in the ooplasm, and to determine if an enzyme, RNA Pol II,[2] which is required during early blastoderm formation and is known to be in high concentration in these oocytes (Kastern *et al.*, 1981), is sequestered in the cortex. Finally, by employing cytochalasin D, an agent which disrupts actin fibers, we attempted to determine whether the patterns of distribution of mRNA and Pol II are dependant upon the integrity of the actin component of the cortical cytoskeleton. We chose silkmoths as an excellent model system for these studies because their ovaries are beautifully organized for temporal studies of oogenesis. The strings of large ovarian follicles develop slowly, and each ovariole represents a smooth gradient with about a 4-hr difference in developmental age from follicle to follicle. The developing oocyte fits the more "classical" mode of oogenesis with no translation or transcription detectable in the ooplasm until after fertilization (Paglia *et al.*, 1976, Kastern *et al.*, 1982). This pattern contrasts sharply with that of higher Dipterans, where oogenesis is speeded up in the penultimate oocyte, and considerable prefertilization translation is detected (Pietruscka and Bier, 1972, Zalockar, 1976).

MATERIALS AND METHODS

Animals

H. cecropia and *A. polyphemus* pupae were purchased from S. E. Ziemer, Kewaunee, Wisconsin and stored at 4°C for 3 months, after which they were removed and placed at room temperature for about 3 weeks in continuous light to allow development of the pharate imago. At this time, developing ovarian follicles and mature, chorionated, unfertilized eggs were excised from females in sterile insect Ringers solution (130 m*M* NaCl, 2.0 m*M* KCl, 1.5 m*M* $CaCl_2$).

Immunohistochemistry

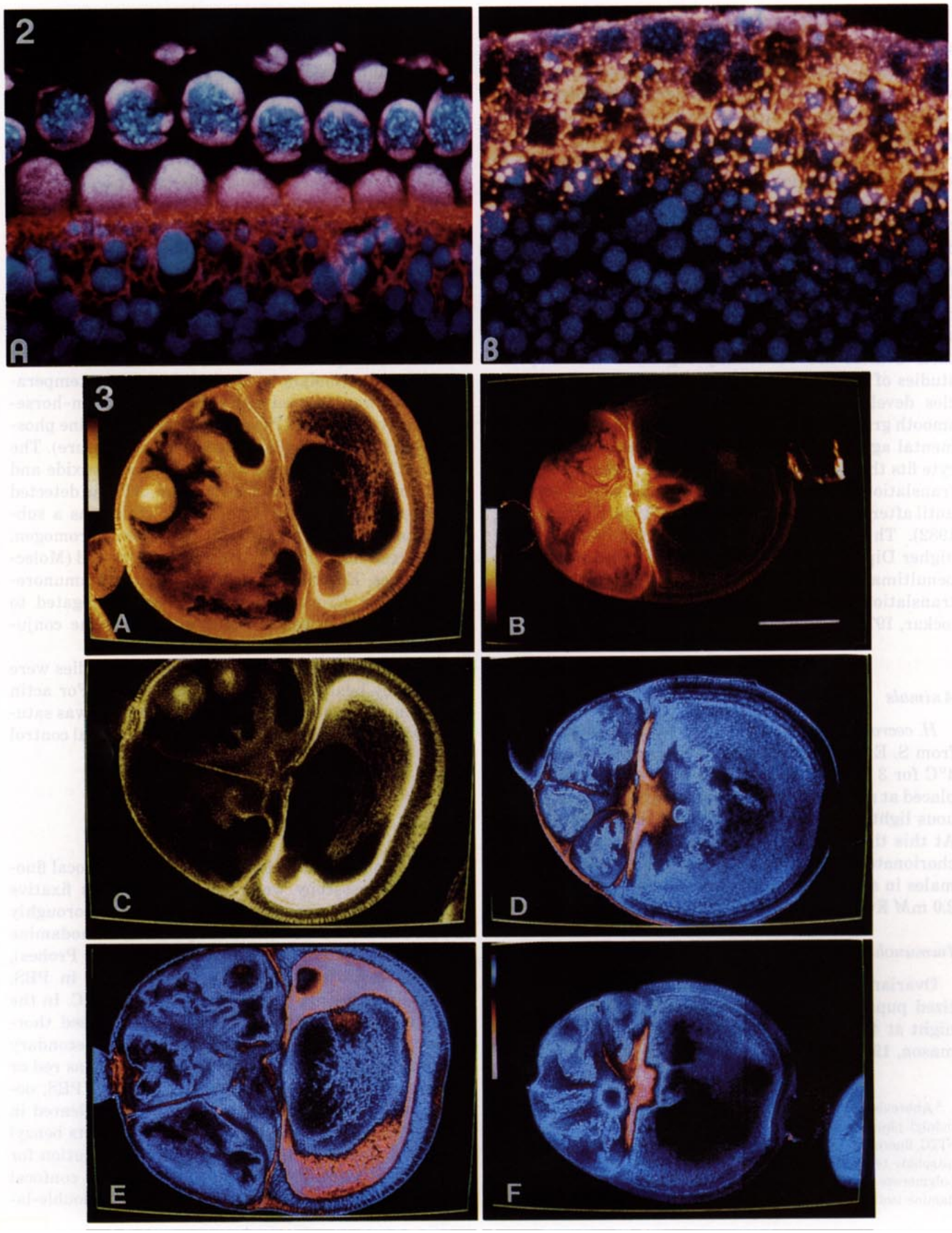
Ovarian follicles were excised from water-anaesthetized pupae under saline and immediately fixed overnight at 4°C in modified Bouin–Hollande fixative (Humason, 1967) containing 0.7% mercuric chloride. Standard histological techniques were used for sample dehydration, embedding in paraplast, sectioning at 4 μm, deparaffinization, and rehydration. The monoclonal anti-actin and polyclonal anti-ferritin antibodies were purchased from Amersham (Arlington Height, IL), and Sigma (St. Louis, MO), respectively. The antibody directed to the largest subunit of *Drosophila* RNA polymerase II (see Weeks *et al.*, 1982) was prepared and kindly made available to us by Prof. Arno L. Greenleaf (Duke University, Durham, NC). Immunological detection was carried out according to the standard protocol supplied with the commercial staining kit (Amersham). Slides with sections were pretreated with 10% normal goat serum in phosphate-buffered saline (PBS) containing 0.1% Triton X-100 (30 min at room temperature) and incubated with primary antibody (overnight at 4°C) and biotinylated secondary antibody (1 hr at room temperature). They were treated with either streptavidin–horseradish peroxidase (HRP) or streptavidin–alkaline phosphatase (AP) complex (1 hr at room temperature). The HRP activity was stained with hydrogen peroxide and 3,3′-diaminobenzidine as a chromogen. AP was detected using bromochloroindolyl phosphate (BCIP) as a substrate and nitroblue tetrazolium (NBT) as a chromogen. For fluorescence confocal microscopy, Texas red (Molecular Probes, Eugene, OR) or Cy3 (Jackson Immunoresearch, West Grove, PA) fluorophores conjugated to streptavidin were used instead of the enzyme conjugates.

In control experiments, the primary antibodies were replaced by heat-inactivated normal serum. For actin detection, the monoclonal anti-actin antibody was saturated with actin before staining as an additional control for its binding specificity.

Confocal Laser Scanning Microscopy

Wholemounts of developing oocytes for confocal fluorescence microscopy were fixed in Altman's fixative (Humason, 1967) overnight at 4°C, washed thoroughly in PBS and incubated with either phalloidin–rhodamine (phalloidin-TRITC, 1 μ*M* in PBS; Molecular Probes), amanitin–fluorescein (amanitin-FITC, 1 μ*M* in PBS; Sigma) or anti-actin antibody overnight at 4°C. In the case of antibody staining, follicles were rinsed thoroughly in PBS, incubated with biotinylated secondary antibody, and, finally, treated with either Texas red or Cy3–streptavidin complex. After washing in PBS, oocytes were dehydrated up to 100% ethanol, cleared in Murray's solution (1 part benzyl alcohol:2 parts benzyl benzoate), and mounted in fresh Murray's solution for direct observation under a Bio-Rad MRC-500 confocal scanning laser microscope, using A.1 and A.2 double-la-

[2] Abbreviations used: AP, alkaline phosphatase; BCIP, bromochloroindolyl phosphate; CD, cytochalasin D; DMSO, dimethyl sulfoxide; FITC, fluorescein isothiocyanate; HRP, horseradish peroxidase; NBT, nitroblue tetrazolium; PBS, phosphate-buffered saline; Pol II, RNA polymerase II; SSC, standard saline citrate; TRITC, tetramethylrhodamine isothiocyanate.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bel filter sets for viewing rhodamine and fluorescein, respectively.

In Situ Hybridization

The follicles for *in situ* hybridization were routinely prepared as described for immunohistochemistry. Serial sections were attached to poly-L-lysine-pretreated microscope slides. Following deparaffinization and rehydration, samples were treated with proteinase K (1 μg/ml in PBS, 30 min, Boehringer-Mannheim) acetylated with 0.5% acetic anhydride in 0.1 *M* triethanolamine, pH 8.0, 10 min (Hayashi *et al.*, 1978), rinsed in 2× SSC, and dehydrated up to 95% ethanol. Hybridization was carried out in solution similar to that described by Lawrence and Singer (1985). [^{3}H]Polyuridylic acid (specific activity 4.5 Ci/mmole; ICN Biochemicals, Costa Mesa, CA) was employed as a hybridization probe. Approximately 1×10^5 cpm was added to each slide in about 25 μl of hybridization buffer. Hybridization was for 12 hr at 42°C. Following hybridization, slides were rinsed three times in 2× SSC for 30 min each, dehydrated up to 95% ethanol, air dried, and coated with NTB-2 radiographic emulsion (Kodak, Rochester, NY). Exposure was for 6 weeks. Slides were developed in Kodak D-19 for 4 min at 15°C, fixed with Kodak fixer, and counterstained with Harris' hematoxylin-eosin (Humason, 1967). Controls (not shown) consisted of pretreatment of sections with pancreatic ribonuclease A followed by ribonuclease T_2 (Jarnot *et al.*, 1988). Grain counts showed control sections to be only slightly above background.

In situ hybridization with nonradioactive labeled probes was carried out under virtually the same conditions as described above for radioactive probe. Oligo(dT) primer (n = 25–30, Pharmacia LKB, Piscataway, NJ) was biotinylated using biotin-21-dUTP oligonucleotide 3′ end labeling system (Clontech, Palo Alto, CA). Following hybridization, sections were incubated with streptavidin-AP complex. The activity of AP was detected using a BCIP/NBT substrate system.

Cytochalasin D Application

Since the cortical cytoskeleton appears to be composed mostly of actin (Jarnot *et al.*, 1988), our experimental procedures included both *in vivo* and *in vitro* treatment of developing follicles with cytochalasin D (CD). This compound is believed to have a strong inhibitory and disruptive effect on actin-based structures (Selden *et al.*, 1980; Schliwa, 1982). CD was initially dissolved in dimethylsulfoxide (DMSO; resulting concentration 5 m*M* CD). Vitellogenic *H. cecropia* females were injected in the abdomen with the CD solution (the final dose of CD amounted to 50 μg per gram of tissue), or strings of follicles were transferred to Grace's insect culture medium containing 10 μ*M* CD. After 4, 8, and 16 hr, follicles were dissected from the injected females under saline or removed from the culture medium, fixed immediately, and sectioned for immunological detection and *in situ* hybridization. Control experiments with *cecropia* females injected with a corresponding dose of pure DMSO or incubated in Grace's medium without CD revealed no detectable effect on developing follicles.

RESULTS

Poly[A]$^+$ Hybridization—Distribution of Maternal Messenger RNA during Oogenesis

Hybridization to [^{3}H]polyuridylic acid or biotinylated oligo (dT) should serve as a rough measure of distribution of mRNA in the egg during oogenesis. In a previous publication (Kastern *et al.*, 1990), we described the cloning of a specific maternal mRNA, *Ec4b*, which was found by *in situ* hybridization to be restricted to the cortex of the mature, unfertilized egg. We followed the pattern of distribution of both a cDNA probe and an anti-sense RNA probe during oogenesis. The pattern of distribution of *Ec4b* was indistinguishable from the poly(U) pattern reported here. Under these conditions, the sense-strand RNA produced no signal above background. We have chosen to present the poly(U) hybridization data because the signal from the specific message was less

FIG. 2. Sections of the cortical region of *A. polyphemus* oocytes viewed under the confocal microscope. (A) Stained with anti-actin antibody coupled to Texas red. Red to orange band represents cortical actin; pink represents cytoplasmic actin in follicle cell cytoplasm. Blue color is caused by autofluorescence of yolk platelets and follicle cell nuclei. (B) Section similar to A, but after 4 hr *in vivo* treatment of follicle with CD. Both sections magnified approximately 870×.

FIG. 3. Confocal images of wholemount follicles of *H. cecropia* before and after exposure *in vivo* to CD for 16 hr. (A) Follicles stained with anti-cytoskeletal actin antibody coupled to Cy3 fluorophore. Note that the antibody, which binds both F- and G-actin, is detected strongly in nurse cell cytoplasm as well as the oocyte cortex and follicle cells. (B) After 16 hr exposure to CD, actin is detected mainly at the ring-canal opening and in the nurse cell cytoplasm. Pink to white represents positive signal of staining; blue color is caused by autofluorescence. (C) Follicles stained with phalloidin-TRITC. Note that little actin is detected in the nurse cell cytoplasm as compared with antibody staining A. A strong signal is observed in the cortex. These results indicate that much of the actin in the nurse cell cytoplasm may be in the G rather than F form. (D) After 16 hr exposure to CD, F-actin is detected only at the ring-canal opening and to a minor extent around the periphery of the nurse cells. (E) Follicles stained with amanitin-FITC. The distribution of amanitin which binds RNA polymerase II specifically is very similar to F-actin distribution in the cortex. (F) After 16 hr exposure to CD, amanitin-FITC is found only at the ring-canal opening.

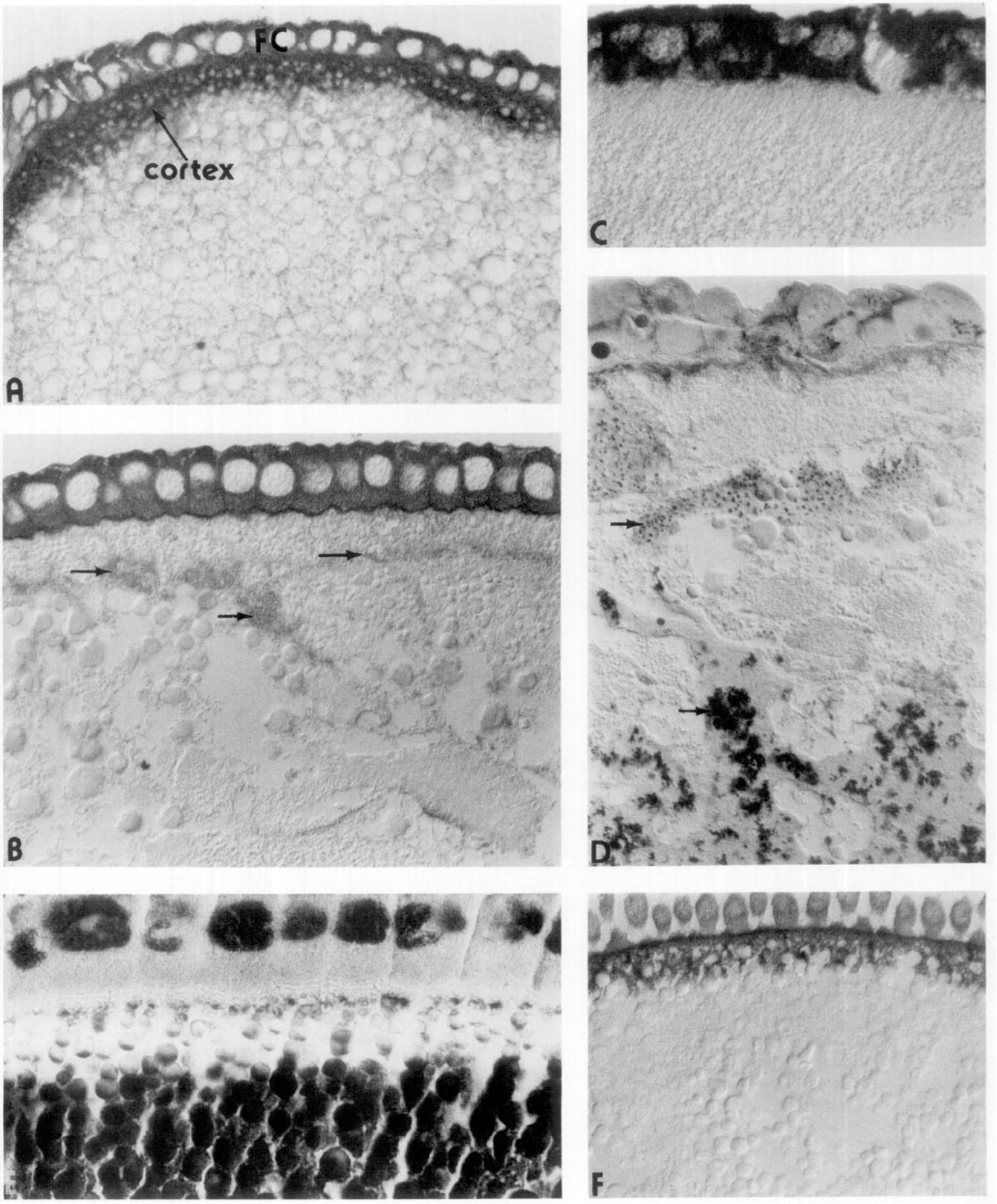

FIG. 4. Effect of cytochalasin D (CD) on the distribution of poly(A)$^+$ RNA and the actin cytoskeleton. (A) Section of an untreated follicle after hybridization to biotinylated oligo(dT). Hybridization signal mainly restricted to the follicle cell cytoplasm and the oocyte cortex (295×). (B)

robust and could not be detected at low magnification, while the pattern of poly(U) hybridization, and thus poly(A) distribution, is apparent in micrographs of the entire follicle.

The results of *in situ* hybridization of poly(U) at different stages of oogenesis are shown in Fig. 1. In early stages, poly(A)$^+$ RNA was detected in the nurse cell nuclei and cytoplasm, but not in the ooplasm (Fig. 1A). Little hybridization was recorded in the follicle cells of the earliest stage (Fig. 1A), but substantial label was observed in later stages. The poly(A)$^+$ RNA began to appear in the ooplasm, particularly near the ring canals and the "delta" of ooplasm which forms a continuum with the nurse cell ooplasm (Fig. 1B). In still later stages a significant portion of the poly(A)$^+$ RNA accumulated in the cortex (Figs. 1C and 1D). A trend, in which poly(A)$^+$ RNA in the central ooplasm moves toward the cortex, is apparent during later stages of vitellogenesis (Figs. 1D and 1E). These results are consistent with the observation of Paglia *et al.* (1976) that the nurse cells are the source of mRNA, and that the cortex of oocyte is a major site for its deposition. Compared to the cortex, only a small amount of poly(A)$^+$ RNA appears to be retained in the deeper ooplasm of the more mature oocyte (Fig. 2E).

The Egg Cytoskeleton—Distribution of Actin

The results of all our experiments are consistent with, and support the hypothesis that, localization of molecules critical for the orderly spatial organization of early development depends on an intact cortical cytoskeleton. Figures 2A, 3A, and 4F illustrate the disposition of a major component of the cortical cytoskeleton, actin, in the late follicle of *H. cecropia.* The positive signal of staining is localized almost exclusively in superficial layers of the oocyte and in the nurse cell cytoplasm. The actin network in the oocyte cortex is retained throughout vitellogenesis and at least up to the point when the egg is fertilized. The sparse staining of the nurse cell cytoplasm by phalloidin-TRITC (Fig. 3C) as opposed to anti-actin antibody (Fig. 3A) indicates that most of the actin is in the G (globular) form, which does not bind phalloidin. Actin in the ooplasm stains heavily with both reagents, indicating that it is in the fibrous form. Actin localization coincides very well with the pattern of poly(U) hybridization.

RNA Polymerase II Localization in the Ooplasm

Since RNA Pol II activity is required during blastoderm formation and is prerequisite for the transcription of the newly formed zygote genome, we examined oocytes to determine whether it was also associated with the cortex. In sections of *H. cecropia* follicles, antibody against Pol II bound to more superficial layers (data not shown) of the cortical cytoskeleton. Wholemount preparations stained with amanitin-FITC also showed a similar binding pattern in the peripheral cortex (Fig. 3E). In the follicles of *A. polyphemus,* anti-Pol II antibody was found to bind to the cortex, but a layer deeper than in *H. cecropia* (not shown).

Cytochalasin D Effects of Disruption of Cytoskeletal Actin on mRNA and Pol II Distribution

Effects of injection into the pupal abdomen or *in vitro* treatment of isolated follicles with cytochalasin D on ovarian follicles are evident after 4 hr. Both phalloidin-TRITC and antibodies against actin detect disorganized masses of actin in the deep cortex and a "disorganization" of the actin cytoskeleton in the superficial cortex (Fig. 2B). By 16 hr after injection or *in vitro* treatment, almost no actin can be detected in the follicles except in an area where the ring canals open into the ooplasm (Figs. 3B and 3D). These effects are not simply pathological and associated with the death of the animals, since the pupae were still alive when dissected, and control animals injected with the same dose of CD were allowed to continue development and were able to complete metamorphosis and emerge as adults. The impression that CD caused the cytoskeleton to dissociate is reinforced by the appearance of blobs of actin on the side of yolk platelets directly under the cortex (Fig. 2B). Amanitin-FITC staining (Figs. 3E and 3F) exhibited the same dissociation pattern as was detected via anti-actin.

When biotinylated oligo (dT) was hybridized to CD-treated follicles, poly(A)$^+$ RNA appeared to be randomly distributed in the ooplasm (Figs. 4B and 4C) rather than localized in the cortex as indicated in controls (Fig. 4A). This dispersion of the mRNA indicates that its localization depends on the integrity of the actin cytoskeleton. After 16 hr treatment, poly(A)$^+$ RNA was so dispersed that it was undetectable, except in small,

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Section of follicle 4 hr after *in vivo* treatment with CD. The structure of the ooplasm is severely disrupted and a small amount of poly(A)$^+$ RNA is present in ooplasm (arrows). Strong hybridization signal is still detectable in the follicle cell perikarya (500×). (C) Section of follicle 16 hr after CD injection. No traces of hybridization are detected (560×). (D) Section similar to C, but stained with anti-actin antibody (550×). Note the disorganized masses of actin in deeper ooplasm (arrows). (E and F) Control sections stained with anti-actin antibody (F, 270×) showing the actin distribution in the cortex of oocyte and antibody against horse ferritin (E, 270×), indicating virtually no positive signal in superficial layers of the follicle.

randomly organized patches (Fig. 4C). Coincidental disruption of cortical actin is evident in Fig. 4D.

DISCUSSION

In this manuscript we report on the time course of deposition of poly(A)$^+$ RNA in the ooplasm, the precise localization of actin and RNA polymerase II using the laser scanning confocal microscope, and the effect of *in vivo* and *in vitro* treatment of developing oocytes with cytochalasin D. In an earlier paper (Jarnot *et al.*, 1988), actin was located using conventional fluorescence microscopy and the mushroom toxin phalloidin. By employing confocal laser scanning microscopy and anti-actin antibodies as well as TRITC-labeled phalloidin, we have been able to compare the distribution of F-actin and G-actin in the follicles. Cytoplasmic actin of nurse cells does not exhibit the complex and intricate three-dimensional organization found in the oocyte cortex. The less intense staining in nurse cell cytoplasm with phalloidin–TRITC, as compared with antibody, indicates that nurse cell actin is less polymerized than cortical actin, because phalloidin does not bind to G-actin, but exclusively to F-actin.

Staining with anti-Pol II antibody and FITC-labeled amanitin was undertaken because we had earlier demonstrated the presence of extraordinary amounts of Pol II activity and binding of [^{3}H]amanitin in the ooplasm (Kastern *et al.*, 1981) Amanitin–FITC and anti-Pol II antibody bound in the cortex in an identical pattern. Both appeared in the superficial cortex in the ooplasm of *H. cecropia*, while in *A. polyphemus* the antibody was located in a slightly deeper layer. We presume that the binding of Pol II to the cortex may localize it in a strategic position to commence transcription when the cellular blastoderm forms after fertilization.

Because the mushroom toxins amanitin and phalloidin and antibodies against both actin and Pol II, as well as a substantial fraction of poly(A)$^+$ RNA, all bind to the cortex in a very similar pattern, we felt it important to eliminate the possibility that the cortex was simply nonspecifically adhesive for these components. We selected an antibody against horse ferritin as a control for nonspecific binding. The results shown in Fig. 4E indicate that the ferritin antibody bound to various structures in the follicle cells and ooplasm, including yolk platelets, but is conspicuously absent from the cortex.

The pattern of hybridization of [^{3}H]polyuridylic acid to poly(A)$^+$ RNA in the follicles is entirely consistent with synthesis of mRNA in the nurse cells and transfer via the ring canals to the ooplasm (Bier, 1970; Paglia *et al.*, 1976). The studies reported here refine the time course of distribution of mRNA in the ooplasm during oogenesis. In early vitellogenic follicles, nurse and follicle cell cytoplasms are heavily labeled, while the ooplasm is virtually unlabeled, except in the region of the germinal vesicle (Fig. 1A). As vitellogenesis progresses, labeling increases in the delta region below the ring canals, and some appears in the cortex (Fig. 1B). During middle and later stages of vitellogenesis, positive signal disperses from the central ooplasm to the periphery where it accumulates (Figs. 1C and 1D). At a time just prior to the involution of the nurse cells, the signal of ^{3}H-labeled poly(U) hybridization is heavily concentrated in the cortex and only sparsely distributed in the central ooplasm (Fig. 1E). The mechanism of this directed dispersal of mRNA is not known, but actin fibers are detected in the delta at early vitellogenesis. The tangle of fibers in the more central ooplasm appears to be continuous with the fibers in the periphery, and thus displacement of the central fibers by arrival of material from the nurse cells could displace the fibers to the cortex.

If the mRNA which had newly entered the ooplasm were bound to the actin fibers, it would also have been carried passively to the cortex. There is no direct evidence that binding to actin occurs at this stage, but the actin cytoskeleton remains intact and the mRNA is still localized in the cortex after mild detergent treatment (Kastern *et al.*, 1990). We employed CD to determine whether the distribution of mRNA and Pol II depend on the integrity of the actin cytoskeleton. CD has been shown to cap the fast growing (barbed) end of actin filaments and also to sever the existing fibers (Selden *et al.*, 1980; Schliwa, 1982). In the presence of CD *in vitro* and *in vivo*, the cortical cytoskeleton showed distinct evidence of disassembly after 4 hr and by 16 hours, no actin could be detected in the cortex by either phalloidin–TRITC or anti-actin antibody. The actin seemed to collect at the opening of the ring canals, and both phalloidin–TRITC and amanitin–FITC produced almost identical patterns of binding in wholemounts of oocytes (Figs. 3D and 3F).

Biotinylated oligo (dT) was employed to detect poly(A)$^+$ RNA in the CD experiments in place of [^{3}H]-polyuridylic acid. The pattern of the oligo (dT) hybridization was identical to that of [^{3}H]poly(U) in untreated follicles. After 4 hr CD treatment *in vivo* and *in vitro*, however, the weak signal of hybridization was found to be randomly distributed in the central ooplasm, and none was found in the cortical region of the oocyte. In contrast, strong hybridization was observed in the follicle cell and nurse cell perikarya. The CD experiment might be interpreted as indicating that actin, rather than other components of the Lepidopteran oocyte cytoskeleton, is involved in the transport, distribution, and localization of maternal mRNA from nurse cell into the oocyte. At a minimum, the CD results demonstrate that

the distribution of both mRNA and Pol II in the cortex depends on the integrity of the actin scaffolding in the cortex. It has been established that Pol II binds actin directly and with very high affinity and is very difficult to separate from actin during enzyme purification (Smith *et al.*, 1979), so that the accumulation of actin and Pol II may be explained by tight binding of the two molecules. The fact that CD simply releases mRNA from the oocyte cortex rather than causing it to migrate to the ring canal region may indicate that the interaction of mRNA and actin is weaker, or that a second molecule is involved in the binding, or that longer stretches of actin filaments are required for mRNA as opposed to protein localization.

The most parsimonious interpretation of our results would be that binding of mRNA and RNA polymerase II are independent events. The obvious relationship between the two entities may be coincidental, except that binding to the cortical cytoskeleton represents strategic localization of these critical elements for the cellularization of the blastoderm.

We dedicate this paper to the memory of two giants of American insect physiology, Howard A. Schneiderman and Carroll M. Williams. We thank Dr. Arno Greenleaf for his gift of anti-Pol II antibody and Dr. Michael Danilchik for his generous assistance with confocal microscopy.

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