Cytokeratin Phosphorylation, Cytokeratin Filament Severing and the Solubilization of the Maternal mRNA Vg1

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Abstract. During meiotic maturation, the cortical cytokeratin filament system of the *Xenopus* oocyte disappears (Klymkowsky, M. W., and L. A. Maynell. 1989. *Dev. Biol.* 134:479). Here we demonstrate that this disappearance results from the severing of cytokeratin filaments into a heterogenous population of oligomers, with S- values ranging from 12S and greater. Cytokeratin filament severing correlates with the hyperphosphorylation of the type II cytokeratin of the oocyte. Both the severing of cytokeratin filaments and cytokeratin hyperphosphorylation are reversed by treatment with cycloheximide. These data suggest that fragmentation of cytokeratin filaments is controlled, at least in part, by the phosphorylation of the type II cytokeratin, and that the cytokeratin kinase activity responsible is biosynthetically labile. Cytokeratin filaments have been suggested to anchor the maternal mRNA Vg1 to the vegetal cortex of the oocyte (Pondel, M., and M. L. King. 1988. *Proc. Natl. Acad. Sci. USA.* 85:7216). By injecting fractions containing active maturation promoting factor or a purified, mutant cyclin protein, we find that the bulk of the Vg1 mRNA in the oocyte can be solubilized under conditions that block the fragmentation of cytokeratin filaments, and that the fragmentation of cytokeratin filaments itself leads to the solubilization of only a minor fraction of the Vg1 mRNA. Thus, at best, cytokeratin filaments directly anchor only a minor fraction of the Vg1 mRNA in the oocyte. Moreover, factors distinct from maturation promoting factor appear to be required for the complete solubilization of Vg1 mRNA during oocyte maturation.

NORMALLY stable, cytoplasmic intermediate filaments (cIFs)[1] can sometimes lose their filamentous morphology. cIFs have been observed to "unravel" in response to cold shock (Schliwa and Euteneur, 1980; Tolle et al., 1987), various drugs (reviewed in Klymkowsky, 1988; Klymkowsky et al., 1989), and during mitosis (Horwitz et al., 1981; Lane and Klymkowsky, 1981; Lane et al., 1982; Franke et al., 1982, 1984; Rosevear et al., 1990). During meiotic maturation of *Xenopus* (Klymkowsky et al., 1987; Klymkowsky and Maynell, 1989), sea urchin (Boyle and Ernst, 1989), and starfish (Schroeder and Otto, 1991) oocytes, cIFs also appear to undergo a dramatic reorganization, although the physical state of the cIFs after this reorganization is less clear.

The "M-phase" reorganization of cIFs shares a number of features in common with the disassembly of the IF-like nuclear lamins. First, both nuclear lamina disassembly and cIF reorganization begins as the cell enters prometaphase. The nuclear lamina reassembles, and cIF proteins reform filaments, as the cell reenters interphase (see Gerace and Blobel, 1980; Lane et al., 1982). Second, both lamins (see Stick, 1987) and cIF subunit proteins (see Sarria and Evans, 1989) are more extensively phosphorylated during mitosis than during interphase. The kinase responsible for lamin hyperphosphorylation, and disassembly (Heald and McKeon, 1990), appears to be maturation (or M-phase) promoting factor (MPF) kinase (Ward and Kirschner, 1990; Peter et al., 1990; Dessev et al., 1991; Enoch et al., 1991).

In the case of the cIF proteins, the link between M-phase hyperphosphorylation and cIF reorganization is less well established. The in vitro phosphorylation of vimentin and desmin-type cIF proteins induces the breakup of cIFs (Inagaki et al., 1987; Geisler and Weber, 1988; Evans, 1988). Chou et al. (1990) found that the mitotic pattern of vimentin phosphorylation can be mimicked in vitro using purified MPF kinase, and that the in vitro phosphorylation of vimentin by MPF kinase induces vimentin filament disassembly. Tolle et al. (1987) reported that either cold-shock or phorbal ester–induced disruption of cytokeratin filament organization in HeLa cells was accompanied by a small, but significant increase in the level of cytokeratin phosphorylation.

On the other hand, there are also a number of reports that argue against the role of cIF phosphorylation in the control of cIF integrity. First, the injection of unregulated protein kinase A into rat embryo fibroblasts leaves cIFs intact, although with a "collapsed" intracellular organization (Lamb et al., 1989). Bravo et al. (1982) found that only a small amount of vimentin becomes "soluble" during mitosis in

1. *Abbreviations used in this paper*: cIF, cytoplasmic intermediate filament; GVBD, germinal vesicle breakdown; MPF, maturation promoting factor.

HeLa cells and that this soluble vimentin was in the dephosphorylated form. Celis et al. (1983) found that the degree of cytokeratin phosphorylation was essentially identical in cells that undergo mitotic cytokeratin filament reorganization and those that do not. Finally, Eckert and Yeagle (1988) reported that the disruption of cytokeratin organization by acrylamide is accompanied by a decrease in cytokeratin phosphorylation.

Perhaps more to the point, while lamin disassembly appears to be a universal feature of mitosis in vertebrate cells, the mitotic reorganization of cIFs is clearly not. Ishikawa et al. (1968) specifically noted the prominence of cIFs in mitotic cells. In fact, in most somatic cell types examined, the cIF system remains intact throughout mitosis, generally "collapsing" to form a cage that surrounds the mitotic spindle (Hynes and Destree, 1978; Gordon et al., 1978; Blose et al., 1979; Zieve et al., 1980; Aubin et al., 1980). Only in the region of the midbody do cIFs appear to be "cut" during the course of cytokinesis, perhaps as a result of their local disassembly (see Blose et al., 1979). Finally, even in cells that do show a mitotic reorganization of cIFs, whether or not this reorganization takes place can sometimes be affected by relatively minor changes in cell culture conditions (Tolle et al., 1987).

The *Xenopus* oocyte is uniquely suited to the study of how cIF organization/assembly is controlled. As isolated from the female, the late stage (stage V/VI) *Xenopus* oocyte is stably arrested in interphase (see Dumont, 1972). The interphase oocyte has a highly organized and asymmetric system of cytokeratin-type cIFs (see Klymkowsky et al., 1987). The egg, in contrast, is arrested in a stable metaphase configuration and contains few, if any, visible cytokeratin filaments (Klymkowsky et al., 1987). The transition between interphase-arrested oocyte and metaphase-arrested egg can be studied in cultured oocytes (Klymkowsky and Maynell, 1989). Such studies reveal that the disappearance of cytokeratin filaments begins as the oocyte enters the first meiotic M-phase, that this disappearance is induced by MPF, is independent of nuclear components, and requires protein synthesis. These studies left unresolved, however, the biochemical mechanism involved in the meiotic disappearance of cytokeratin filaments.

Here we show that the meiotic disappearance of cytokeratin filaments involves their fragmentation (or severing) into a heterogeneous population of soluble oligomers; that the type II cytokeratin of the oocyte is hyperphosphorylated during maturation; that both cytokeratin hyperphosphorylation and the severing of cytokeratin filaments are reversed by treating the oocyte with cycloheximide; and that the kinase involved in cytokeratin hyperphosphorylation appears to be distinct from MPF kinase. These data strongly suggest that cytokeratin filament fragmentation is controlled by a biosynthetically labile cytokeratin kinase activity whose translation is regulated by MPF kinase. Cytokeratin filament severing activity is one of a number of activities controlled at the translational level by MPF (see also McGrew and Richter, 1990), and we provide evidence that the formation of the meiotic spindle is also controlled, in part, at the translational level.

Finally, Pondel and King (1988) found that the maternal, vegetally localized mRNA Vg1 (Weeks and Melton, 1987; Melton, 1987) is associated with the insoluble, cytokeratin-rich fraction of oocytes, but is soluble in eggs. They suggested that the disappearance of cytokeratin filaments during oocyte maturation might be directly responsible for the solubilization of Vg1 mRNA. Using the injection of MPF-containing fractions and purified CYCΔ90 protein, we demonstrate that cytokeratin filaments can be responsible for anchoring, at most, only a minor fraction of the Vg1 mRNA within the oocyte, and that the factor responsible for releasing the bulk of the Vg1 mRNA during maturation is itself distinct from MPF kinase.

Materials and Methods

Eggs, Oocytes, Oocyte Maturation, and Drug Treatments

Eggs were isolated from hormonally primed females. Oocytes were isolated by collagenase treatment of dissected ovaries (see Klymkowsky et al., 1987). Stage V/VI oocytes, recognized by their size and unpigmented equatorial zones, were matured at room temperature using 5 μg/ml progesterone. We used batches of oocytes in which animal poles appeared in >80% of the oocytes between 3 to 5 h after the addition of progesterone or within 2 to 3 h of the injection of MPF or CYCΔ90 (see below).

For radioactive phosphate labeling, oocytes were cultured in the presence of 0.5mCi/ml carrier-free radioactive phosphate (Amersham Corp., Arlington Heights, IL) overnight at 16°C and then matured at room temperature. To block protein synthesis, oocytes were incubated with 100–500 μg/ml cycloheximide. At these concentrations, cycloheximide blocked protein synthesis by >90%, as determined by the inhibition of the incorporation of radioactive methionine into protein (data not shown). Ammonium sulfate fractions containing active *Xenopus* MPF (Lohka et al., 1988) (obtained from Guy Vigers and Manfred Lohka, UC Health Sciences Center, Denver, CO) or bacterially synthesized CYCΔ90 (Murray et al., 1989) protein (obtained from Michael Glotzer and Marc Kirschner, University of California at San Francisco), were injected into oocytes as described previously (Klymkowsky and Maynell, 1989).

mABs and Immunocytochemistry

The monoclonal antilamin antibody 14a9 and the monoclonal anti-β-tubulin antibody E7 were used to visualize the breakdown of the nuclear envelope and the formation of the meiotic spindle during oocyte maturation. E7 was also used in two-dimensional Western blots to locate β-tubulin as a position marker (see below). The monoclonal anti-type II cytokeratin antibody 1h5 (Klymkowsky et al., 1987) was used to visualize cytokeratins in both whole-mount immunocytochemistry; 1h5 and the monoclonal anti-type I cytokeratin antibody AE1 (Woodcock-Mitchell et al., 1982) were used in Western blot analyses (see below).

Xenopus oocytes are reported to contain a single type II cytokeratin and two type I cytokeratins of 46 and 42 kD (Franz et al., 1983; Gall and Karsenti, 1987). AE1 reacts only weakly with the type I cytokeratins. 1h5 reacts strongly with the type II cytokeratin and with two soluble proteins of ~90 kD (see Fig. 5, *a* and *b*). The nature of these soluble, 1h5-reactive proteins is unknown. They are present throughout oogenesis and embryogenesis and they do not react with other anti-IF antibodies, suggesting that they are not IF proteins. They do, however, provide a convenient marker, together with β-tubulin, on two-dimensional gels since neither the 90-kD 1h5-reactive proteins, nor β-tubulin are posttranslationally modified during oocyte maturation (see Fig. 5).

Whole-mount immunocytochemistry was carried out following the protocols in Klymkowsky and Hanken (1991), derived with minor modifications from Klymkowsky et al. (1987) and Dent et al. (1989). 14a9, E7, and 1h5 are available through the Developmental Studies Hybridoma Bank (Ames, Iowa).

Biochemical Analyses

Two methods were used to prepare insoluble residues from oocytes. In the first, oocytes were homogenized directly in a high salt buffer (XEX buffer: 1.5M KCl, 300mM sucrose, 50mM NaF, 10mM Tris-base, 0.5% NP-40, 10mM EGTA pH 7.4; modified from Franz et al., 1983). Typically, 20–40 oocytes were homogenized in 2 ml of XEX buffer by passing them through

a pasteur pipette until no macroscopic pieces were visible. The resulting homogenate was then centrifuged at 13,000 *g* for 15 min at 4°C. The yolk, floating on the top of the solution, and the supernatant were removed by aspiration and the pellet was resuspended in XEX buffer and the insoluble fraction was again recovered by centrifugation. Insoluble material was solubilized in either SDS-sample buffer for protein analysis or was digested with proteinase K to isolate associated RNA (see below).

During the analysis of the solubility properties of a mutant cytokeratin expressed in the oocyte, we found that XEX buffer could "salt out" the mutant protein, making it appear insoluble (Mansour and Klymkowsky, unpublished observations). We therefore adopted a second method for preparing detergent and salt-insoluble residues. In this method, oocytes were first homogenized in a more physiological buffer (SOL buffer; 140 mK KCl, 2 mM $MgCl_2$, 5 mM EGTA, 10 mM $NaPO_4$, 5 mM NaF, 0.5% Triton, pH 7.0, with NaOH). The homogenates were then centrifuged at 13,000 *g* for 15 min at 4°C, and the resulting pellets were washed once with XEX buffer, as described above.

Electrophoretic Analyses

To prepare total oocyte protein for two-dimensional gel analysis, 2–10 oocytes were solubilized in 50 μL of 9 M urea, 4% NP-40, 2% 9–11 ampholines. Two-dimensional IEF/SDS-polyacrylamide gel analysis was carried out as described in Ausubel et al. (1987). One-dimensional SDS-PAGE/Western blotting analysis was carried out as described in Klymkowsky et al. (1987). HRP-conjugated secondary antibodies (Bio-Rad Laboratories, Cambridge, MA) and either 4-chloro-1-napthol or diaminobenzidine as substrates were used for Western blots. Radioactively labeled samples were autoradiographed at −70°C on Kodak XAR film.

Gradient Analysis

Control and matured oocytes (300 each) were homogenized in an equal volume of 140 mM NaCl, 10 mM Tris-HCl, 5 mM EDTA, pH 7.4, and protease inhibitors (pepstatin, Nα-*p*-tosyl-L-arginine methyl ester, benzamidine, leupeptin and soybean trypsin inhibitor). Large aggregates were removed by centrifugation at 19,000 rpm for 40 min at 4°C in a Sorvall SS34 rotor. The soluble material was then loaded onto a 4.2-ml 5–30% wt/vol sucrose gradient and centrifuged for 18 h at 40,000 rpm in a Beckman SW60 rotor at 4°C, as described by Soellner et al. (1985). The gradient was fractioned and each fraction was analyzed by SDS-PAGE/Western blot using the monoclonal anticytokeratin antibody lh5. Cytochrome c (2S), catalase (11S), and thyroglobulin (19S) were used as sedimentation velocity markers.

RNA Isolation and Analysis

RNA was isolated from whole oocytes and insoluble oocyte residues following methods communicated to us by D. A. Melton (Harvard University). For whole oocyte RNA, 10–20 oocytes were homogenized in 1 ml proteinase K buffer (150 U/ml proteinase K, 50 mM Tris-HCl, 5 mM EDTA, 0.5% SDS, pH 7.5) and then incubated at 55°C for 1 h. The solution was extracted twice with phenol/chloroform and the aqueous phase then made 4 M LiCl. After 1 h on ice the precipitated material was collected by centrifugation (13,000 *g* for 10 min). The pelleted material was washed first with 100% ethanol and then with 70% ethanol, dried, resuspended in 50 μl RNAase-free water, and then stored at −70°C. To isolate RNA associated with insoluble oocyte residues, 10–40 oocytes were used. Oocytes were homogenized in buffers containing 10 mM vanadyl ribonucleoside complex. After the isolation of insoluble material, the pellet was digested with proteinase K, extracted with phenol/chloroform, precipitated with lithium chloride, as described for total oocyte RNA (see above).

The presence of Vg1 mRNA was assayed by Northern blot. Between 0.5 to 2 μg/lane of RNA was electrophoresed on formaldehyde/agarose gels. Gels were either stained with ethidium bromide or blotted onto immobilon N paper. Blots were probed with an antisense Vg1 RNA probe made from a plasmid supplied by Doug Melton (Harvard University) and then reprobed using an antisense DNA probe against histone H3 mRNA, made by random primer extension of an isolated H3 cDNA supplied by Mary Lou King (University of Miami Medical School). Blots were prehybridized for 1–6 h and hybridized overnight (at least 15 h). Blots were prehybridized and hybridized in 45% formamide, 0.2 M sodium phosphate, pH 7.0, 1% BSA, 7% SDS, and 1 mM EDTA. After hybridization, blots were washed twice at the hybridization temperature in 2× SSC, 1% SDS for 10 min each time and then twice with 0.2× SSC, 0.1% SDS for 30 min each time. Autoradiograms were exposed at −70°C using Kodak XAR film.

Results

Xenopus oocytes, isolated from adult females, are found at various points in phosphase of meiosis I. The late stage (Dumont stages V/VI) oocyte has a substantial, polarly organized, cortical cytokeratin filament system (Klymkowsky et al., 1987). Exposure to progesterone leads to the resumption of meiosis (see Sato and Koide, 1987). The oocytes complete meiosis I, pass through a short interphase, and arrest in metaphase of meiosis II (Fig. 1, adapted from Murray and Kirschner, 1989*a*). Entry of the oocyte into meiosis I is heralded by the appearance of the animal pole, an unpigmented region in the animal hemisphere caused by the movement of the nucleus toward the cortex, and followed by the breakdown of the oocyte nucleus, known as germinal vesicle breakdown (GVBD). The disappearance of cytokeratin filaments begins at GVBD and is complete in most batches of oocytes by 6–8 h after the addition of progesterone (at 22°C) (Klymkowsky and Maynell, 1989). Whole-mount immunocytochemistry and antilamin and antitubulin antibodies indicates that by 8 h after progesterone addition, the oocytes are in metaphase of meiosis II (data not shown).

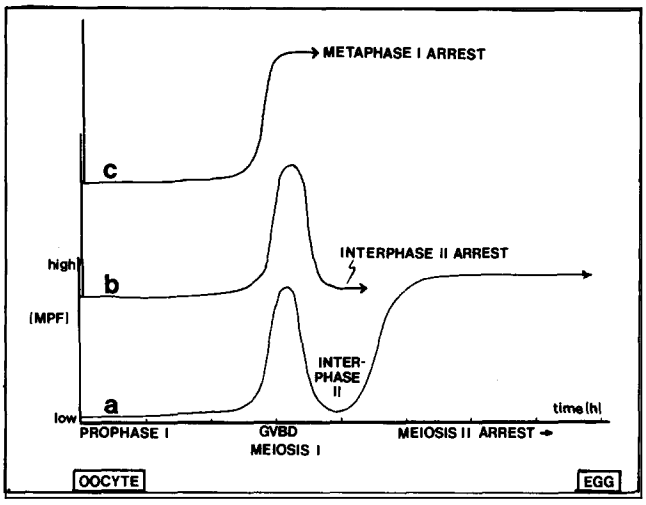

Figure 1. Major landmarks and arrest points during *Xenopus* oocyte maturation. (*a*) Normal maturation. In response to progesterone, cyclin is synthesized from maternal mRNA during "prophase I". This leads to the activation of MPF kinase that, in turn, induces GVBD and the entry into meiosis I. The rise in MPF activity, in turn, induces cyclin degradation, a decrease in MPF activity, the completion of meiosis I. The maturing oocyte then passes through an interphase-like state ("interphase II"). As cyclin is resynthesized MPF kinase is reactivated and the maturing oocyte passes into meiosis II where it arrests until fertilization. The breakdown of the cytokeratin filament system begins as the oocyte enters meiosis I. In MPF-injected oocytes there is a similar progression of events, except that GVBD occurs within 1.5 to 2 h of injection. The time scale is in hours after the addition of progesterone (time 0). (*b*) Interphase II arrest can be induced in progesterone-matured oocytes by treating them with cycloheximide shortly before GVBD (*arrow*), or by injecting oocytes with an MPF-containing fraction in the presence of cycloheximide. (*c*) Arrest in metaphase I of meiosis I can be induced by injecting oocytes with the mutant cyclin CYCΔ90. A similar arrest occurs in CYCΔ90-injected oocytes in the presence of cycloheximide, but no spindle forms (see text).

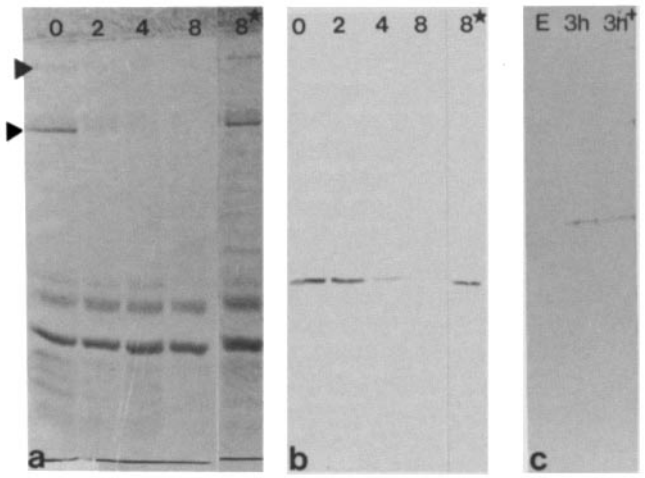

Figure 2. Cytokeratin solubilization during oocyte maturation. Oocytes were homogenized and insoluble material was collected by a 12,000-*g* 15-min centrifugation at various times (0, 2, 4, 8 h) after the addition of progesterone. This insoluble fraction was analyzed by SDS-PAGE (*a*) and Western blot (*b*) using the monoclonal anti-type II cytokeratin antibody 1h5. In addition to the type II cytokeratin, a number of other polypeptides become soluble during maturation (marked by *triangles* to the left of *a*). If cycloheximide (100 μg/ml) is added to oocytes at 6 h after exposure to progesterone, insoluble cytokeratin reappears by 8 h (lanes *8**). A similar analysis of eggs (*c*, lane *E*) indicates that cytokeratins are still "soluble". However, by 3 h (lane *3h*) after fertilization, cytokeratin has reappeared in the insoluble fraction. Addition of 0.5 mg/ml cycloheximide to fertilized eggs has little effect on the reappearance of cytokeratin in the insoluble fraction (lane *3h+*), even though it blocks first cleavage (not shown).

During Meiotic Maturation Cytokeratin Becomes Soluble

To determine the fate of the oocyte's cytokeratin filament system when it disappears during meiotic maturation, we analyzed detergent- and salt-insoluble residues of oocytes by SDS-PAGE and Western blot. Cytokeratin polypeptides, together with a number of other proteins, moved from the "insoluble" to the "soluble" fraction during the course of maturation (Fig. 2, *a* and *b*). A similar analysis of eggs indicates that cytokeratin is not present in the "insoluble" fraction (Fig. 2 *c*). After the fertilization or activation of the egg by exposure to the calcium ionophore A23187, cytokeratin reappears in the insoluble fraction (Fig. 2 *c*) and cytokeratin filaments reappear, as visualized by whole-mount immunocytochemistry (Klymkowsky et al., 1987). The reappearance of cytokeratin in the insoluble fraction during early embryogenesis occurs in the presence of cycloheximide (Fig. 2 *c*), which suggests that it is due to the reassembly of maternal cytokeratin protein, rather than to the assembly of newly synthesized cytokeratin.

Under the centrifugation conditions used to prepare insoluble residues from oocytes, i.e., 12,000 *g* for 10–15 min, a structure must have an S value of ~3,000 to pellet. To define the exact nature of the "soluble" cytokeratins, we analyzed soluble (S < 800S) fractions of both control and progesterone-matured oocytes by velocity sedimentation, SDS-PAGE, and Western blot analysis. In untreated stage V/VI oocytes, we found little cytokeratin in the 5 to 40S size range (Fig. 3, *a* and *b*). By 8 h after exposure to progesterone, a time when cytokeratin has become soluble as judged by low-speed centrifugation (Fig. 2 *b*), there was a substantial increase in the amount of cytokeratin with S values of 12 and higher (Fig. 3, *c* and *d*). The heterogenous size range of the cytokeratins in matured oocytes indicates that the cytokeratin filaments are fragmenting, more or less randomly along their length.

The Fragmentation of Cytokeratin Filaments Is Reversible

When cycloheximide is added to progesterone-treated oocytes at the time of GVBD, oocytes complete meiosis I and arrest in interphase-II of meiosis (Fig. 1). Under these conditions, not only is the fragmentation of cytokeratin filaments blocked but cytokeratin filament organization becomes more intricate and extensive than that found before the addition of progesterone (data not shown). In maturing oocytes in which cytokeratin filaments have disappeared, as monitored by whole-mount immunocytochemistry, the addition of cycloheximide induces the reappearance of a substantial cytokeratin filament system within 2 h (Fig. 4) and cytokeratin reappears in the insoluble fraction of the oocytes (Fig. 2, *a* and *b*). The effect of cycloheximide appears to be specific to maturing oocytes; treating interphase-I arrested oocytes with cycloheximide had no apparent effect on cytokeratin filament organization (data not shown).

Fragmentation of Cytokeratin Filaments Correlates with Cytokeratin Phosphorylation

Gall and Karsenti (1987) reported that the cytokeratins of the *Xenopus* oocyte and the egg are phosphorylated to the same extent. We were therefore surprised to discover that the type II cytokeratin of the oocyte consistently becomes more acidic during oocyte maturation (Fig. 5, *a–b*, and *d–f*). Labeling maturing oocytes with radioactive phosphate reveals that the maturation-induced acidification of the type II cytokeratin is due to its hyperphosphorylation (Fig. 5 *c*). The degree of type I cytokeratin phosphorylation during maturation changes very little (data not shown; see Gall and Karsenti, 1987). To determine whether the reassembly of cytokeratin filaments, induced by cycloheximide (Figs. 2, *a* and *b*, and 4), is accompanied by the dephosphorylation of the type II cytokeratin, oocytes were treated with cycloheximide for 2 h, beginning at 6 h after the addition of progesterone, and then analyzed by two-dimensional Western blot (Fig. 5, *f* and *g*). The result is a clear dephosphorylation of the type II cytokeratin in response to cycloheximide treatment.

The Relationship between MPF, Cytokeratin Filament Fragmentation, and the Insolubility of Vg1 mRNA

When fractions containing active MPF kinase are injected into oocytes they induce entry into active meiosis (Gerhart et al., 1984), disrupt cytokeratin filament organization (Klymkowsky and Maynell, 1989), and induce the solubilization of the maternal mRNA Vg1 (Fig. 6, *a* and *b*). When this same MPF-containing fraction was injected into oocytes in the presence of cycloheximide, which blocks the fragmentation of cytokeratin filaments (Klymkowsky and Maynell,

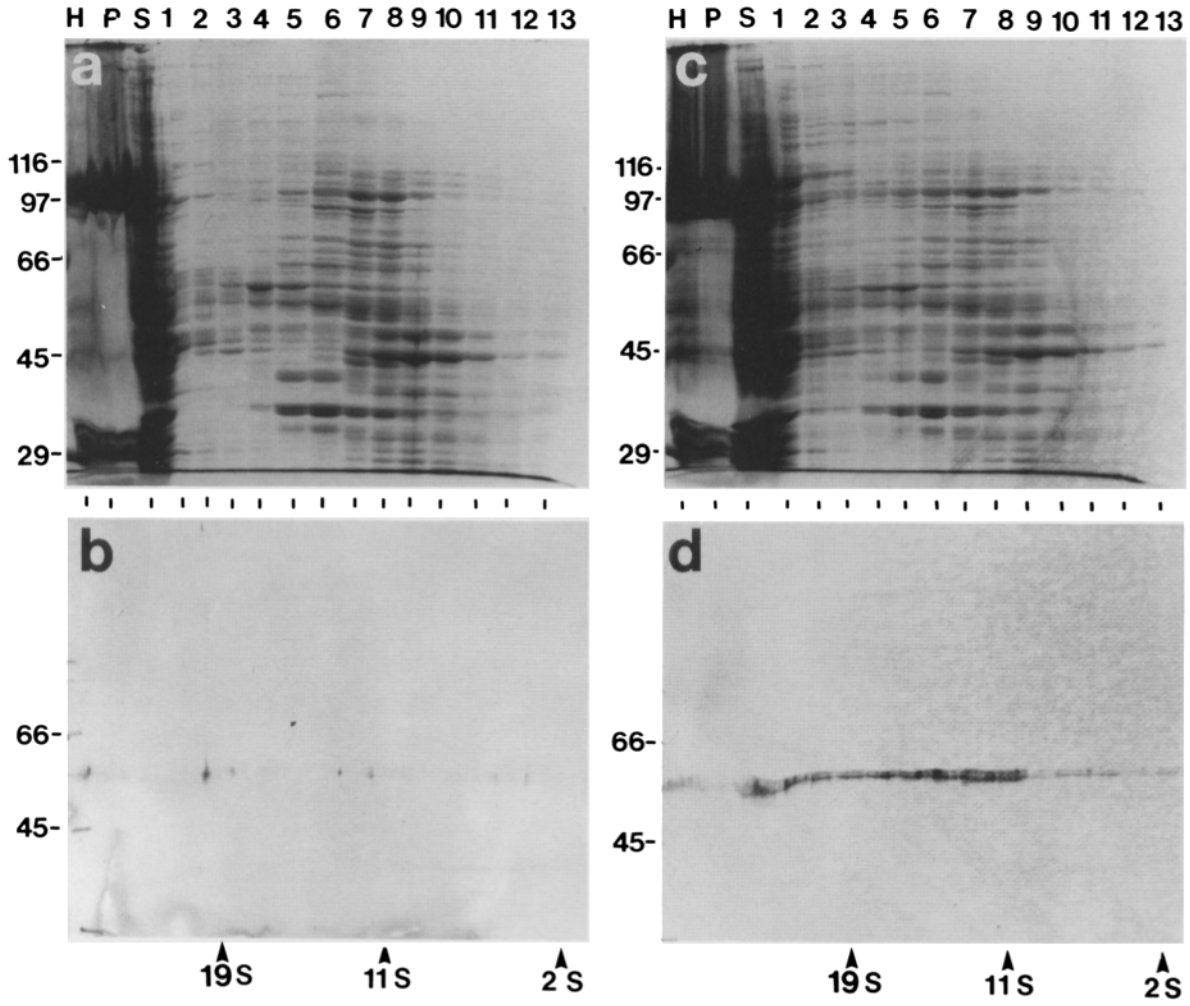

Figure 3. Gradient analysis of maturation-solubilized cytokeratin. Control (*a* and *b*) and 8-h progesterone-treated (*c* and *d*) oocytes were homogenized, a soluble fraction (S < 800S) was prepared and analyzed by velocity sedimentation. After the fractionation of the gradient, each fraction was analyzed by SDS-PAGE (*a* and *c*) and by Western blot (*b* and *d*) using the mAb 1h5. In addition, the original homogenate (*H*), the low speed pellet (*P*) and S < 800S supernatant (*S*) fractions were also analyzed on the same gel. The sedimentation patterns of the major soluble proteins in the oocyte were unchanged by maturation (compare *a* and *c*). There was, however, a dramatic increase in the amount of soluble cytokeratin, particularly in the range of 12S and greater in progesterone-matured (*d*) compared with control oocytes (*b*). Molecular weight markers are noted on the left side of *a* and *b*. Cytochrome B (2S), catalase (11S), and thyroglobulin (19S) were used as sedimentation size markers and their positions are marked along the bottom panels of *b* and *d*.

1989), there was still a substantial, but not complete, release of Vg1 mRNA from the insoluble fraction (Fig. 6 *c*). Whether the incompleteness of Vg1 mRNA release under these conditions was due to factors distinct from MPF, or to the fact that in the presence of cycloheximide, MPF-injected oocytes arrest in interphase-II of meiosis (see Fig. 1) with low MPF kinase activity (Gerhart et al., 1984) was unclear. To circumvent this problem, we injected oocytes with a bacterially synthesized mutant form of a sea urchin B-type cyclin, CYCΔ90. CYCΔ90 has its NH_2-terminal 90 amino acids deleted and is resistant to proteolytic inactivation (Murray et al., 1989). In cycling egg extracts, CYCΔ90 induces the extract to arrest in a metaphase configuration with high MPF kinase activity (Murray et al., 1989). In the oocyte, CYCΔ90 induces GVBD, metaphase I-arrest (Fig. 7, *a* and *b*), the disappearance of cytokeratin filaments (Fig. 7 *d*), and the solubilization of cytokeratin (Fig. 7 *e*). CYCΔ90-injected oocytes have levels of active MPF kinase, as monitored by histone H1 kinase levels, similar to that of progesterone-treated oocytes (Table I). However, only a small amount of the Vg1 mRNA within the oocyte was released from the insoluble fraction in response to CYCΔ90 injection (Fig. 6, *d* and *e*).

The effects of CYCΔ90 on cytokeratin filament organization were inhibited by cycloheximide (Fig. 7 *f*); cycloheximide also inhibits the formation of a meiotic spindle in CYCΔ90-injected oocytes; GVBD, however, occurs normally (Fig. 7 *c*). The release of Vg1 mRNA by CYCΔ90 was not significantly effected by the presence of cycloheximide (Fig. 6, *d* and *f*).

Discussion

In those cases where cytokeratin filaments have been found

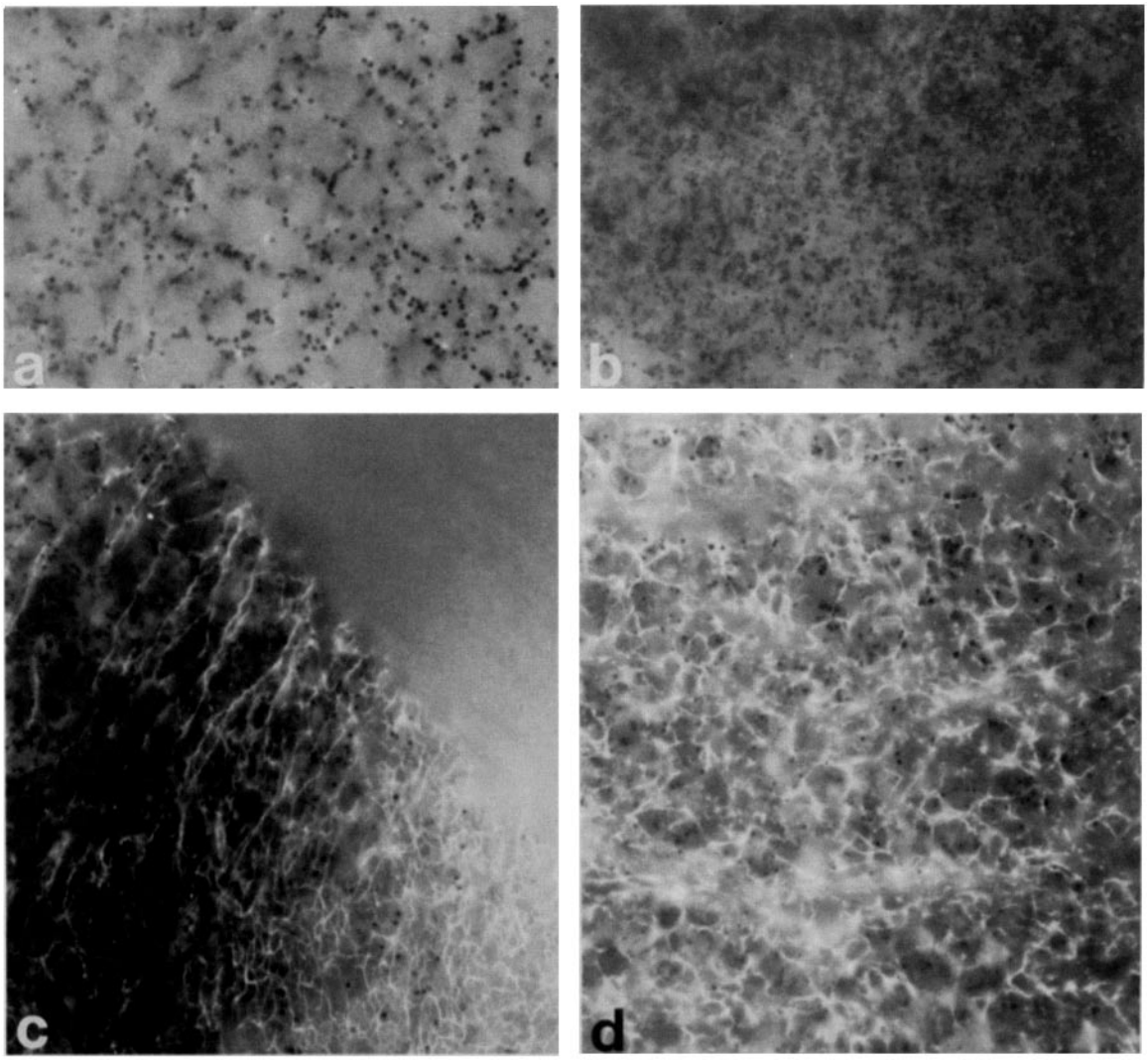

Figure 4. Cytokeratin filaments reassemble in response to cycloheximide. Oocytes were matured with progesterone for 6 h (GVBD occurring between 3 to 4 h) and then cultured for another 2 h in modified ringers alone (*a* and *b*) or in the presence of 0.5 mg/ml (*c* and *d*). Oocytes were then fixed and stained in whole-mount immunocytochemistry with the monoclonal anticytokeratin antibody 1h5. In this particular experiment, cytokeratin filaments had completely disappeared by 6 h after the addition of progesterone (not shown). In the presence of cycloheximide, a robust cytokeratin filament system reappears in both the animal (*c*) and the vegetal (*d*) hemispheres. In oocytes that had not been treated with progesterone, treatment with cycloheximide had no significant effect on the apparent organization of the cytokeratin filament system (data not shown). Note: the grey areas in this figure and Fig. 7 are due to the autofluorescence of the yolk mass; specifically-stained cytokeratin filaments appear white. Bar, 10 μm.

to reorganize in response to cold shock, drugs, or during mitosis, the reorganized cytokeratin remains in an insoluble form as dense aggregates of cytokeratin protein. The reorganization of cytokeratin filaments during *Xenopus* oocyte maturation appears to be distinctly different in that the cytokeratin filaments appear to fragment into soluble oligomers (Fig. 2). The heterogenous size distribution of these cytokeratin filament fragments (Fig. 3) argues that this process is not a true depolymerization, but rather is due to the severing of cytokeratin filaments along their length.

The mechanism by which cytokeratin filaments are severed appears to be related to, or at least correlated with, the hyperphosphorylation of the type II cytokeratin of the oocyte (Fig. 5). This cytokeratin is hyperphosphorylated as cytokeratin filaments fragment, and it is dephosphorylated when cytokeratins are induced to reassemble in response to the protein synthesis inhibitor cycloheximide (Figs. 4 and 5). Our results contradict those of Gall and Karsenti (1987) who reported that while the subcortical cytokeratin filament system of the oocyte appears to be somewhat fragmented in eggs, the cortical cytokeratin filament system remained intact and that there was only a small increase in cytokeratin solubility and no change in the phosphorylation state of cytokeratins between oocytes and laid eggs. Since they did not directly assay cortical cytokeratin filament organization, they may have been working with eggs that had been unintentionally activated, and so had reassembled their cytokeratin filament systems (see Klymkowsky et al., 1987).

The correlation between cytokeratin filament fragmentation and cytokeratin phosphorylation in maturing *Xenopus*

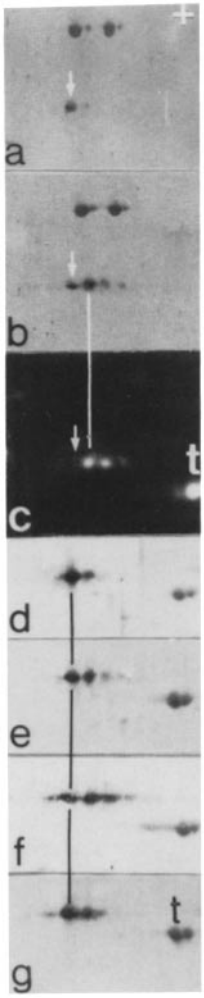

Figure 5. Cytokeratin phosphorylation and dephosphorylation during fragmentation and reassembly. Shown here are regions of 1EF/SDS-PAGE gels/Western blots of total oocyte protein (five oocytes per gel) stained with the monoclonal anticytokeratin antibody 1h5. *a* and *d* show control oocytes, *b* and *f* are blots of maturing oocytes 8 h after progesterone addition. There is a clear increase in acidic isoforms during maturation (acidic end of gels marked by "+" in *a*). In the experiment illustrated in *b*, the oocytes had been labeled with radioactive phosphate; autoradiography of the blot shown in *b* and *c* indicates that the acid cytokeratin variants are due to the phosphorylation of the type II cytokeratin. (Small arrow marks unphosphorylated cytokeratin in *a–c*; line between *b* and *c* marks position of first acidic variant. Arrowhead in *a* marks 90-kD 1h5-reactive, soluble proteins. These proteins do not appear to be phosphorylated, at least under these conditions.) To determine whether the type II cytokeratin was dephosphorylated when cytokeratin filament reassembly was induced with cycloheximide, oocytes were matured for 6 *h* (part *e*), and then incubated for a further 2 h in the presence (*g*) or the absence (*f*) of cycloheximide (0.5 mg/ml). There was a clear dephosphorylation of the cytokeratin in cycloheximide-treated oocytes (line connecting *d–g* marks position of the unphosphorylated type II cytokeratin). β-tubulin (recognized by the mAb E7) is phosphorylated in the oocyte (*t* in *C*), but its degree of phosphorylation appears unaffected by either maturation or cycloheximide treatment.

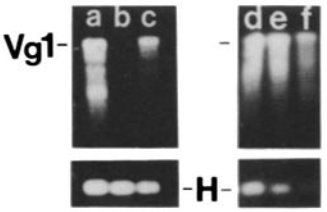

Figure 6. Effects of MPF and CYCΔ90 on Vg1 mRNA solubility. Insoluble RNA was prepared from oocytes injected with 15 nl of either buffer alone (lane *a*), an active MPF fraction (lane *b*), MPF in the presence of 100 μg/ml cycloheximide (lane *c*), CYCΔ90 (lane *e*), CYCΔ90 in the presence of cycloheximide (lane *f*), or uninjected, cycloheximide-treated oocytes (lane *d*). Animal poles appeared in both MPF and CYCΔ90-injected oocytes by 1.5 to 2 h after injection, whether or not cycloheximide was present (data not shown). RNA was prepared at 4 h after injection and 1 μg of total RNA was loaded per gel lane. After blotting, the blots were probed first with an antisense Vg1 RNA probe (*Vg1*) and then reprobed with an antisense H3 RNA probe (*H*). MPF-injection lead to the complete release of Vg1 mRNA from the cytoskeletal fraction (lane *b*); in the presence of cycloheximide, a substantial amount of Vg1 mRNA (>50%) became soluble (lane *c*). The injection of buffer alone had no effect on Vg1 mRNA's association with the cytoskeletal fraction (lane *a*). CYCΔ90 injection, in the presence or absence of cycloheximide, released little Vg1 mRNA from the insoluble fraction (lanes *e* and *f*, note: differences between lanes *e* and *f* are due to different loading, as monitored by the level of H3 mRNA). None of the conditions affected the amount of histone H3 mRNA associated with the insoluble fraction. Treatment of oocytes with cycloheximide alone had little if any effect on Vg1 mRNA's association with the cytoskeletal fraction (lane *d*). Note: figure is an autoradiograph.

oocytes is striking. The only previous report that cytokeratin phosphorylation correlated with the loss of filamentous organization of cytokeratins was that of Tolle et al. (1987) who found that cytokeratin phosphorylation increased by a factor of 1.2 to 1.6 under conditions that induce cytokeratin filament reorganization, i.e., hypotonic buffers and cold shock, or phorbal esters. Based on our results, the following conclusions concerning the cytokeratin kinase activity in the maturing *Xenopus* oocyte can be drawn. First, while MPF kinase has been implicated in the M-phase hyperphosphorylation of nuclear lamins and vimentin (see above), it is unlikely to be the cytokeratin kinase of oocytes. There are no MPF kinase consensus phosphorylation sites, i.e., a serine or threonine followed by a proline (see Pines and Hunter, 1990) in the type II cytokeratin expressed in the oocyte (Franz and Franke, 1986). Moreover, under conditions where MPF kinase activity is expected to be high, e.g., in oocytes injected with CYCΔ90 in the presence of cycloheximide, cytokeratin filaments remain intact (see Fig. 7) and the type II cytokeratin is not hyperphosphorylated (data not shown). Second, whatever the nature of the cytokeratin kinase, the rapid reassembly of cytokeratin filaments after exposure to cycloheximide suggests that it is biosynthetically labile, at least within the intact oocyte. Third, since cytokeratins have been found to be substrates for both cAMP-dependent and cAMP-independent protein kinases (see Gilmartin et al., 1984), there remain a large number of potential candidate kinases.

Nevertheless, it is clear that MPF kinase controls cytokeratin kinase activity at the translational level (Klymkowsky and Maynell, 1989; Fig. 7). In this way, cytokeratin kinase activity is similar to the activity involved in the MPF-induced cytoplasmic polyadenylation of specific mRNAs that occurs during *Xenopus* oocyte maturation (McGrew and Richter, 1990). It may also be that the inability of the meiotic spindle to form in CYCΔ90-injected, cycloheximide-treated oocytes (Fig. 7) indicates that MPF also regulates the translation of components involved in spindle assembly.

Does Cytokeratin Phosphorylation Directly Induce the Severing of Cytokeratin Filaments?

Our results are compatible with two models of cytokeratin

Table I. MPF (Histone H1) Kinase Levels

Sample	cpm incorporated*	cpm precipitated‡
Control	850	378
(DMSO)	1,390	407
Buffer	1,191	406
Injected	1,032	334
Progesterone	8,177	9,729
(8 h)	10,512	9,920
CYCΔ90	5,146	6,648
(4 h)	6,524	8,901

* Counts per minute incorporated into histone H1. Assay conditions were as described in Lohka et al. (1988).
‡ Counts per minute precipitated by p13-beads, otherwise the assay conditions were identical to those described in Lohka et al. (1988).

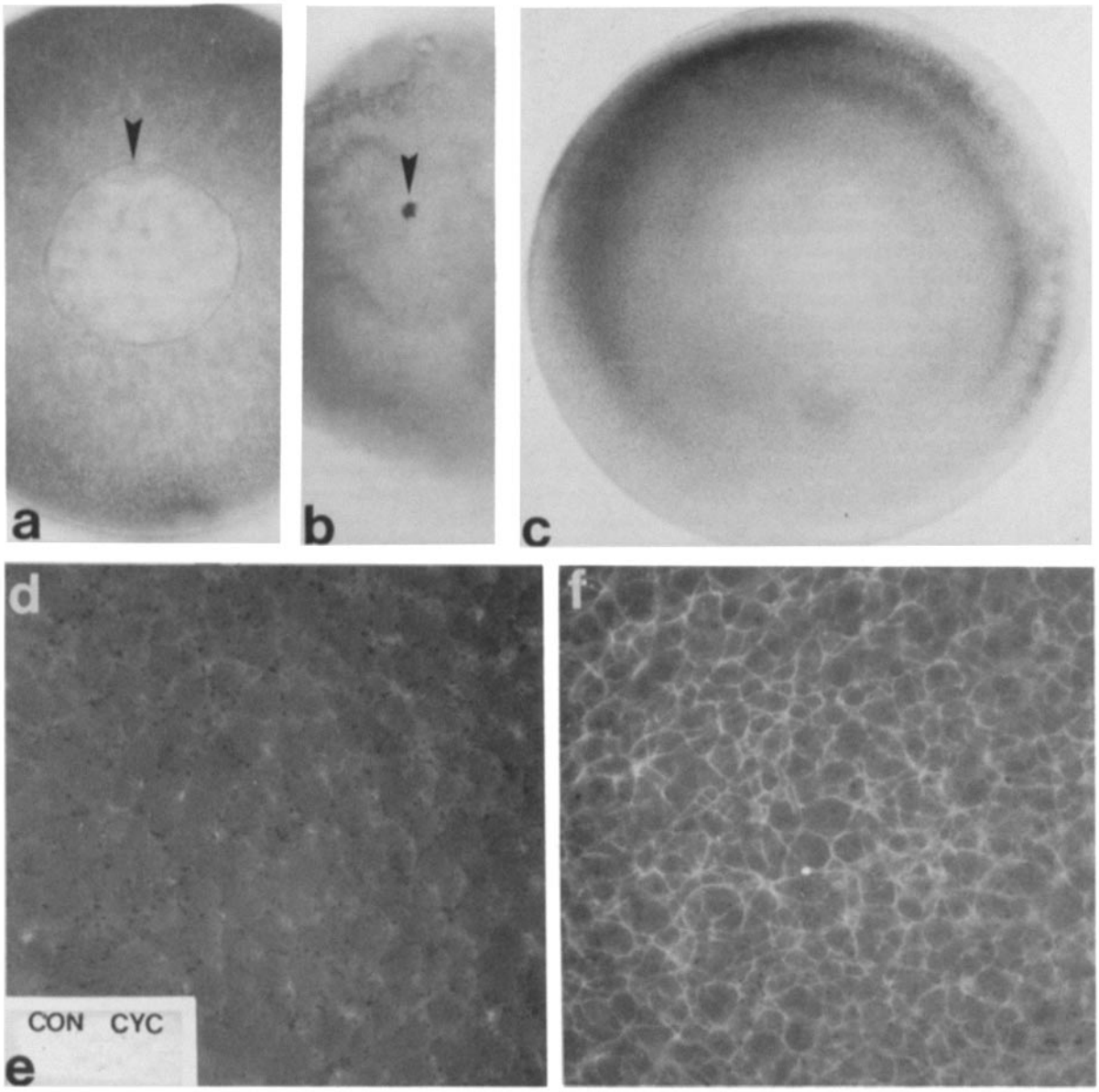

Figure 7. CYCΔ90 effects on the oocyte. The effects of injecting CYCΔ90 into oocytes were monitored by whole-mount immunocytochemistry using the antilamin antibody 14a9 and the antitubulin antibody E7 (*a–c*), cortical whole-mount immunocytochemistry using the anticytokeratin antibody 1h5 (*d* and *f*), and Western blot analysis of insoluble fractions of oocytes (*e*) using 1h5. Buffer-injected oocytes have a large intact nucleus (*a*, stained with antilamin, nucleus marked by *arrowhead*). In contrast, CYCΔ90-injected oocytes contain a prominent metaphase spindle (*b*, stained with antitubulin, spindle marked by *arrowhead*). Oocytes injected with CYCΔ90 in the presence of cycloheximide have undergone GVBD, but no spindle has been formed (*c*, stained with antitubulin). Cortical whole-mount immunocytochemistry with anticytokeratin of CYCΔ90-injected oocytes reveals only diffuse, somewhat punctate staining (*d*). Western blot analysis (*e*) of the insoluble fraction of control (*CON*) and CYCΔ90-injected (*CYC*) oocytes reveals that the cytokeratin has become soluble in response to CYCΔ90 injection. CYCΔ90-injected, cycloheximide-treated oocytes possess a robust cytokeratin filament system (*f*). Bars: (*a–c*) 100 μm; (*d* and *f*) 10 μm.

filament fragmentation. First, the phosphorylation of the type II cytokeratin could directly render the cytokeratin filament fragile, that is, more susceptible to mechanical fragmentation or local disassembly. Alternatively, the hyperphosphorylation of the type II cytokeratin could serve to target to the cytokeratin filament a "severing factor" that would then actively cut the filament. By defining the exact sites phosphorylated in the maturing oocyte, and then examining the effects of phosphorylating these sites in vitro, we should be able to distinguish between these mechanisms. In any case, it is clear that either cytokeratin phosphorylation or cytokeratin filament severing activity is actively required to maintain cytokeratin oligomers in the fragmented state. This cytokeratin filament severing activity bears some resemblance to the microtubule-severing activity recently described by Vale (1991). Given the evidence that microfilament organization is also reorganized during oocyte maturation (reviewed by Dent and Klymkowsky, 1989) it is clear

that the transition between the oocyte and the egg/early embryo involves the active reorganization of the entire cytoskeleton.

The soluble cytokeratin oligomers that form during meiotic maturation are clearly able to reassemble, either by annealing with one another, or by some other mechanism. One can ask whether there is any relationship between these cytokeratin oligomers and the normal assembly intermediates that form during the de novo synthesis and assembly of cIFs? The answer to this question is difficult to determine at present. In vitro studies of cytokeratin assembly (Hatzfeld and Weber, 1990; Columbe and Fuchs, 1990) indicate that the basic building block of cytokeratin filaments is a remarkably stable dimer of a type I and a type II cytokeratin. This dimeric species of cytokeratin has never, to our knowledge, been identified in living cells. Cytokeratin dimers are then thought to assemble into tetramers. Soluble tetrameric cytokeratin has been observed (Franke et al., 1987); however, whether this is a true assembly intermediate remains unclear. In the case of vimentin, soluble tetrameric vimentin could not be "chased" into the insoluble, cIF-containing fraction (Soellner et al., 1985) (see Isaacs et al., 1989 for discussion). Both Blikstad and Lazarides (1980) and Black et al. (1986) found that newly synthesized vimentin and neurofilament-type cIF proteins pass through a distinct soluble phase before their assembly into the insoluble, cIF-containing fraction. The exact form of this soluble cIF assembly intermediate has, however, not yet been determined. In addition to the assembly of soluble forms, there also appears to be a component of cIF assembly that involves a "cotranslational" process (see Isaacs et al., 1989), that is, in which the newly synthesized cIF protein never becomes truly soluble within the cell. The mechanism of this cotranslational assembly is obscure.

Do Cytokeratin Filaments Play a Role in the Insolubility of Vg1 mRNA?

Given the active nature of cytokeratin filament fragmentation during the maturation of the *Xenopus* oocyte, it is natural to ask whether the fragmentation of cytokeratin filaments plays an active role in *Xenopus* development. Pondel and King (1988) have proposed that the meiotic fragmentation of cytokeratin filaments may be required for the redistribution of the maternal mRNA Vg1 that accompanies oocyte maturation. In the late stage *Xenopus* oocyte Vg1 mRNA is localized to the vegetal cortex of the oocyte (Melton, 1987) and is associated with the insoluble component of the oocyte; during oocyte maturation Vg1 mRNA becomes soluble (Pondel and King, 1988; Yisraeli et al., 1990). This leads to the concentration of Vg1 mRNA in the vegetal blastomeres of the early embryo, which in turn results in a localized source of Vg1 protein (Dale et al., 1989; Tannahill and Melton, 1989). The asymmetric distribution of Vg1 protein may act as a modifier of inductive signals during embryonic development.

We have been able to test whether the fragmentation of cytokeratin filaments is either necessary or sufficient for the release of Vg1 mRNA into the soluble phase. In cycloheximide-treated oocytes injected with MPF-containing fractions, a significant amount of the Vg1 mRNA can be released even though cytokeratin filaments remain intact (Fig. 6, *a–c*). Based on this result, we expected to find a similar result when the mutant cyclin, CYCΔ90, was injected into oocytes. CYCΔ90 has been shown to activate MPF kinase and induce entry into M-phase in cycling oocyte extracts (Murray et al., 1989). The removal of NH_2-terminal domain of the CYCΔ90 protein renders it relatively resistant to proteolytic degradation (Murray et al., 1989). Since degradation of the cyclin is required for the inactivation of MPF kinase (Murray and Kirschner, 1989*a*,*b*; Murray et al., 1989; Draetta et al., 1989), CYCΔ90 induces the arrest of egg extracts in a stable metaphase-like state. In the oocyte, CYCΔ90 injection induces the activation of MPF kinase, as monitored by H1 kinase activity (Table I) and the metaphase arrest of the oocyte (Fig. 7, *a* and *b*). CYCΔ90 induces the fragmentation of cytokeratin filaments and the solubilization of cytokeratin (Fig. 7, *d* and *e*), and yet induces the release of only a minor fraction of the bound Vg1 mRNA (Fig. 6, *d–f*). Together, these results indicate that the fragmentation of the cytokeratin filament system of the oocyte is neither necessary nor sufficient to release the bulk of the Vg1 mRNA in the oocyte into the soluble phase.

Yisraeli et al. (1990) had previously reported that treating oocytes with cytochalasin B, which should specifically affect microfilament organization, induced the solubilization of ~50% of the insoluble Vg1 mRNA in the oocyte. Given these results, it seems likely that Vg1 mRNA is anchored by interaction primarily with actin filaments and that cytokeratin filaments play only a relatively minor, if any, role.

A second conclusion can be drawn from these results, namely that while MPF kinase may directly induce the release of a small fraction of the Vg1 mRNA in the oocyte, the bulk of the Vg1 mRNA is released by factors distinct from MPF kinase. The fact that crude fractions of MPF derived from maturing oocytes are capable of releasing Vg1 mRNA efficiently, suggests that the Vg1 mRNA releasing factor develops in parallel to MPF kinase during the early (pre-GVBD) period of maturation.

Based on the apparent independence of cytokeratin filament fragmentation and Vg1 mRNA solubilization (see above), we favor a model in which the true function of the maturation-induced fragmentation of cytokeratin filaments is to prepare for the assembly of a new type of cytokeratin filament system, namely that of the early embryo. The oocyte is a passive cell, intent on maintaining its own internal organization for extended periods of time. Its cytokeratin filament system presumably aids in that goal. In contrast, the early embryo is highly dynamic, first passing through a period of rapid cell division and then a process of intense morphogenic movement, culminating in gastrulation and neurulation. The distinctive embryonic cytokeratin filament system (Klymkowsky et al., 1987; Dent and Klymkowsky, 1989) presumably plays an important role in these events. Since the cytokeratin filament systems of the oocyte and the early embryo are significantly different from one another, not in composition but in organization (see Klymkowsky et al., 1987), the induced fragmentation of the oocyte's cytokeratin filaments may serve to facilitate the assembly of the embryonic system.

We thank Tracey Smith, Guy Vigers, and Manfred Lohka for performing the histone H1 kinase activity measurements and for supplying MPF fractions; Mike Glotzer and Marc Kirschner for supplying CYCΔ90 protein; Mary Lou King for histone H3 cDNA; Doug Melton for Vg1 cDNA; and Henry Sun for antibodies. We thank Karla Kirkegaard, Susan Dutcher, and Bob Boswell for their comments on the manuscript.

This work was supported by grant DCB89-0522 from the National

Science Foundation and a Pew Biomedical Scholars Award to M. W. Klymkowsky.

Received for publication 10 April 1991 and in revised form 5 May 1991.

References

Aubin, J., M. Osborn, and K. Weber. 1980. Intermediate filaments of the vimentin-type and the cytokeratin-type are distributed differently during mitosis. *Exp. Cell Res.* 129:149–165.

Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl. 1987. Current Protocols in Molecular Biology. J. Wiley & Sons, New York. 10.4.1–10.4.5.

Black, M. M., P. Keyser, and E. Sobel. 1986. Interval between the synthesis and assembly of cytoskeletal proteins in cultured neurons. *J. Neurosci.* 5:1004–1012.

Blikstad, I., and E. Lazarides. 1983. Vimentin filaments are assembled from a soluble precursor in avian erythroid cells. *J. Cell Biol.* 96:1803–1808.

Blose, S. H. 1979. Ten-nanometer filaments and mitosis: maintenance of structural continuity in dividing endothelial cells. *Proc. Natl. Acad. Sci. USA.* 76:3372–3376.

Boyle, J. A., and S. G. Ernst. 1989. Sea urchin oocytes possess elaborate cortical arrays of microfilaments, microtubules and intermediate filaments. *Dev. Biol.* 134:72–84.

Bravo, R., J. V. Small, S. J. Fey, P. Mose-Larsen, and J. E. Celis. 1982. Architecture and polypeptide composition of HeLa cell cytoskeletons. Modifications of cytoarchitectural proteins during mitosis. *J. Mol. Biol.* 154: 121–142.

Celis, J. E., P. Mose-Larsen, S. J. Fey, and A. Celis. 1983. Phosphorylation of keratin and vimentin polypeptides in normal and transformed mitotic human epithelial amnion cells: behavior of keratin and vimentin filaments during mitosis. *J. Cell Biol.* 97:1429–1434.

Chou, Y.-H., J. R. Bischoff, D. Beach, and R. D. Goldman. 1990. Intermediate filament reorganization during mitosis is mediated by *p* 34^{cdc2} phosphorylation of vimentin. *Cell.* 62:1063–1071.

Columbe, P. A., and E. Fuchs. 1990. Elucidating the early stages of keratin filament assembly. *J. Cell Biol.* 111:153–169.

Dale, L., G. Matthews, L. Tabe, and A. Colman. 1989. Developmental expression of the protein product of Vg1, a localized maternal mRNA in the frog Xenopus laevis. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:1057–1065.

Dent, J. A., and M. W. Klymkowsky. 1989. Whole-mount immunocytochemical analysis of cytoskeletal function during oogenesis and early embryogenesis in Xenopus. *In* The Cell Biology of Fertilization. H. Shatten and G. Shatten, editors. Academic Press, New York. 63–103.

Dent, J. A., A. G. Polson, and M. W. Klymkowsky. 1989. A whole-mount immunocytochemical analysis of the expression of the intermediate filament protein vimentin in Xenopus. *Development (Camb.).* 105:61–74.

Dessev, G., C. Iovcheva-Dessev, J. R. Bischoff, D. Beach, and R. Goldman. 1991. A complex containing *p* 34^{cdc2} and cyclin B phosphorylates the nuclear lamin and disassembles nuclei of clam oocytes in vitro. *J. Cell Biol.* 112:523–533.

Dumont, J. A. 1972. Oogenesis in *Xenopus laevis* (Daudin) I. Stages of oocyte development in laboratory maintained animals. *J. Morphol.* 136:153–180.

Eckert, B. S., and P. L. Yeagle. 1988. Acrylamide treatment of PtK1 cells causes dephosphorylation of keratin polypeptides. *Cell Motil. Cytoskeleton.* 11:24–30.

Enoch, T., M. Peter, P. Nurse, and E. A. Nigg. 1991. *p* 34^{cdc2} acts as a lamin kinase in fission yeast. *J. Cell Biol.* 112:797–807.

Evans, R. M. 1988. Cyclic cAMP-dependent protein kinase induced vimentin filament disassembly involves modification of the N-terminal domain of intermediate filament subunits. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 234: 73–78.

Franke, W. W., E. Schmid, C. Grund, and B. Geiger. 1982. Intermediate filamentous structures: transient disintegration and inclusion of subunit proteins in granular aggregates. *Cell.* 30:103–113.

Franke, W. W., C. Grund, C. Kuhn, V. P. Lehto, and I. Virtanen. 1984. Transient change of organization of vimentin filaments during mitosis as demonstrated by a monoclonal antibody. *Exp. Cell Res.* 154:567–580.

Franke, W. W., S. Winter, E. Schmid, P. Soellner, G. Hammerlin, and T. Achtstatter. 1987. Monoclonal cytokeratin antibody recognizing a heterotypic complex: immunological probing of conformational states of cytoskeletal proteins in filaments and in solution. *Exp. Cell Res.* 173:17–37.

Franz, J. K., and W. W. Franke. 1986. Cloning of cDNA and amino acid sequence of a cytokeratin expressed in oocytes of *Xenopus laevis*. *Proc. Natl. Acad. Sci. USA.* 83:6475–6479.

Franz, J. K., L. Gall, M. A. Williams, B. Picheral, and W. W. Franke. 1983. Intermediate-size filaments in a germ cell: expression of cytokeratins in oocytes and eggs of the frog Xenopus. *Proc. Natl. Acad. Sci. USA.* 80:6254–6258.

Gall, L., and E. Karsenti. 1987. Soluble cytokeratins in Xenopus laevis oocytes and eggs. *Biol. Cell.* 61:33–38.

Geisler, N., and K. Weber. 1988. Phosphorylation of desmin in vitro inhibits formation of intermediate filaments; identification of three kinase A sites in the aminoterminal head domain. *EMBO (Eur. Mol. Biol. Organ.) J.* 7: 15–20.

Gerace, L., and G. Blobel. 1980. The nuclear envelope lamina is reversibly depolymerized during mitosis. *Cell.* 19:277–287.

Gerhart, J., M. Wu, and M. Kirschner. 1984. Cell cycle dynamics of an M-phase-specific cytoplasmic factor in *Xenopus laevis* oocytes and eggs. *J. Cell Biol.* 98:1247–1255.

Gilmartin, M. E., J. Mitchell, A. Vidrich, and I. M. Feedberg. 1984. Dual regulation of intermediate filament phosphorylation. *J. Cell Biol.* 98:1144–1149.

Gordon, W. E., A. Bushnell, and K. Burridge. 1978. Characterization of the intermediate (10nm) filaments of cultured cells using an autoimmune rabbit antiserum. *Cell.* 13:249–261.

Hatzfeld, M., and K. Weber. 1990. The coiled coil of in vitro assembled keratin filaments is a heterodimer of type I and type II keratins: use of site specific mutagenesis and recombinant protein expression. *J. Cell Biol.* 110:1199–1210.

Heald, R., and F. McKoen. 1990. Mutations of phosphorylation sites in lamin A that prevent nuclear lamina disassembly in mitosis. *Cell.* 61:579–589.

Horwitz, B., H. Kupfer, Z. Eshhar, and B. Geiger. 1981. Reorganization of arrays of prekeratin filaments during mitosis: Immunofluorescence microscopy with multiclonal and monoclonal prekeratin antibodies. *Exp. Cell Res.* 134:281–290.

Hynes, R. O., and A. T. Destree. 1978. 10nm filaments in normal and transformed cells. *Cell.* 13:151–163.

Inagaki, M., Y. Nishi, K. Nishizawa, M. Matsuyama, and C. Sato. 1987. Site-specific phosphorylation induces disassembly of vimentin-filaments in vitro. *Nature (Lond.).* 328:649–652.

Isaacs, W. B., R. K. Cook, J. C. Van Atta, C. M. Redmond, and A. B. Fulton. 1989. Assembly of vimentin in cultured cells varies with cell type. *J. Biol. Chem.* 264:17953–17960.

Ishikawa, H., R. Bischoff, and H. Holtzer. 1968. Mitosis and intermediate sized filaments in developing skeletal muscle. *J. Cell Biol.* 38:538–555.

Klymkowsky, M. W. 1988. Metabolic inhibitors and intermediate filaments organization in human fibroblasts. *Exp. Cell Res.* 174:282–290.

Klymkowsky, M. W., and L. A. Maynell. 1989. MPF-induced breakdown of the cytokeratin filament system during oocyte maturation involves the translation of maternal mRNAs. *Dev. Biol.* 134:479–485.

Klymkowsky, M. W., and J. Hanken. 1991. Whole-mount staining of Xenopus and other vertebrates. Xenopus laevis: Practical uses in cell and molecular biology. *Methods Cell Biol.* In press.

Klymkowsky, M. W., L. A. Maynell, and A. G. Polson. 1987. Polar asymmetry in the organization of the cortical cytokeratin system of Xenopus laevis oocytes and embryos. *Development (Camb.).* 100:543–557.

Klymkowsky, M. W., J. B. Bachant, and A. Domingo. 1989. Functions of intermediate filaments. *Cell Motil. Cytoskeleton.* 14:309–331.

Lamb, N. J. C., A. Fernandez, J. R. Feramisco, and W. J. Welch. 1988. Modulation of vimentin containing intermediate filament distribution and phosphorylation in living fibroblasts by cAMP-dependent protein kinase. *J. Cell Biol.* 108:2409–2422.

Lane, E. B., and M. W. Klymkowsky. 1981. Epithelial tonofilaments: investigating their form and function using monoclonal antibodies. *Cold Spring Harbor Symp. Quant. Biol.* 46:387–402.

Lane, E. B., S. L. Goodman, and L. K. Trejdosiewicz. 1982. Disruption of the keratin filament network during epithelial cell division. *EMBO (Eur. Mol. Biol. Organ.) J.* 1:1365–1372.

Lohka, M. J., M. K. Hayes, and J. L. Maller. 1988. Purification of maturation promoting factor, an intracellular regulator of early mitotic events. *Proc. Natl. Acad. Sci. USA.* 85:3009–3013.

McGrew, L. L., and J. D. Richter. 1990. Translational control by cytoplasmic polyadenylation during Xenopus oocyte maturation: characterization of cis and trans elements and regulation of cyclin/MPF. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:3743–3751.

Melton, D. A. 1987. Translocation of a localized maternal mRNA to the vegetal pole of Xenopus oocytes. *Nature (Lond.).* 328:80–83.

Murray, A. W., and M. W. Kirschner. 1989*a*. Dominoes and clocks: The union of two views of the cell cycle. *Science (Wash. DC).* 246:614–621.

Murray, A. W., and M. W. Kirschner. 1989*b*. Cyclin synthesis drives the early embryonic cell cycle. *Nature (Lond.).* 339:275–280.

Murray, A. W., M. J. Solomon, and M. W. Kirschner. 1989. The role of cyclin synthesis and degradation in the control of maturation promoting factor activity. *Nature (Lond.).* 339:280–286.

Pines, J., and T. Hunter. 1990. *p* 34^{cdc2}: The S and M kinase? *New Biol.* 2:389–401.

Peter, M., J. Nakagawa, M. Doree, J. C. Labbe, and N. A. Nigg. 1990. In vitro disassembly of the nuclear lamina and M phase-specific phosphorylation of lamins by cdc2 kinase. *Cell.* 61:591–602.

Pondel, M., and M. L. King. 1988. Localized maternal mRNA related to transforming growth factor β is concentrated in a cytokeratin-enriched fraction from Xenopus oocytes. *Proc. Natl. Acad. Sci. USA.* 85:7612–7616.

Rosevear, E., M. McReynolds, and R. Goldman. 1990. Dynamic properties of intermediate filaments: disassembly and reassembly during mitosis in baby hamster kidney cells. *Cell Motil. Cytoskeleton.* 17:150–166.

Sarria, A. J., and R. M. Evans. 1989. Intermediate filaments in biology and

disease. *RBC Cell Biol. Rev.* 22:1–77.
Sato, E., and S. S. Koide. 1987. Biochemical transmitters regulating arrest and resumption of meiosis in oocytes. *Int. Rev. Cytol.* 106:1–33.
Schliwa, M., and U. Eutenuer. 1979. Structural transformation of epidermal tonofilaments upon cold treatment. *Exp. Cell Res.* 122:93–101.
Schroeder, T. E., and J. J. Otto. 1991. Snoods: a periodic network containing cytokeratin in the cortex of starfish oocytes. *Dev. Biol.* 144:240–247.
Soellner, P., R. Quilan, and W. W. Franke. 1985. Identification of a distinct soluble subunit of an intermediate filament protein: tetrameric vimentin from living cells. *Proc. Natl. Acad. Sci. USA.* 82:7929–7933.
Stick, R. 1987. Dynamics of the nuclear lamina during mitosis and meiosis. *In* Molecular Regulation of Nuclear Events during Mitosis and Meiosis. R. A. Schlegel, M. S. Halleck, and P. N. Roa, editors. Academic Press, New York. 43–66.
Tannahill, D., and D. Melton. 1989. Localized synthesis of the Vg1 protein during early Xenopus development. *Development (Camb.).* 106:775–785.
Tolle, H. G., K. Weber, and M. Osborn. 1987. Keratin filament disruption in interphase and mitotic cells—how is it induced? *Eur. J. Cell Biol.* 43:35–47.
Vale, R. D. 1991. Severing of stable microtubules by a mitotically activated protein in *Xenopus* egg extracts. *Cell.* 64:827–839.
Ward, G. E., and M. W. Kirschner. 1990. Identification of cell cycle regulated phosphorylation sites on nuclear lamin C. *Cell.* 61:561–577.
Weeks, D. L., and D. A. Melton. 1987. A maternal mRNA localizing to the vegetal hemisphere in Xenopus eggs codes for a growth factor related to TGFβ. *Cell.* 51:861–867.
Woodcock-Mitchell, J., R. Eichner, W. G. Nelson, and T. T. Sun. 1982. Immunolocalization of keratin polypeptides in human epidermis using monoclonal antibodies. *J. Cell Biol.* 95:580–588.
Yisraeli, J. K., S. Sokol, and D. A. Melton. 1990. A two-step model for the localization of maternal mRNA in *Xenopus* oocytes: involvement of microtubules and microfilaments in the translocation and anchoring of Vg1 mRNA. *Development (Camb.).* 108:289–298.
Zieve, G. W., S. R. Heidemann, and J. R. McIntosh. 1980. Isolation and partial characterization of a cage of filaments that surround the mammalian mitotic spindle. *J. Cell Biol.* 87:160–169.