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 heterogeneous population of oligomers, with S-values ranging from 12S and greater. Cytokeratin filament severing correlates with the hyperphosphorylation of the type H 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.

**N**ormally stable, cytoplasmic intermediate filaments (cIFs) 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; Deseve 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 phorbol 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
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 (Töll 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 VgI (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 VgI mRNA. Using the injection of MPF-containing fractions and purified CYCA90 protein, we demonstrate that cytokeratin filaments can be responsible for anchoring, at most, only a minor fraction of the VgI mRNA within the oocyte, and that the factor responsible for releasing the bulk of the VgI 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 CYCA90 (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 CYCA90 (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 1449 and the monoclonal anti-α-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 cotransformation Western blots to locate β-tubulin as a position marker (see below). The monoclonal anti-type II cytokeratin antibody lh5 (Klymkowsky et al., 1987) was used to visualize cortekinins in both whole-mount immunocytochemistry; lh5 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 Kar sens, 1987). AE1 reacts only weakly with the type I cytokeratins. lh5 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, lh5-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 lh5-reactive proteins, nor β-tubulin are posttranslationally modified during oocyte maturation (Fig. 5).

Whole-mount immunocytochemistry was carried out following the protocols in Klymkowsky and Hanksen (1991), derived with minor modifications from Klymkowsky et al. (1987) and Dent et al. (1989). 1449, E7, and lh5 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 (NEX 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 NEX buffer by passing them through...
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 mM KCl, 2 mM MgCl₂, 5 mM EGTA, 10 mM NaPO₄, 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 amphotolines. 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-naphthol 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 (pepsatin, 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% w/v sucrose gradient and centrifuged for 18 h at 40,000 rpm in a Beckman SW60 rotor at 4°C, as described by Soelner et al. (1985). The gradient was fractioned and each fraction was analyzed by SDS-PAGE/Western blot using the monoclonal anti-cytokeratin antibody h5. Cytochrome c (25k), catalase (11k), 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 protease K buffer (150 U/ml protease 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 down to 2 μg/lane of RNA was electrophoresed on formaldehyde/agarose gels. Autoradiograms were exposed at –70°C using Kodak XAR film.

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 cyclerin 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 CYCA90. A similar arrest occurs in CYCA90-injected oocytes in the presence of cycloheximide, but no spindle forms (see text).
by SDS-PAGE (a) and Western blot (b) using the monoclonal anti-
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 1b5. In addition to the type II cytokerat-
in, a number of other polypeptides become soluble during matura-
tion (marked by triangles to the left of a). If cycloheximide (100 
µg/ml) is added to oocytes at 6 h after exposure to progester-
one, 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 reap-
peared in the insoluble fraction. Addition of 0.5 mg/ml cyclohexi-
mide 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 sys-
tem when it disappears during meiotic maturation, we ana-
lized detergent- and salt-insoluble residues of oocytes by
SDS-PAGE and Western blot. Cytokeratin polypeptides, to-
gether with a number of other proteins, moved from the “in-
soluble” to the “soluble” fraction during the course of matu-
ration (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 immuno-
cytochemistry (Klymkowsky et al., 1987). The reap-
pearance of cytokeratin in the insoluble fraction during early em-
byrogenesis 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 in-
soluble 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 ana-
lized 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 proges-
terone, a time when cytokeratin has become soluble as judged
by low-speed centrifugation (Fig. 2 b), there was a substan-
tial 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 ran-
domly along their length.

**The Fragmentation of Cytokeratin Filaments Is Reversible**

When cycloheximide is added to progesterone-treated oo-
cytes at the time of GVBD, oocytes complete meiosis I and
arrest in interphase-II of meiosis (Fig. 1). Under these condi-
tions, not only is the fragmentation of cytokeratin filaments
blocked but cytokeratin filament organization becomes more
in 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 cyclo-
heximide induces the reappearance of a substantial cytokera-
tin filament system within 2 h (Fig. 4) and cytokeratin reap-
pears 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 cytoskeleton 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). La-
beling maturing oocytes with radioactive phosphate reveals
that the maturation-induced acidification of the type II cy-
tokeratin is due to its hyperphosphorylation (Fig. 5 c).
The degree of type I cytokeratin phosphorylation during matura-
tion changes very little (data not shown; see Gall and Kar-
senti, 1987). To determine whether the reassembly of cy-
tokeratin 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 cyclohexi-
mide for 2 h, beginning at 6 h after the addition of progester-
one, 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 treat-
ment.

**The Relationship between MPF, Cytokeratin Filament
Fragmentation, and the Insolubility of VGl 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 solubi-
ilization of the maternal mRNA VGl (Fig. 6, a and b). When
this same MPF-containing fraction was injected into oocytes
in the presence of cycloheximide, which blocks the fragmenta-
tion 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 lh5. 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 Vgl mRNA from the insoluble fraction (Fig. 6 c). Whether the incompleteness of Vgl 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, CYCA90. CYCA90 has its N-terminal 90 amino acids deleted and is resistant to proteolytic inactivation (Murray et al., 1989). In cycling egg extracts, CYCA90 induces the extract to arrest in a metaphase configuration with high MPF kinase activity (Murray et al., 1989). In the oocyte, CYCA90 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). CYCA90-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 Vgl mRNA within the oocyte was released from the insoluble fraction in response to CYCA90 injection (Fig. 6, d and e).

The effects of CYCA90 on cytokeratin filament organization were inhibited by cycloheximide (Fig. 7 f); cycloheximide also inhibits the formation of a meiotic spindle in CYCA90-injected oocytes; GVBD, however, occurs normally (Fig. 7 c). The release of Vgl mRNA by CYCA90 was not significantly effected by the presence of cycloheximide (Fig. 6, d and f).

Discussion

In those cases where cytokeratin filaments have been found
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 heterogeneous 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*...
be substrates for both cAMP-dependent and cAMP-independent oocyte. Third, since cytokeratins have been found to be cytoplasmic after exposure to cycloheximide, the nature of the cytokeratin kinase, the rapid reassembly remains intact (see Fig. 7) and 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 CYCA90 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 CYCA90-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

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**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           |
| CYCA90       | 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 lh5 (d and f), and Western blot analysis of insoluble fractions of oocytes (e) using lh5. 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.

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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 (Soeller et al., 1985) (see Isaacs et al., 1989 for discussion). Both Blikstad and Lazariades (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 blastomers 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 NH2-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, 1989a,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 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 morphogenetic 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.

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