Host Cell Factors Controlling Vimentin Organization in the *Xenopus* Oocyte

Joseph A. Dent, Robert B. Cary, Jeffrey B. Bachant, Alberto Domingo, and Michael W. Klymkowsky

Department of Molecular, Cellular, and Developmental Biology, University of Colorado at Boulder, Boulder, Colorado 80309-034

Abstract. To study vimentin filament organization in vivo we injected *Xenopus* oocytes, which have no significant vimentin system of their own, with in vitro-synthesized RNAs encoding *Xenopus* vimentins. Exogenous vimentins were localized primarily to the cytoplasmic surface of the nucleus and to the subplasma membrane "cortex." In the cortex of the animal hemisphere, wild-type vimentin forms punctate structures and short filaments. In contrast, long anastomosing vimentin filaments are formed in the vegetal hemisphere cortex. This asymmetry in the organization of exogenous vimentin is similar to that of the endogenous keratin system (Klymkowsky, M. W., L. A. Maynell, and A. G. Polson. 1987. *Development (Camb.).* 100:543-557), which suggests that the same cellular factors are responsible for both. Before germinal vesicle breakdown, in the initial stage of oocyte maturation, large vimentin and keratin filament bundles appear in the animal hemisphere. As maturation proceeds, keratin filaments fragment into soluble oligomers (Klymkowsky, M. W., L. A. Maynell, and C. Nislow. 1991. *J. Cell Biol.* 114:787-797), while vimentin filaments remain intact and vimentin is hyperphosphorylated. To examine the role of MPF kinase in the M-phase reorganization of vimentin we deleted the conserved proline of vimentin's single MPF-kinase site; this mutation had no apparent effect on the prophase or M-phase behavior of vimentin. In contrast, deletion of amino acids 19–68 or 18–61 of the NH_2-terminal "head" domain produced proteins that formed extended filaments in the animal hemisphere of the prophase oocyte. We suggest that the animal hemisphere cortex of the prophase oocyte contains a factor that actively suppresses the formation of extended vimentin filaments through a direct interaction with vimentin's head domain. During maturation this "suppressor of extended filaments" appears to be inactivated, leading to the formation of an extended vimentin filament system.

In the interphase cell, cytoplasmic intermediate filaments (IFs)[1] form an extensive system that encircles the nucleus and extends out to the cell periphery. The interphase organization of IFs has been shown to depend upon interactions with microtubules (see Gyovea and Gelfand, 1991), microfilaments (Hollenbeck et al., 1989; Tint et al., 1991), and the plasma membrane (Magneat and Burridge, 1985). Interactions with the nuclear envelope–nuclear lamina have also been suggested to play a role in the control of IF organization (Georgatos and Blobel, 1987*a*,*b*; Georgatos et al., 1987; Djabali et al., 1991; Papamarcaki et al., 1991).

Given the extensive interaction between IFs and other cytoplasmic components, it is not particularly surprising that during mitosis there is a dramatic reorganization of IFs. In many cell types the IF system "collapses" toward the cell center to form a cage that surrounds the mitotic spindle (Ishikawa et al., 1968; Blose, 1979; Hynes and Destree, 1978; Gordon et al., 1978; Aubin et al., 1980; Zieve et al., 1980; Blose and Bushnell, 1982; Jones et al., 1985). In the maturing *Xenopus* oocyte, IFs are transformed into soluble oligomers (Klymkowsky et al., 1991), while in a number of cultured somatic cell types IFs are transformed into nonfilamentous, insoluble aggregates (Horwitz et al., 1981; Lane et al., 1982; Franke et al., 1982, 1984; Brown et al., 1983; Kitajima et al., 1985; Chou et al., 1989; Rosevear et al., 1990). Whether the IF system "collapses" or "fragments" during M-phase is affected by seemingly minor changes in culture conditions (see Tolle et al., 1987).

Three observations implicate the phosphorylation of IF proteins in the control of the M-phase reorganization of IFs. First, IF proteins are commonly phosphorylated (Cabral and Gottesman, 1979; Gard and Lazarides, 1982; Steinert et al., 1982) and their level of phosphorylation increases during M-phase (Evans and Fink, 1982; Celis et al., 1983; Evans, 1984, 1989; Klymkowsky et al., 1991). Second, the in vitro phosphorylation of IF proteins blocks the ability of the protein to polymerize or induces the disassembly of preassembled IFs (Inagaki et al., 1987, 1988, 1990; Geisler and Weber, 1988; Evans, 1988; Ando et al., 1989; Geisler et al., 1989; Kitamura et al., 1989; Chou et al., 1990; Yano et al.,

1. *Abbreviations used in this paper*: IF, intermediate filament; MPF, maturation promoting factor; OLIGOS, oligonucleotides; PCR, polymerase chain reaction.

1991). Finally, induction of protein kinases by drugs, growth factors (Coca-Prados, 1985; Baribault et al., 1989; Escribano and Rozengurt, 1988; Huang et al., 1988; Ciesielski-Treska et al., 1991), or the direct injection of active kinase (Lamb et al., 1989) induces IF reorganization.

The *Xenopus* oocyte is a uniquely accessible system in which to study cellular factors that affect both interphase and M-phase IF organization. As isolated from the female frog, oocytes are arrested in a stable interphase-like meiotic prophase. The prophase oocyte can be induced to reenter active meiosis by treatment with progesterone, injection of maturation (or M-phase) promoting factor (MPF)-containing fractions or injection of purified cyclin protein (Maller, 1990). Moreover, these treatments can be combined with the protein synthesis inhibitor cycloheximide to arrest the oocyte at various defined points in the meiotic pathway (see Klymkowsky et al., 1991).

The *Xenopus* oocyte has a cytoplasmic keratin-type IF system (Gall et al., 1983; Franz et al., 1983; Godsave et al., 1984*a*) composed of a single type II keratin and two type I keratins (Franz et al., 1983). The oocyte has also been reported to contain a subcortical vimentin filament system (Godsave et al., 1984*b*; Torpey et al., 1990, 1992) although others have failed to find evidence for the presence of vimentin in the oocyte (Franz et al., 1983; Hermann et al., 1989; Dent, 1992—see below). In the cortex of the oocyte, keratin filaments display a marked asymmetry in their organization; short keratin filaments and punctate structures are found in the animal hemisphere while an almost geodesic system of extended keratin filaments is typical of the vegetal hemisphere (Klymkowsky et al., 1987). During oocyte maturation, the entire oocyte keratin filament system fragments into soluble oligomers that, after fertilization, reassemble into the embryonic keratin filament systems (see Klymkowsky et al., 1987, 1991).

Using the oocyte system we have examined how vimentin, assembled from protein translated from injected RNA, behaves in both the prophase and M-phase *Xenopus* oocyte. Vimentin and keratins do not copolymerize when expressed in the same cell (see Aubin et al., 1980; Henderson and Weber, 1981; Guidice and Fuchs, 1987) although they may well interact (Klymkowsky, 1982). The absence of an endogenous vimentin filament system in the cortex of the *Xenopus* oocyte permits the analysis of potentially "recessive" mutant polypeptides, uncomplicated by the presence of wild-type proteins.

In the oocyte we find evidence for a factor, localized to the cortex of the animal hemisphere, that blocks the formation of extended vimentin filaments. Based on the behavior of mutant vimentins, it appears that this factor interacts with vimentin's NH_2-terminal head domain. We also find that phosphorylation of vimentin during oocyte maturation does not induce vimentin filament disassembly, again emphasizing the fact that cell type–specific factors play an important role in determining how vimentin filaments behave during M-phase.

Materials and Methods

Antibodies, Immunofluorescence, and Immunoelectron Microscopy

The murine monoclonal antivimentin antibody 14h7 (Dent et al., 1989), the anti–type II keratin antibody 1h5, and the anti-β-tubulin antibody E7 (Klymkowsky et al., 1987) are available through the Developmental Biology Hybridoma Bank (Iowa City, Iowa). Anti-*myc* tag antibody 9E10 hybridoma cells (Evan et al., 1985) can be obtained from the Amer. Type Culture Collection (Rockville, MD). Prophase and matured oocytes (see below) were fixed and stained in whole mount (Dent et al., 1989) and examined either cortically or in section. For section-based analysis, oocytes were dehydrated, embedded in LR White, and sectioned (1–2 μm thick) using a "histo-knife" (Diatome-US, Fort Washington, PA). For immunoelectron microscopy we used a method developed to examine the effects of mutant vimentin proteins on muscle structure in *Xenopus* (Cary, R. B., and M. W. Klymkowsky, manuscript in preparation). Oocytes were fixed with 4% paraformaldehyde, 0.1% glutaraldehyde, 10 mM $MgCl_2$, 10 mM $CaCl_2$, in 150 mM sodium cacodylate, pH 7.8, overnight at 4°C. After fixation the oocytes were rinsed twice in 150 mM glycine in 150 mM sodium cacodylate (pH 7.8), dehydrated, and then infiltrated overnight with 100% LR White. The following day, oocytes were transferred to fresh LR White for 1 h, and then placed in gelatin capsules filled with LR White and polymerized for 24 h at 50°C in a vacuum oven. Sections, cut with a diamond knife, were transferred with a platinum loop onto a poly-lysine (Sigma Chemical Co., St. Louis, MO)–coated coverslip and allowed to dry at 50°C. To identify oocytes containing exogenous vimentin, 1-μm-thick sections were stained with 9E10 and fluorescein-conjugated secondary antibody and examined. Thin sections (20–50 nm) were then collected on nickel slot grids coated with a 0.5% formvar film and labeled with 9E10 and 10-nm gold–conjugated goat anti–mouse antibodies. After antibody staining, the sections were stained with uranyl acetate and lead citrate and examined. Fluorochrome-conjugated secondary antibodies were purchased from Boehringer-Mannheim Corp. (Indianapolis, IN) or Sigma Chemical Co. Peroxidase- and alkaline phosphatase–conjugated secondary antibodies were purchased from Bio-Rad Laboratories (Richmond, CA); colloidal gold–conjugated antibody was purchased from Amersham Corp. (Arlington Heights, IL).

Construction of Epitope-tagged and Mutant Vimentins

A simple scheme was used to construct plasmids for in vitro RNA synthesis (Domingo et al., 1992). *X. laevis* vimentin 1 and vimentin 2 cDNAs were amplified by polymerase chain reaction (PCR) using oligonucleotides (OLIGOS) 1 and 2, which introduce a 5′ NdeI site and a 3′ XbaI site into the amplified sequence (Fig. 1, *B* and *C*). For the PCR reaction, 1 μg of DNA and 1 μg of each OLIGO were incubated with Taq polymerase and reaction buffer (Promega Corp., Madison, WI). The samples were incubated for 3 min at 94°C, and then cycled 20 times for 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C. The reaction was then incubated at 94°C for 1 min, 72°C for 2 min for two cycles, and then 72°C for 5 min to fill in the ends and anneal the complementary strands. The vimentin 1 and vimentin 2 PCR products were glass bead purified from agarose gels (Vogelstein and Gillespie, 1979), digested with NdeI and XbaI and cloned into either pSP6.tag or pSP6.stop, modified forms of the pSP64T plasmid (Melton et al., 1984) (pSP64T supplied by D. A. Melton, Harvard University) (Fig. 1 *A*). When the amplified vimentin 1 sequence is cloned into pSP6.tag, the original vimentin start codon is reconstituted and a sequence encoding the amino acid sequence recognized by the murine mAb 9E10 (anti-*myc*) is added "in-frame" to the original COOH-terminal end (Fig. 1 *B*). In the case of vimentin 2, the final tagged product had a COOH-terminal sequence identical to that of the vimentin 1.tag construct (Fig. 1 *C*). To generate an untagged form of vimentin 1, a vimentin 1 PCR product was subcloned into pSP6.stop (Fig. 1 *A*). Δ19↔68.vimentin (V5N49) (numbers refer to amino acid sequence, with the start methionine = 1) was supplied by Jan Christian and Randy Moon (University of Washington, Seattle) (Christian et al., 1990) (Fig. 1 *D*). To create Δ18↔61.vimentin 1.tag, vimentin 1 cDNA was amplified by PCR (see above) using OLIGOS 2 and 3 (Fig. 1 *D*). OLIGO 3 anneals to bases 183–198 of the coding region of vimentin and includes a StyI site 5′ to the region homologous to vimentin. The amplified DNA was restricted with StyI and XbaI and then subcloned into pSP6.vimentin 1.tag restricted with StyI, which cuts after base pair 46, and XbaI. The resulting plasmid encodes a vimentin 1 coding region with amino acids 18 to 61 deleted (Fig. 1 *D*). Proline-54 was deleted from a vimentin 1 cDNA by site-directed mutagenesis as follows: single-stranded pBS SK^+ vector containing a full-length vimentin 1 clone was transformed into the *ung*$^-$, *dut*$^-$, *E. coli* strain CJ236. The plasmid was then isolated as single-stranded DNA; OLIGO 4 (Fig. 1 *E*) was annealed to it and used to prime replication of the plasmid with T4 polymerase (Un. States Biochem. Corp., Cleveland, OH). The resulting double-stranded DNA was transformed into the *E. coli* strain JM109. Plasmid DNA from the resulting colonies was sequenced for the presence of the appropriate mutation using OLIGO 1 to prime the se-

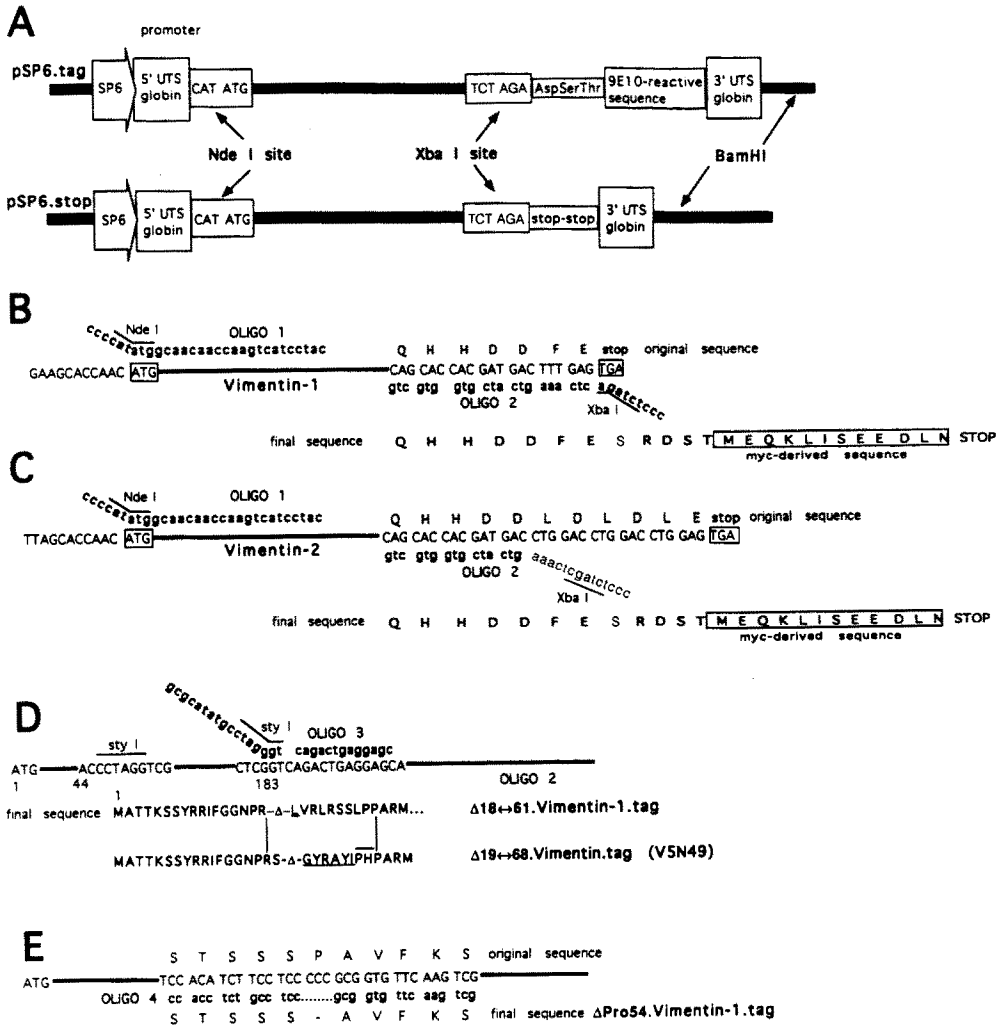


Figure 1. Vimentin constructs. To generate RNA encoding vimentin, vimentin coding regions were cloned into either of two plasmids: pSP6.tag and pSP6.stop (*A*). Both are based on the pSP64T plasmid (Melton et al., 1984); pSP6.tag has an added XbaI cloning site and an amino acid sequence recognized by the mAb 9E10; pSP6.stop has two "in-frame" stop codons between the XbaI site and the epitope tag sequence. Vimentin-coding cDNAs were amplified using OLIGOS 1 and 2 (*B* and *C*), restricted with NdeI and XbaI and cloned into pSP6.tag. This reconstitutes the start ATG codon and adds the tagging sequence "in-frame" to the COOH terminus of vimentin-1 (*B*). In the case of vimentin 2, the COOH-terminal-most six amino acids are deleted and replaced with the tagging sequence (*C*). To generate an untagged version of vimentin 1, the amplified DNA was restricted with NdeI and XbaI and cloned into pSP6.stop. This replaces the original stop codon with the sequence S.R.stop.stop. To generate the 18↔61 deletion, vimentin 1 cDNA was amplified with OLIGOS 3 and 2 (*D*); the amplified DNA was then restricted with StyI and XbaI and subcloned into the pSP6.vimentin 1.tag plasmid that had been restricted with StyI and XbaI. At the StyI junction a single new amino acid, a leucine (*underlined*) was introduced. For reference, the sequence of the 19↔68 deletion (V5N49 of Christian et al., 1990) is included—amino acids 19–68 have been deleted and six new amino acids have been inserted (*underlined*). In addition, Δ19↔68.vimentin differs from the vimentin 1 sequence characterized by Hermann et al. (1989) at two sites immediately adjacent to the deleted region (*overline*). In *E*, OLIGO 4, which was used to construct the ΔPro_{54}-deleted version of vimentin 1, is shown together with the final sequence.

quencing reaction. The mutated cDNA insert was then released from Bluescript and cloned into the NdeI and XbaI sites of pPSP6.tag to produce the pSP6.ΔPro_{54}.vimentin 1.tag plasmid (Fig. 1 *E*).

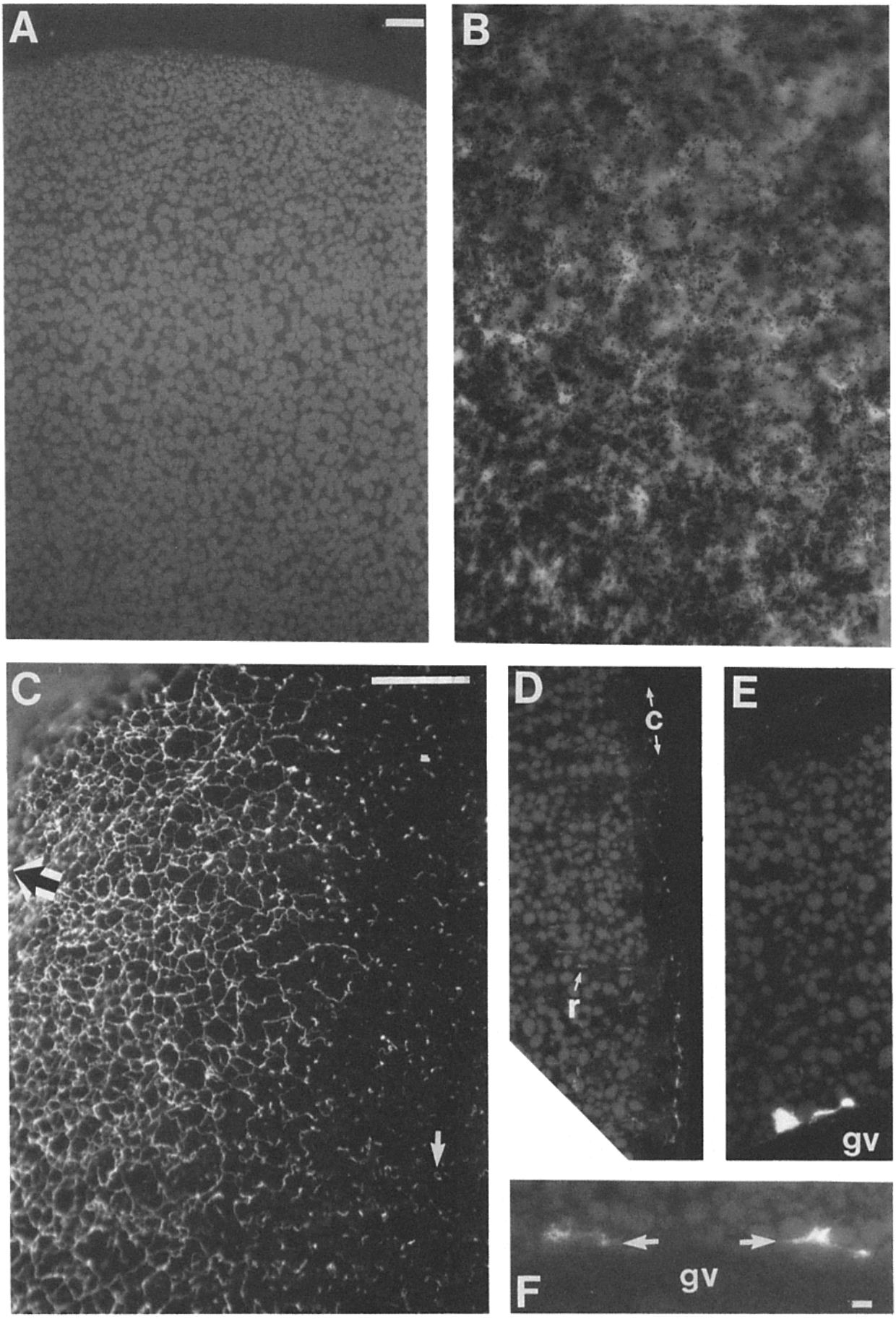
pSP6 plasmids were grown in the *E. coli* strain JM103 and purified on CsCl gradients according to Ausubel et al. (1987). To generate RNA for injection into oocytes, purified plasmid DNA was linearized by digestion with BamHI. Capped RNA was transcribed using SP6 polymerase (Ambion, Inc., Austin, TX) and cap analog (7mG[5']ppp[5']G; New England Biolabs, Beverly, MA) (Krieg and Melton, 1987). Free nucleotides were removed using a Quick Spin Sephadex G-50 column (5'→3' Inc., Boulder, CO).

Oocyte Isolation, Culture, and Manipulation

Oocytes were released from their connective tissue sheath using collagenase (Klymkowsky et al., 1987). Large (stage V/VI; Dumont, 1972) oocytes were cultured in modified Ringer's (110 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, $CaCl_2$, $NaHCO_3$, 5 mM Hepes, pH 7.8, supplemented with either 50 μg/ml gentamycin sulfate or 100 U each of penicillin and streptomycin). Oocytes were injected using a pressure-driven microneedle system (Domingo, A., manuscript in preparation) with 20–50 nl of a 0.15–0.6-μg/μl RNA solution. After culture for 14–18 h in Ringer's at 16–18°C, the oocytes were induced to mature (at room temperature, 22–25°C) by exposure to 5 μg/ml progesterone. Under these conditions oocytes typically enter into active meiosis 4–6 h after the addition of progesterone. Entry into active meiosis was judged by the appearance of the cortical white spot, although we have found cohorts of oocytes in which germinal vesicle breakdown occurs without the appearance of the white spot. Oocytes were fractionated into soluble and insoluble fractions, and insoluble fractions were analyzed by two-dimensional gel electrophoresis as described previously (Klymkowsky et al., 1991). For immunoblot analysis 14h7 and 9E10 tissue culture supernatants were used neat. Bound primary antibody was visualized using affinity-purified goat anti-mouse immunoglobulin antibody conjugated to alkaline phosphatase (diluted 1:1,000–1:2,000) and the nitrotetrazolium blue/5-bromo-4-chloro-3-indolylphosphate reaction. Blots were routinely reprobed with 1h5 (5 μg/ml) or 1h5 and the anti-β-tubulin antibody E7 (5 μg/ml) and peroxidase-conjugated anti-mouse Ig and visualized using DAB.

Results

In addition to its asymmetrically organized cortical keratin filament system (Klymkowsky et al., 1987), the late stage *Xenopus* oocyte has been reported to contain a distinct, subcortical vimentin filament system (Godsave et al., 1984*b*; Torpey et al., 1990, 1992). On the other hand, neither Franz et al. (1983) nor Hermann et al. (1989) found evidence for significant amounts of vimentin in the oocyte. We had previ-


A
B
C
D
c
r
E
gv
F
gv

ously characterized a monoclonal antibody, 14h7, that reacts with *Xenopus* vimentin (Dent et al., 1989). In collaboration with Don Sakaguchi (University of California, San Diego), we used 14h7 antibody to screen a λgt11 expression library constructed from *X. laevis* XR1 cell cDNA. Eight clones were plaque purified and subcloned into the EcoRI site of pBSII SK+ (Stratagene Inc., La Jolla, CA). Sequence analysis of the 3′ and 5′ ends of the clones with the largest inserts, clones 7 and 9, revealed that clone 7 is highly homologous to the VIM1 cDNA; clone 9 is homologous to the VIM4 clone characterized by Hermann et al. (1989) (Dent, 1992). In an in vitro transcription/translation reaction, the VIM1-like clone 7 encoded a 55-kD polypeptide while the VIM4-like clone 9 encoded a 57-kD polypeptide. These polypeptides comigrated on one- and two-dimensional gels with the 55-kD and 57-kD 14h7-reactive proteins of *Xenopus* A6 cells previously identified as vimentin (Dent, 1992). Since these two vimentins were present in every animal we have examined ($n > 20$) they appear to be the products of distinct vimentin genes (see Graf and Kobel, 1991) and we refer to them as vimentin 1 (55 kD) and vimentin 2 (57 kD).

Western blot analysis of late stage oocytes failed to reveal the presence of either vimentin 1 or vimentin 2 proteins (Dent, 1992). Similarly, immunocytochemical analysis of late-stage oocytes with 14h7 showed no staining of cortical or subcortical structures in either the animal or vegetal hemispheres (Fig. 2, *A* and *B*). When vimentin was expressed in the oocyte (see below), 14h7 reaction was observed in Western blots, whole mount, and section-based immunocytochemistry (see below).

A substantial vimentin system can be generated in the *Xenopus* oocyte by injecting oocytes with in vitro–synthesized, 5′-capped vimentin RNA. Three different vimentin RNAs were used in our studies: RNA encoding vimentin 1 (Fig. 1 *A*), or RNAs encoding epitope-tagged forms of vimentin 1 or vimentin 2 (Fig. 1, *B* and *C*). Both the untagged vimentin 1, and the epitope tagged vimentin 1 (vimentin 1.tag) and vimentin 2 (vimentin 2.tag) proteins readily incorporate into preexisting vimentin filament systems when expressed in cultured *Xenopus* or mammalian cells (data not shown). Moreover, when expressed in a cell without an endogenous vimentin filament system, they form into filaments on their own (data not shown).

To generate a vimentin filament system in the oocyte, oocytes were injected with 20–50 nl of a 0.15–0.6-mg/ml vimentin RNA solution and incubated in modified Ringer's overnight at 16°C. At this point oocytes were either analyzed or were maintained at room temperature (22–24°C) for an additional 6–8 h before analysis. Maintenance of oocytes at room temperature had no apparent effect on vimentin organization. Oocytes injected in the animal hemisphere with RNAs encoding vimentin 1 were examined using the anti-vimentin antibody 14h7; oocytes injected with RNAs encoding epitope-tagged vimentins were examined using the anti-tag antibody 9E10. Vimentin synthesized from injected RNA is not uniformly distributed in the oocyte, but rather was concentrated in a limited region, approximately one-fourth to one-fifth of the oocyte surface area (Fig. 2 *C*). Thick section analysis (1–2 μm) reveals that exogenous wild-

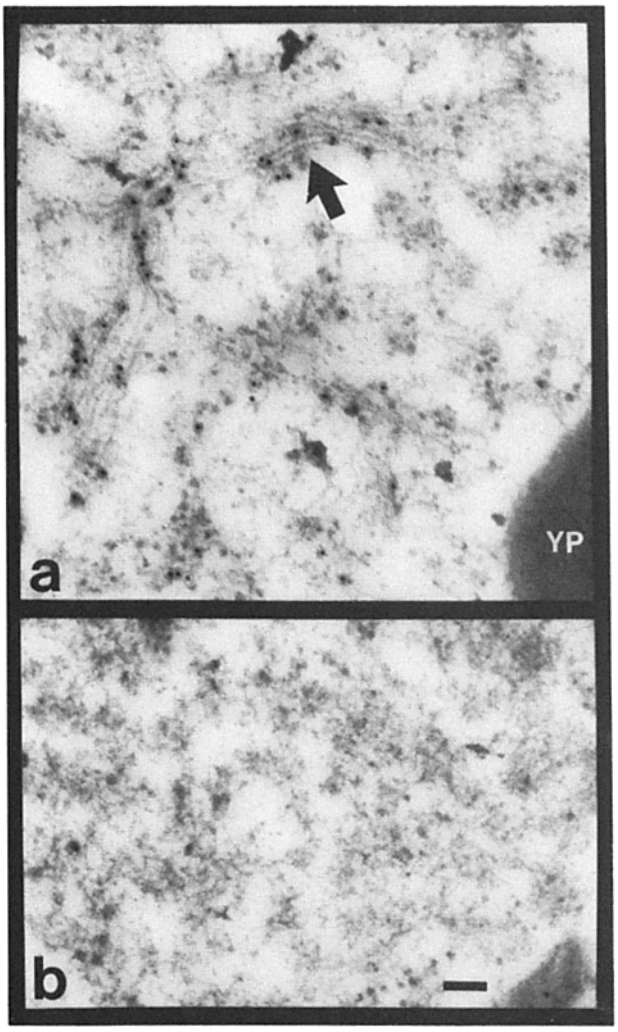


Figure 3. Immunoelectron microscopy analysis. Prophase oocytes, injected with vimentin 1.tag RNA, were examined by immunogold electron microscopy using the antitag antibody 9E10. Gold-stained filaments (*arrow*) are clearly visible in the perinuclear regions (*a*). In more superficial cytoplasmic regions (not shown) or within the nucleus (*b*) there is a very low level of nonspecific staining. *YP* marks yolk platelet in *a*. Bar, 100 nm.

Figure 2. Antibody specificity and the distribution of exogenous vimentins. Neither the anti-vimentin 1 and 2 antibody 14h7 (*A* and *B*) or the anti-tag antibody 9E10 (data not shown) stained uninjected *Xenopus* oocytes. (*A*) A 1-μm LR White section; (*B*) cortical whole mount specimen. (Note: Gray regions in *B* are due to yolk autofluorescence unshielded by cortical pigment.) When RNA encoding vimentin was injected into the oocyte, the newly synthesized protein was consistently restricted to a limited region of the oocyte cortex (*C*). (Note: This image is of a matured oocyte because it is easier to visualize the exogenous protein at lower magnification under these conditions—see below). A gradient of exogenous protein was centered on the apparent site of injection (*large arrow*). Circular structures are often visible in peripheral regions (*small arrow*). Section analysis revealed that exogenous vimentin was concentrated in the oocyte cortex (*D*; marked *c*) and at the cytoplasmic surface of the germinal vesicle (*E* and *F*; marked *gv*). Radial vimentin filaments (*r*) are also visible. (*F*) Arrows mark nuclear envelope/cytoplasmic boundary. Bars: (*A, B, D,* and *E*) 10 μm; (*C*) 50 μm; (*F*) 10 μm.

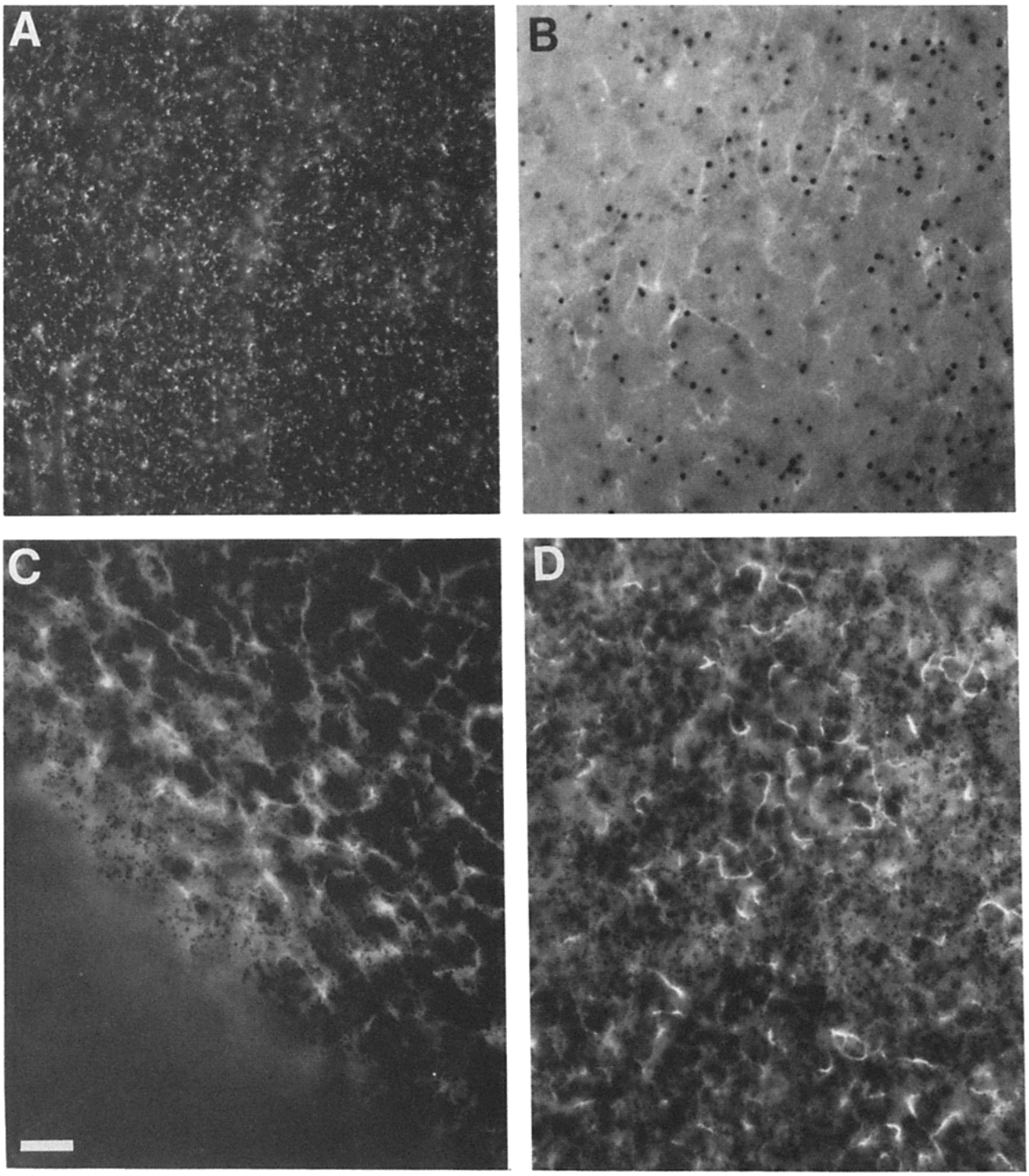


Figure 4. Asymmetric organization of exogenous vimentin. When RNA encoding vimentin 1 was injected into the animal hemisphere of an oocyte (*A*), the cortically localized vimentin formed punctate structures (see also Fig. 5, *A* and *C*). The same RNA injected into the vegetal hemisphere (*B*) produced extended vimentin filaments. When RNA encoding either Δ19↔68.vimentin (*C*) or Δ18↔61.vimentin 1.tag (*D*) was injected into the animal hemisphere, extended vimentin filaments were formed. Bar, 10 μm.

type vimentin is concentrated in the oocyte cortex (Fig. 2 *D*) and near the cytoplasmic surface of the nuclear envelope (Fig. 2, *E* and *F*). Radial filaments in the intervening region were also found but were relatively rare (Fig. 2 *D*). Immunogold electron microscopy analysis of prophase oocytes injected with vimentin RNA indicates that the aggregates of vimentin protein associated with the nucleus are in a filamentous form (Fig. 3). We have not been able to clearly visualize cortical vimentin using immunoelectron microscopy, probably because the small size of these structures makes them difficult to stain and to recognize when stained.

When RNA encoding vimentin 1 or vimentin 1.tag was in-

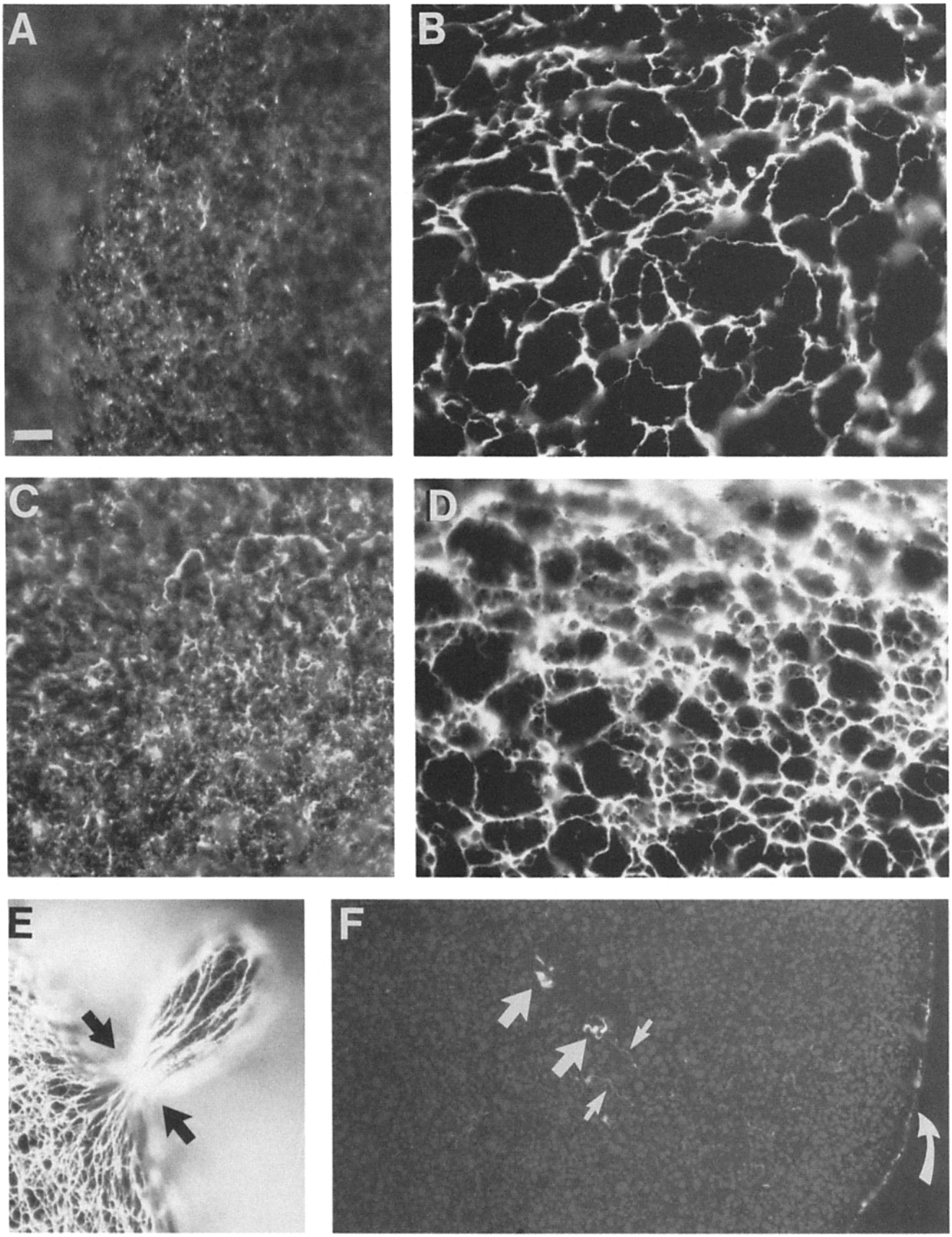


Figure 5. Maturation-induced reorganization of exogenous vimentins. In the prophase oocyte, vimentin 1.tag (*A*) and vimentin 2.tag (*C*) formed punctate structures and short filaments; upon maturation the vimentin protein reorganized into an extensive and interconnected filamentous system (*B* and *D*). During maturation, a bleb often forms at the apparent site of injection. (*E*) These blebs were densely packed with vimentin filaments (arrows mark the "neck" of the bleb). (*F*) Section analysis of matured, vimentin 2.tag RNA-injected oocytes illustrates the disappearance of the germinal vesicle and presence of large vimentin aggregates (*large arrows*), presumably derived from the nuclear-associated vimentin (see Fig. 2, *E* and *F*). Radial vimentin filaments (*small arrows*) are also present in the M-phase oocyte. The localization of the cortical vimentin appears unchanged (*curved arrow*). Bar, 10 μm.

jected into the animal hemisphere, the exogenous vimentin formed cortical structures that ranged from punctate to short filaments (Fig. 4 *A*). In contrast these same RNAs, injected into the vegetal hemisphere, produced extended, interconnected vimentin filaments (Fig. 4 *B*). This asymmetry in vimentin filament organization was similar to that seen in the endogenous keratin filament system (see Klymkowsky et al., 1987).

Christian et al. (1990) reported that the region of the vimentin NH_2-terminal head domain between amino acids 19 and 68 could be deleted without affecting the ability of the mutant protein to form filaments in the *Xenopus* embryo. We generated a similar mutant from vimentin 1.tag in which amino acids 18 through 61 have been deleted (Δ18↔61.vimentin 1.tag) (Fig. 1 *D*). When RNA encoding either Δ18↔61.vimentin 1.tag or Δ19↔68.vimentin was injected into the animal hemisphere of the prophase oocyte, the mutant polypeptides formed extensive cortical vimentin filament systems (Fig. 4, *C* and *D*) similar to the extended filaments formed by wild-type vimentin in the vegetal hemisphere (Fig. 4 *B*).

Maturation-induced Changes in Vimentin Organization

When oocytes injected in the animal hemisphere with vimentin 1, vimentin 1.tag or vimentin 2.tag RNA were matured by exposure to progesterone, there was a dramatic reorganization of vimentin. In place of the short scattered filaments and punctate structures observed in the prophase oocyte (Fig. 5, *A* and *C*), massive filament bundles were found (Fig. 5, *B*, *D*, and *E*). The M-phase system of vimentin filament bundles is similar to that formed by the endogenous keratin system before its fragmentation (data not shown). Section analysis reveals that deep within the oocyte there are large aggregates and radial fibers of vimentin (Fig. 5 *F*), presumably derived from the germinal vesicle–associated vimentin present in the prophase oocyte (see Fig. 2, *E* and *F*).

Western blot analyses of two-dimensional gels of prophase and matured oocytes revealed a clear acidification of the exogenous vimentin during maturation (Fig. 6, *A* and *B*). Autoradiographic analysis of $^{32}PO_4$-labeled oocytes indicates that this acidification is due to phosphorylation (Fig. 6, *B* and *C*). Fractionation of vimentin RNA-injected oocytes reveals the presence of a substantial soluble pool of both 7S (i.e., tetrameric; Soellner et al., 1985) vimentin (data not shown) and of insoluble vimentin (Fig. 6, *D* and *E*). During maturation there was no consistent increase in either the amount of insoluble (Fig. 6, *D* and *E*) or total (data not shown) vimentin. Differences in staining intensities can be seen (Figs. 6, *A* and *B*, and 7, *A* and *B*) but are not reproducible from experiment to experiment and may be due to variations in extraction and solubilization of the protein. Typically the amount of vimentin before and after maturation is similar (i.e., within twofold) as determined by the intensity of the Western blot reaction. Moreover, the level of phosphorylation of the insoluble vimentin is quite similar to that seen for total (i.e., soluble + insoluble) vimentin (compare Figs. 6 *B* and 7 *b* with 6 *D*), indicating that neither soluble nor insoluble forms are preferentially phosphorylated. It remains possible, however, that the sites of phosphorylation on the two forms are not identical.

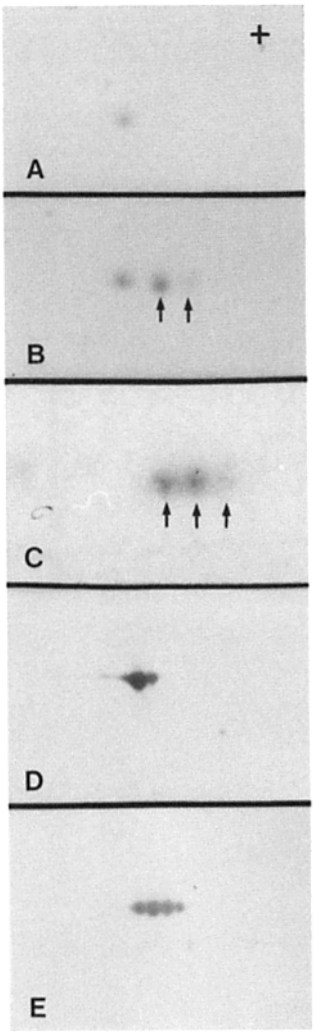


Figure 6. Western blot analysis of vimentin modification. Oocytes were injected with RNA encoding vimentin 2.tag, cultured in 0.5 mCi/ml carrier-free $^{32}PO_4$ for 12 h, and then matured with progesterone. After 6 h, both control and matured oocytes (five each) were analyzed by two-dimensional gel electrophoresis/Western blot. In the prophase oocytes (*A*) there was a single 9E10-reactive polypeptide visible. In contrast, in matured oocytes (*B*) a number of more acidic isoforms were present. (*C*) Autoradiography of the blot in *B* revealed that these acidic isoforms contained ^{32}P. No radioactivity was observed in the single vimentin isoform present in *A* (data not shown) or in the most basic isoform visible in *B*. (*D* and *E*) In a second experiment, oocytes were injected with vimentin 2.tag RNA. Control and progesterone-matured oocytes were fractionated into soluble and insoluble fractions; the insoluble fraction was analyzed by two-dimensional gel electrophoresis/Western blot. In the prophase oocytes (*D*) the bulk of the vimentin was unphosphorylated. Upon maturation (*E*) the amount of unphosphorylated vimentin decreased and two new, acidic vimentin isoforms appeared.

Role of Phosphorylation in Vimentin Reorganization

Chou et al. (1990) reported that phosphorylation of hamster vimentin by MPF kinase induces the structural transformation of vimentin filaments in vitro. In hamster vimentin, this M-phase phosphorylation occurs primarily at a single conserved site, $serine_{55}$-$proline_{56}$ (Chou et al., 1991). A similar sequence exists in both *X. laevis* vimentins ($serine_{53}$-$proline_{54}$) (see Hermann et al., 1989) and is the only minimal MPF kinase consensus sequence (Maller, 1990; Moreno and Nurse, 1990; Pines and Hunter, 1990) present. We removed $proline_{54}$ from *X. laevis* vimentin 1 by site-directed mutagenesis (Fig. 1 *E*); the ΔPro_{54}.vimentin 1.tag protein forms short filaments and punctate structures in the animal hemisphere of oocytes; these structures appear identical to those formed by wild-type vimentin (compare Fig. 7 *e* with Figs. 3 *a* and 5 *A*). During maturation, the ΔPro_{54}.vimentin 1.tag filaments undergo a change in organization identical to that observed for wild-type vimentin (Fig. 7 *f*). Western blot analysis of two-dimensional gels indicated that the degree of ΔPro_{54}.vimentin 1.tag's phosphorylation in the M-phase oocyte was markedly less than that seen for wild-type vimentins (Fig. 7, *c* and *d*). The mutant vimentins Δ19↔68.vimen-

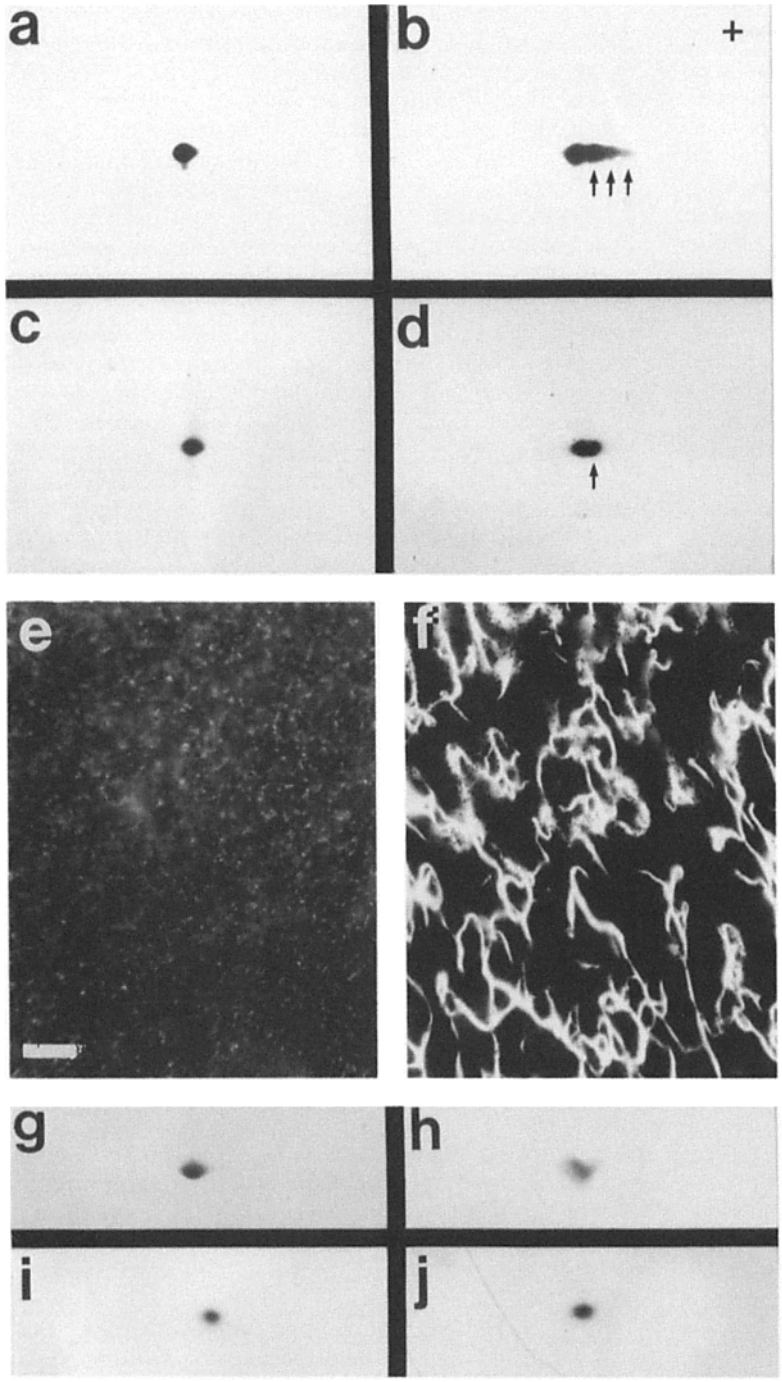


Figure 7. Mutational analysis of vimentin phosphorylation. In these experiments, oocytes injected with vimentin 1.tag RNA (*a* and *b*), ΔPro_{54}.vimentin 1.tag RNA (*c–f*), Δ19↔68.vimentin (*g* and *h*), or Δ18↔61.vimentin-1.tag (*i* and *j*) were matured and analyzed by either two-dimensional gel electrophoresis/Western blot (*a–d* and *g–j*) or whole mount immunocytochemistry (*e* and *f*). As in the case of vimentin 2.tag, vimentin 1 became hyperphosphorylated upon maturation (*a*, prophase; *b*, matured). The extent of the M-phase hyperphosphorylation of vimentin was reduced by the deletion of proline-54 (*c*, prophase; *d*, M-phase). Nevertheless, the ΔPro_{54}.vimentin 1.tag protein behaves in essentially the same way as the wild-type protein, as visualized by whole mount immunocytochemistry (*e*, prophase; *f*, matured). The extent of phosphorylation of both the Δ19↔68.vimentin and Δ18↔61.vimentin 1.tag proteins changed little during maturation (*g* and *i*, prophase; *h* and *j*, matured). Since both formed extensive filament systems in the prophase oocyte (see Fig. 3, *C* and *D*), it was difficult to determine whether any further reorganization occurred during oocyte maturation. Bar, (*e* and *f*) 10 μm.

tin and Δ18↔61.vimentin 1.tag also have the MPF kinase site deleted, together with most of their other known phosphorylation sites (see Christian et al., 1990). During oocyte maturation, both failed to be hyperphosphorylated (Fig. 7, *G–J*). However, since both Δ19↔68.vimentin and Δ18↔61.vimentin 1.tag form extensive filaments in the animal hemisphere of the prophase oocyte (Fig. 4, *C* and *D*) changes in their organization during maturation were necessarily less dramatic than those seen for the wild-type vimentins. We were therefore unable to unambiguously discern maturation-induced changes in the organization of Δ19↔68.vimentin and Δ18↔61.vimentin 1.tag filaments.

Discussion

The fact that in vitro IF proteins readily assemble from purified, denatured protein has, we think, led to a relative neglect of the role of host cell factors in the control IF organization in vivo. The behavior of vimentins, expressed in the cortex of the *Xenopus* oocyte, provides a dramatic illustration of the interaction between IF proteins and host cell factors. The large size of the oocyte and its high degree of cellular asymmetry (for review see Dent and Klymkowsky, 1989) makes it possible to identify specific regional differences in the behavior of exogenous vimentin. First, there is a clear

localization of exogenous vimentin to the cortex of the oocyte and to the surface of the nuclear envelope (Fig. 2). This localization is due, presumably, to the interaction between vimentin and specific cellular components localized to these regions. The nature of these components is not yet completely clear, but strong candidates can be proposed (for review see Klymkowsky et al., 1989). For example, spectrin (Magneat and Burridge, 1985), ankyrin (Georgatos and Marchesi, 1985; Georgatos et al., 1985, 1987), filensin (Merdes et al., 1991), and an as yet unnamed lamin B–cross reactive protein (Cartaud et al., 1990) are associated with the plasma membrane and have been found to interact with vimentin. They could act to direct or to anchor vimentin to the cortex. Similarly, there are ultrastructural data that IFs interact laterally with as yet unidentified components of the nuclear surface (Carmo-Fonseca et al., 1987) and *in vitro* data that nuclear lamins interact with IF proteins (Georgatos and Blobel, 1987*a*,*b*; Georgatos et al., 1987; Djabali et al., 1991; Papamarcaki et al., 1991). In addition, there is clear evidence that IFs are moved around in the cell through interactions with microtubules and microfilaments (see Gyoeva and Gelfand, 1991; Hollenbeck et al., 1989; Tint et al., 1991). Such transport movements, by themselves, could generate much of the subcellular localization of IFs seen in the oocyte.

The oocyte provides evidence for a distinctly different type of factor that influences the organization, rather than just the intracellular distribution, of IFs. The vimentin that localizes to the cortex of the animal hemisphere is organized into short filaments and punctate structures (Figs. 4 *A* and 5, *A* and *C*). In the vegetal hemisphere cortex, in contrast, exogenous vimentin forms large extended filaments (Fig. 4 *B*). This asymmetry in the organization of exogenous vimentin is similar to that seen in the oocyte's endogenous keratin filament system (Klymkowky et al., 1987) and suggests that the same, cortically localized cellular factor(s) affects both vimentin and keratin filament systems.

Three lines of evidence indicate that the difference in the organization of IFs in the animal and vegetal hemispheres is due to the active suppression of extended IF organization in the animal hemisphere. First, it is clear that the wild-type vimentins used in this study are quite capable of forming extended filaments. For example, in the vegetal hemisphere cortex (Fig. 4 *B*) and in association with the oocyte nucleus (Fig. 3) exogenous vimentin forms extended filaments. Second, mutations in vimentin that remove internal regions of the NH_2-terminal head domain form extended filament systems when expressed in the embryo (Christian et al., 1990) and in the animal hemisphere of the oocyte (Fig. 4, *C* and *D*). Finally, during oocyte maturation there is a dramatic appearance of extended vimentin filaments (Figs. 2 *C* and 5) in the animal hemisphere. Biochemical analysis of prophase and matured oocytes indicates that there is no consistent increase in the amount of total or insoluble vimentin (Figs. 6 and 7) during oocyte maturation, arguing that the apparent increase in filaments is not due to the de novo synthesis of vimentin or the assembly of soluble vimentin into filaments, but rather to the reorganization of preexisting insoluble forms of vimentin into extended filaments. Taken together, these data indicate that in the animal hemisphere cortex of the prophase oocyte the formation of extended vimentin and keratin filaments is actively inhibited and that during maturation this inhibition is released. Moreover, the behavior of the $\Delta 19 \leftrightarrow 68$ and $\Delta 18 \leftrightarrow 61$ vimentins suggests that the integrity of the NH_2-terminal head domain is required for suppressing the formation of extended vimentin filaments.

In many aspects, the behavior of keratin in the oocyte is similar to that of vimentin. Keratin organization is also asymmetric, with punctate structures and short filaments present in the animal hemisphere cortex and extended keratin filament bundles in the vegetal hemisphere (Klymkowsky et al., 1987). During the first stage of oocyte maturation, large keratin filament cables appear in the animal hemisphere (data not shown). The difference between keratin and vimentin filament behavior during oocyte maturation is that keratin filaments disassemble into soluble oligomers as maturation continues (Klymkowsky and Maynell, 1989; Klymkowsky et al., 1991), while vimentin filaments do not.

During *Xenopus* oocyte maturation, MPF kinase is activated. Chou et al. (1990) had previously reported that MPF kinase phosphorylates hamster vimentin and induces vimentin filament disassembly in vitro. They had suggested that MPF kinase was the direct cause of the M-phase reorganization of vimentin filaments observed in vivo. Given that MPF kinase is activated to high levels during *Xenopus* oocyte maturation (see Kuang et al., 1991), we expected vimentin filaments to be disassembled in the M-phase oocyte. Surprisingly, vimentin filaments remain intact during maturation (Fig. 5), even though vimentin is hyperphosphorylated (Fig. 6). The decreased level of M-phase phosphorylation in the mutant vimentin ΔPro_{54}.vimentin 1.tag (Fig. 7, *a–d*), in which the conserved proline of the single MPF kinase phosphorylation site has been deleted (Fig. 1 *E*), argues that vimentin is a substrate for MPF kinase (or a kinase with MPF-like specificity). Phosphorylation of vimentin by MPF kinase, however, does not induce vimentin filament disassembly or otherwise affect the M-phase behavior of vimentin (Figs. 5 and 7), at least in the *Xenopus* oocyte. In the course of our studies, we have found batches of oocytes in which the maturation-induced reorganization of vimentin filaments occurred without a concomitant increase in vimentin phosphorylation; keratin hyperphosphorylation and keratin filament disassembly, on the other hand, did occur in these oocytes (data not shown). Whether phosphorylation of vimentin plays any role in the M-phase reorganization of vimentin *in vivo*, therefore, remains very much an open issue.

In summary, the formation of an extensive vimentin filament system in the animal hemisphere of the maturing *Xenopus* oocyte appears to be due to the inactivation of a factor that normally suppresses the formation of extended IFs in the prophase oocyte. Based on the ability of mutant vimentins, with deletions in the NH_2-terminal head region, to form extended filaments in the animal hemisphere of the oocyte, we would argue that it is likely that this "suppressor of extended filaments" interacts directly with the head domain of vimentin. It has previously been shown that anti-IF antibodies (see Klymkowsky et al., 1983), the adenovirus-encoded protein E1B (White and Cipriani, 1989), and the E1-E4 protein of human papillomavirus (Doorbar et al., 1991) can bind to and disrupt normal IF organization. The suppressor factor of the *Xenopus* oocyte may act in a similar manner. Modification of the factor during oocyte maturation presumably leads to its inactivation and the subsequent formation of extended

IFs. We have begun in vitro studies using cytoplasmic extracts from prophase and M-phase oocytes and purified bacterially synthesized vimentin to attempt to develop an assay that could lead to the purification of this suppressor factor.

We thank Don Sakaguchi, Bill Harris, and Mike Crepeau for isolating and help in the initial characterization of the original λgt11 clones; Jan Christian and Randy Moon for the vimentin mutant, V5ND49; Doug Melton for pSP64T plasmid; and Bob Evans and Susan Dutcher for their helpful comments on the manuscript.

This work was supported by grants DCB89-0522 and DCB91-05523 from the National Science Foundation (NSF) and a grant from the Colorado Chapter of the American Heart Association. A portion of this work was submitted as part of a Ph.D. thesis at the University of Colorado at Boulder (Dent, 1992); J. A. Dent was supported by an NSF predoctoral fellowship and a training grant from the National Institutes of Health.

Received for publication 20 May 1992 and in revised form 14 August 1992.

References

Ando, S., K. Tanabe, Y. Gonda, C. Sato, and M. Inagaki. 1989. Domain- and sequence-specific phosphorylation of vimentin induces disassembly of the filament structure. *Biochemistry.* 28:2974–2979.

Aubin, J. E., M. Osborn, W. W. Franke, 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.

Baribault, H., R. Blouin, L. Bourgon, and N. Marceau. 1989. Epidermal growth factor–induced selective phosphorylation of cultured rat hepatocyte 55-kD cytokeratin before filament reorganization and DNA synthesis. *J. Cell Biol.* 109:1665–1676.

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.

Blose, S. H., and A. Bushnell. 1982. Observations on the vimentin–10-nm filaments during mitosis in BHK21 cells. *Exp. Cell Res.* 142:57–62.

Brown, D. T., B. H. Anderton, and C. C. Wylie. 1983. Alterations in the organization of cytokeratin filaments in normal and malignant human colonic epithelial cells during mitosis. *Cell Tissue Res.* 233:619–628.

Cabral, F., and M. M. Gottesman. 1979. Phosphorylation of the 10-nm filament protein from Chinese hamster ovary cells. *J. Biol. Chem.* 254:6203–6206.

Carmo-Fonseca, M., A. J. Cidadao, and J. F. David-Ferreira. 1987. Filamentous cross-bridges link intermediate filaments to the nuclear pore complex. *Eur. J. Cell Biol.* 45:282–290.

Cartaud, A., M. A. Ludosky, J. C. Courvalin, and J. Cartaud. 1990. A protein antigenically related to nuclear lamin B mediates the association of intermediate filaments with desmosomes. *J. Cell Biol.* 111:581–588.

Celis, J. E., P. M. 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., E. Rosevear, and R. D. Goldman. 1989. Phosphorylation and disassembly of intermediate filaments in mitotic cells. *Proc. Natl. Acad. Sci. USA.* 86:1885–1889.

Chou, Y.-H., J. R. Bischoff, D. Beach, and R. D. Goldman. 1990. Intermediate filament reorganization during mitosis is mediated by $p34^{cdc2}$ phosphorylation of vimentin. *Cell.* 62:1063–1071.

Chou, Y.-H., K.-L. Ngai, and R. D. Goldman. 1991. The regulation of intermediate filament reorganization in mitosis: $p34^{cdc2}$ phosphorylates vimentin at a unique N-terminal site. *J. Biol. Chem.* 266:7325–7328.

Christian, J. L., N. G. Edelstein, and R. T. Moon. 1990. Over-expression of wild-type and dominant negative vimentin subunits in developing *Xenopus* embryos. *New Biol.* 2:700–711.

Ciesielski-Treska, J., G. Ulrich, and D. Aunis. 1991. Protein kinase C–induced redistribution of the cytoskeleton and phosphorylation of vimentin in cultured brain macrophages. *J. Neurosci. Res.* 29:362–378.

Coca-Prados, M. 1985. Regulation of protein phosphorylation of the intermediate-sized filament vimentin in the ciliary epithelium of the mammalian eye. *J. Biol. Chem.* 260:10328–10332.

Dent, J. A. 1992. Expression and organization of vimentin filaments in oocytes and embryos of *Xenopus laevis*. Ph.D. thesis. University of Colorado at Boulder.

Dent, J. A., and M. W. Klymkowsky. 1989. Whole-mount analyses of cytoskeletal reorganization and function during oogenesis and early embryogenesis in *Xenopus*. *In* The Cell Biology of Fertilization. H. and G. Shatten, editors. Academic Press Inc., Orlando, FL. pp. 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.

Djabali, K., M.-M. Portier, F. Gros, G. Blobel, and S. D. Georgatos. 1991. Network antibodies identify nuclear lamin B as a physiological attachment site for peripherin intermediate filaments. *Cell.* 64:109–121.

Domingo, A., S. Sarria, R. Evans, and M. W. Klymkowsky. 1992. Studying intermediate filaments. *In* The Cytoskeleton: A Practical Approach. K. and C. Carraway, editors. Oxford University Press, New York. pp. 223–255.

Doorbar, J., S. Ely, J. Sterling, C. McLean, and L. Crawford. 1991. Specific interaction between HPV-16 E1-E4 and cytokeratins results in collapse of the epithelial cell intermediate filament network. *Nature (Lond.).* 352: 824–827.

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

Escribano, J., and E. Rozengurt. 1988. Cyclic AMP increasing agents rapidly stimulate vimentin phosphorylation in quiescent cultures of Swiss 3T3 cells. *J. Cell. Physiol.* 137:223–234.

Evan, G. I., G. K. Lewis, G. Ramsey, and J. M. Bishop. 1985. Isolation of monoclonal antibodies specific for human c-*myc* proto-oncogene product. *Mol. Cell. Biol.* 5:3610–3616.

Evans, R. M. 1984. Peptide mapping of phosphorylated vimentin. Evidence for a site-specific alteration in mitotic cells. *J. Biol. Chem.* 259:5372–5375.

Evans, R. M. 1988. Cyclic AMP-dependent protein kinase–induced vimentin filament disassembly involves modification of the NH_2-terminal domain of intermediate filament subunits. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 234: 73–78.

Evans, R. M. 1989. Phosphorylation of vimentin in mitotically selected cells: in vitro cyclic AMP–independent kinase and calcium-stimulated phosphatase activities. *J. Cell Biol.* 108:67–78.

Evans, R., and L. M. Fink. 1982. An alteration in the phosphorylation of vimentin-type intermediate filaments is associated with mitosis in cultured mammalian cells. *Cell.* 29:43–52.

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.

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., B. Picheral, and P. Gounon. 1983. Cytochemical evidence for the presence of intermediate filaments and microfilaments in the egg of *Xenopus laevis*. *Biol. Cell.* 47:331–342.

Gard, D. L., and E. Lazarides. 1982. Analysis of desmin and vimentin phosphopeptides in cultured avian myogenic cells and their modulation by 8-bromo-adenosine 3′,5′-cyclic monophosphate. *Proc. Natl. Acad. Sci. USA.* 79:6912–6916.

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

Geisler, N., M. Hatzfeld, and K. Weber. 1989. Phosphorylation in vitro of vimentin by protein kinases A and C is restricted to the head domain. Identification of the phosphoserine sites and their influence on filament formation. *Eur. J. Biochem.* 183:441–447.

Georgatos, S. D., and G. Blobel. 1987*a*. Two distinct attachment sites for vimentin along the plasma membrane and the nuclear envelope in avian erythrocytes: a basis for vectorial assembly of intermediate filaments. *J. Cell Biol.* 105:105–115.

Georgatos, S. D., and G. Blobel. 1987*b*. Lamin B constitutes an intermediate filament attachment site at the nuclear envelope. *J. Cell Biol.* 105:117–125.

Georgatos, S. D., and V. T. Marchesi. 1985. The binding of vimentin to human erythrocyte membranes: a model system for the study of intermediate filament–membrane interactions. *J. Cell Biol.* 100:1955–1961.

Georgatos, S. D., D. C. Weaver, and V. T. Marchesi. 1985. Site specificity in vimentin–membrane interactions: intermediate filament subunits associate with the plasma membrane via their head domains. *J. Cell Biol.* 100:1962–1967.

Georgatos, S. D., K. Weber, N. Geisler, and G. Blobel. 1987. Binding of two desmin derivatives to the plasma membrane and the nuclear envelope of avian erythrocytes: evidence for a conserved site specificity in intermediate filament–membrane interactions. *Proc. Natl. Acad. Sci. USA.* 84:6780–6784.

Godsave, S. F., C. C. Wylie, E. B. Lane, and B. H. Anderton. 1984*a*. Intermediate filaments in the *Xenopus* oocyte: the appearance and distribution of cytokeratin-containing filaments. *J. Embryol. Exp. Morphol.* 83:157–167.

Godsave, S. F., B. H. Anderton, J. Heasman, and C. C. Wylie. 1984*b*. Oocytes and early embryos of *Xenopus laevis* contain intermediate filaments which react with anti-mammalian vimentin antibodies. *J. Embryol. Exp. Morphol.* 83:169–184.

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

Graf, J.-D., and H. R. Kobel. 1991. Genetics of *Xenopus laevis*. *Methods Cell Biol.* 36:19–34.

Guidice, G. J., and E. Fuchs. 1987. The transfection of epidermal keratin genes into fibroblasts and simple epithelial cells: evidence for inducing a type I keratin by a type II gene. *Cell.* 48:453–463.

Gyoeva, F. K., and V. I. Gelfand. 1991. Coalignment of vimentin intermediate filaments with microtubules depends on kinesin. *Nature (Lond.).* 353:445–448.

Henderson, D., and K. Weber. 1981. Immunoelectron microscopical identification of the two types of intermediate filaments in established epithelial cells. *Exp. Cell Res.* 132:297–311.

Hermann, H., B. Foquet, and W. W. Franke. 1989. Expression of intermediate filament proteins during development of *Xenopus laevis*. I. cDNA clones of vimentin. *Development (Camb.).* 105:279–298.

Hollenbeck, P. J., A. D. Bershadsky, O. Y. Pletjushkina, I. S. Tint, and J. M. Vasiliev. 1989. Intermediate filament collapse is an ATP-dependent and actin-dependent process. *J. Cell Sci.* 92:621–631.

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.

Huang, C. K., J. F. Devanney, and S. P. Kennedy. 1988. Vimentin, a cytoskeletal substrate of protein kinase C. *Biochem. Biophys. Res. Commun.* 150:1006–1011.

Hynes, R. O., and A. T. Destree. 1978. 10-nm 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.

Inagaki, M., Y. Gonda, M. Matsuyama, K. Nishizawa, Y. Nishi, and C. Sato. 1988. Intermediate filament reconstitution in vitro: the role of phosphorylation on the assembly-disassembly of desmin. *J. Biol. Chem.* 263:5970–5978.

Inagaki, M., Y. Gonda, K. Nishizawa, S. Kitamura, C. Sato, S. Ando, K. Tanabe, K. Kikuchi, S. Tsuiki, and Y. Nishi. 1990. Phosphorylation sites linked to glial filament disassembly in vitro locate in a non-α-helical head domain. *J. Biol. Chem.* 265:4722–4729.

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

Jones, J. C., A. E. Goldman, H. Y. Yang, and R. D. Goldman. 1985. The organizational fate of intermediate filament networks in two epithelial cell types during mitosis. *J. Cell Biol.* 100:93–102.

Kitajima, Y., S. Inoue, K. Yoneda, S. Mori, and H. Yaoita. 1985. Alteration in the arrangement of the keratin-type intermediate filaments during mitosis in cultured human keratinocytes. *Eur. J. Cell Biol.* 38:219–225.

Kitamura, S., S. Ando, M. Shibata, K. Tanabe, C. Sato, and M. Inagaki. 1989. Protein kinase C phosphorylation of desmin at four serine residues within the non-α-helical head domain. *J. Biol. Chem.* 264:5674–5678.

Klymkowsky, M. W. 1982. Vimentin and keratin intermediate filament systems in culture PtK_2 epithelial cells are interrelated. *EMBO (Eur. Mol. Biol. Organ.) J.* 1:161–165.

Klymkowsky, M. W., and L. A. Maynell. 1989. MPF-induced breakdown of cytokeratin filament organization during oocyte maturation in *Xenopus* depends upon the translation of maternal mRNA. *Dev. Biol.* 134:479–485.

Klymkowsky, M. W., R. H. Miller, and E. B. Lane. 1983. Morphology, behavior, and interaction of cultured epithelial cells after the antibody-induced disruption of keratin filament organization. *J. Cell Biol.* 96:494–509.

Klymkowsky, M. W., L. A. Maynell, and A. G. Polson. 1987. Polar asymmetry in the organization of the cortical cytokeratins 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.

Klymkowsky, M. W., L. A. Maynell, C. Nislow. 1991. Cytokeratin phosphorylation, cytokeratin filament severing and the solubilization of the maternal mRNA Vg1. *J. Cell Biol.* 114:787–797.

Krieg, P. A., and D. A. Melton. 1987. In vitro RNA synthesis with SP6 RNA polymerase. *Methods Enzymol.* 155:397–415.

Kuang, J., J. E. Penkala, C. L. Ashorn, D. A. Wright, G. F. Saunders, and P. N. Rao. 1991. Multiple forms of maturation-promoting factor in unfertilized *Xenopus* eggs. *Proc. Natl. Acad. Sci. USA.* 88:11530–11534.

Lamb, N. J. C., A. Fernandez, J. R. Feramisco, and W. J. Welch. 1989. Modulation of vimentin containing intermediate filament distribution and phosphorylation in living fibroblasts by the cAMP-dependent protein kinase. *J. Cell Biol.* 108:2409–2422.

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.

Magneat, P. H., and K. Burridge. 1985. Immunoprecipitation of non-erythrocyte spectrin within live cells following microinjection of specific antibodies: relation to cytoskeletal structures. *J. Cell Biol.* 98:16363–1377.

Maller, J. L. 1990. Xenopus oocytes and the biochemistry of cell division. *Biochemistry.* 29:3157–3166.

Melton, D. A., P. A. Kreig, M.R. Rebagliati, T. Maniatis, K. Zinn, and M. R. Green. 1984. Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacterial SP6 promoter. *Nucleic Acids Res.* 12:7035–7056.

Merdes, A., M. Brunkener, H. Horstmann, and S. D. Georgatos. 1991. Filensin: a new vimentin-binding, polymerization-competent, and membrane-associated protein of the lens fiber cell. *J. Cell Biol.* 115:397–410.

Moreno, S., and P. Nurse. 1990. Substrates for $p34^{cdc2}$: in vivo veritas? *Cell.* 61:549–551.

Nurse, P. 1990. Universal control mechanism regulating onset of M-phase. *Nature (Lond.).* 344:503–508.

Papamarcaki, T., P. D. Kouklis, T. E. Kreis, and S. D. Georgatos. 1991. The "lamin B-fold." Anti-idiotypic antibodies reveal a structural complementarity between nuclear lamin B and cytoplasmic intermediate filament epitopes. *J. Biol. Chem.* 266:21247–21251.

Pines, J., and T. Hunter. 1990. $p34^{cdc2}$: the S and M kinase? *New Biol.* 2: 389–401.

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.

Soellner, P., R. Quinlan, 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.

Steinert, P. M., M. L. Wantz, and W. W. Idler. 1982. O-phosphoserine content of intermediate filament subunits. *Biochemistry.* 21:177–183.

Tint, I. S., P. J. Hollenbeck, A. B. Vershovsky, I. G. Surgucheva, and A. D. Bershadsky. 1991. Evidence that intermediate filament reorganization is induced by ATP-dependent contraction of the actomyosin cortex in permeabilized fibroblasts. *J. Cell Sci.* 98:375–384.

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.

Torpey, N. P., J. Heasman, and C. C. Wylie. 1990. Identification of vimentin and novel vimentin-related proteins in *Xenopus* oocytes and early embryos. *Development (Camb).* 110:1185–1195.

Torpey, N. P., J. Heasman, and C. C. Wylie. 1992. Distinct distribution of vimentin and cytokeratin in *Xenopus* oocytes and early embryos. *J. Cell Sci.* 101:151–160.

Vogelstein, B., and D. Gillespie. 1979. Preparative and analytical purification of DNA from agarose. *Proc. Natl. Acad. Sci. USA.* 76:615–619.

White, E., and R. Cipriani. 1989. Specific disruption of intermediate filaments and the nuclear lamina by the 19-kDa product of the adenovirus E1B oncogene. *Proc. Natl. Acad. Sci. USA.* 86:9886–9890.

Yano, T., T. Tokui, Y. Nishi, K. Nishizawa, M. Shibata, K. Kikuchi, S. Tsuiki, T. Yamauchi, and M. Inagaki. 1991. Phosphorylation of keratin intermediate filaments by protein kinase C, by calmodulin-dependent protein kinase and by cAMP-dependent protein kinase. *Eur. J. Biochem.* 197:281–290.

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.