A Fractionated Cell-free System for Analysis of Prophase Nuclear Disassembly

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Abstract. We describe a cell-free system in which a postribosomal supernatant (s140) from metaphase Chinese hamster ovary (CHO) cells induces prophase-like changes in isolated CHO cell nuclei, including chromatin condensation, and nuclear envelope and lamina disassembly. These events are strongly promoted by γ-S-ATP and an ATP-regenerating system, and do not take place with an s140 derived from G2-phase cells. The metaphase cell s140 also induces disassembly of an isolated nuclear lamina fraction that is depleted of membranes, chromatin, and nuclear pore complexes. Disassembly of the isolated lamina is accompanied by phosphorylation of the major lamina proteins (lamins A, B, and C) to levels characteristic of metaphase cells. Kinetic analysis of lamina depolymerization indicates that cooperativity may be involved in this process. The biochemical properties of in vitro lamina disassembly suggest that the activity that depolymerizes the lamina during mitosis is soluble in metaphase cells, and support the notion that this activity is a lamin protein kinase.

THE onset of mitosis in eukaryotic cells apparently is controlled by diffusible, cytosolic factor(s) that become active toward the end of interphase. When cytoplasm from late G2-phase or mitotic cells is introduced into interphase recipients by means of cell fusion (Johnson and Rao, 1970) or microinjection (Wasserman and Smith, 1978; Sunkara et al., 1979), chromosome condensation and nuclear envelope (NE)[1] disassembly are induced to occur in the interphase nuclei, even in the absence of protein synthesis (Halleck et al., 1984). Prophase-inducing cytoplasmic factors also are present in meiotic cells, as shown by injection of meiotic cell extracts into immature oocytes (Masui and Markert, 1971; Gerhart et al., 1984) and G2-arrested embryonic cells (Miake-Lye et al., 1983). Recently, it has been shown that early mitotic events (including chromosome condensation and disassembly of the NE and lamina) can take place in vitro when nuclei from amphibian sperm or somatic cells (Lohka and Masui, 1983; Lohka and Maller, 1985), or from mammalian tissue culture cells (Miake-Lye and Kirschner, 1985), are incubated with postnuclear supernatants of amphibian eggs. This type of system should permit direct biochemical analysis of mechanisms by which mitotic events are controlled.

Because the structure of the NE and its dynamics during mitosis have been characterized extensively, the process of NE disassembly affords a useful model for studies of mitotic regulation. During prophase, disassembly of the NE coincides with depolymerization of the nuclear lamina, a meshwork of intermediate-type filaments (Aebi et al., 1986; Fisher et al., 1986; McKeon et al., 1986) lining the nucleoplasmic surface of the NE which is composed primarily of three proteins (lamins A, B, and C) in mammalian somatic cells (Gerace et al., 1978; Krohne et al., 1978; Gerace and Blobel, 1980). Reformation of the NE during telophase closely follows reassembly of the lamina at the surfaces of chromosomes. Since the lamina physically interacts with both the inner nuclear membrane and with chromatin during interphase, and because lamins are required for telophase NE assembly (Burke and Gerace, 1986), reversible depolymerization of the lamina may directly regulate disassembly and reformation of the NE during mitosis (Gerace et al., 1978; Gerace et al., 1984). There is good evidence that the structural dynamics of the lamina are controlled by reversible enzymatic phosphorylation of lamins. Lamina disassembly is temporally correlated with a four- to sevenfold increase in the steady-state level of lamin phosphorylation (Gerace and Blobel, 1980; Ottaviano and Gerace, 1985; Miake-Lye and Kirschner, 1985), and conditions that inhibit lamin dephosphorylation (γ-S-ATP) block telophase-like assembly of the lamina in vitro (Burke and Gerace, 1986).

In this report, we describe a cell-free system in which a soluble extract from metaphase Chinese hamster ovary (CHO) cells induces a prophase-like state in isolated CHO cell nuclei. Moreover, we demonstrate that the extract also causes disassembly of an isolated rat liver lamina fraction comprised primarily of lamins A, B, and C, and leads to metaphase-like lamin hyperphosphorylation. The biochemical characteristics of cell-free lamina disassembly suggest that a soluble lamina-depolymerizing activity is present in metaphase CHO cells, and that this activity may be a lamin protein kinase.

1. *Abbreviation used in this paper*: NE, nuclear envelope.

Materials and Methods

Cell Culture and Synchrony

CHO cells were maintained in monolayer culture at 37°C in Joklik's spinner medium containing 10% FCS (HyClone Laboratories, Logan, UT), 20 mM Hepes, 100 U/ml penicillin, and 100 μg/ml streptomycin (complete medium). For synchronization, cells were grown in 24.5 × 24.5 cm tissue culture dishes (Nunc Co., Thousand Oaks, CA) as described previously (Burke and Gerace, 1986), except that 40 ng/ml nocodazole was added to the cultures 2.5 h after removal of thymidine. 1 h later, loosely attached cells were detached by selective mechanical "shake off" (Tobey et al., 1967) and were discarded. After further incubation for 4 h in complete medium containing 40 ng/ml nocodazole (Zieve et al., 1980), a population of metaphase cells was harvested by selective shake off. Cells that remained attached to the culture dishes were incubated for 30 min in complete medium containing 40 ng/ml nocodazole and 10 μM cytochalasin B. After a final shake off, adherent cells were washed with 75 ml ice-cold PBS (Dulbecco's formulation supplemented with 1 mM $MgCl_2$ [MgPBS]) and were scraped into 100 ml MgPBS, yielding an interphase population enriched in G2-phase cells. Typically, at least 95% of the cells in this population exhibited interphase morphology characterized by decondensed chromatin, prominent nucleoli, and an intact nuclear lamina (based on immunofluorescence microscopy). At least 95% of the metaphase population possessed highly condensed mitotic chromosomes and lacked both an NE and perichromosomal anti-lamin immunofluorescence (data not shown).

Subcellular Fractionation

For preparation of a postribosomal supernatant (s140), cells were washed with 40 vol of ice-cold KPM (50 mM KCl/50 mM Pipes-KOH, pH 7.0/10 mM EGTA/1.92 mM $MgCl_2$) and 40 vol of KPM containing 1 mM dithiothreitol (DTT) and 20 μM cytochalasin B (complete KPM) by centrifuging for 5 min at 300 *g*. Washed cell pellets were resuspended in 1 vol of complete KPM and were lysed (on ice) using 20–50 passes of a tight-fitting dounce homogenizer. The lysate was centrifuged for 40 s in an Eppendorf microfuge, yielding a postnuclear supernatant. An s140 was obtained by centrifuging 1-ml aliquots of the postnuclear fraction for 20 min at 140,000 *g* in a Beckman SW-41 rotor at 0°C (Beckman Instruments, Inc., Palo Alto, CA).

For isolation of CHO cell nuclei, interphase cells were washed with 40 vol of 10 mM KCl/10 mM Hepes-KOH, pH 7.4/1.5 mM $MgCl_2$/1 mM DTT/10 μM cytochalasin B/0.5 mM PMSF/1 μg per ml CLAP (chymostatin/leupeptin/antipain/pepstatin A). Washed cells were resuspended in 3 vol of this formulation, swelled for 20–30 min (on ice) and gently lysed using 20–50 passes of a dounce homogenizer. Subsequently, homogenates of up to 10^8 cells were layered over 4.5 ml 30% sucrose/10 mM KCl/10 mM Hepes-KOH, pH 7.4/1.5 mM $MgCl_2$/1 mM DTT (contained in a 40-ml capacity round-bottom tube) and were centrifuged for 2 min at 500 *g*. The pellet was resuspended to 10^8 nuclei/ml in complete KPM.

A lamina-enriched fraction was isolated from "crude" rat liver NEs (Dwyer and Blobel, 1976) using a modified version of procedures described by Gerace et al. (1984). Aliquots of "salt-washed" NEs containing ~0.3 mg protein were layered over 400 μl of 30% sucrose/10 mM triethanolamine-HCl, pH 7.4/0.1 mM $MgCl_2$/1 mM DTT in a 1.5-ml Eppendorf tube and were centrifuged for 15 min at 4,000 *g* (JS5.2 rotor; Beckman Instruments, Inc.) onto a 40-μl cushion consisting of 50% sucrose in MMD (10 mM Mes-KOH, pH 6.0/0.1 mM $MgCl_2$/1 mM DTT). Resuspended pellets were extracted for 5 min with MMD containing 2% Triton X-100 and 0.3 M KCl and were centrifuged for 15 min at 1,000 *g* through 400 μl of 20% sucrose in MMD onto 40 μl of 50% sucrose in MMD. The lamina-enriched fraction was resuspended as a 4× stock (usually 0.66 mg/ml lamins) in complete KPM using a dounce homogenizer. Our protocol typically resulted in a 60% recovery of lamins initially present in the crude NEs (Gerace et al., 1984).

Cell-free Nuclear Disassembly

3 vol of s140 were mixed with 1 vol of nuclei (or lamina fraction) in Sigmacote-treated tubes (Sigma Chemical Co., St. Louis, MO) at 0°C. Adenosine-5'-0-(3-thiotriphosphate) (γ-S-ATP) (Boehringer Mannheim Biochemicals; Indianapolis, IN) and pyruvate kinase were added to final concentrations of 1 mM and 10 U/ml, and disassembly was initiated by warming in a 33°C water bath. After 10 min, phosphoenol pyruvate was added to a concentration of 20 mM and the incubations were mixed every 20 min for a period of 2 h.

It is likely that our cell-free incubations initially contain ~1 mM ATP, arising from a 2.7-fold dilution of the intracellular pool (2.5–3.0 mM; Chapman et al., 1971). In the absence of a regenerating system, >90% of this ATP is hydrolyzed in 10 min at 33°C (Burke and Gerace, 1986). In contrast, thin layer chromatographic analysis of incubations containing 1 mM exogenous ATP (including tracer α-^{32}P-ATP) and the ATP-regenerating system indicate that 65% of the tracer is present as ATP after 90 min (data not shown). γ-S-ATP is hydrolyzed at a substantially lower rate than ATP; such that if 1 mM γ-S-ATP is present initially, 0.5 mM remains after 45 min and 0.4 mM remains after 75 min at 33°C (based on thin layer chromatography of cell-free reactions containing γ-^{35}S-ATP; data not shown).

Disassembly of the lamina was quantitated by mixing 35–100 μl of cell-free reaction with 1 ml ice-cold PNX (100 mM NaCl/10 mM Pipes-NaOH, pH 7.0/1 mM DTT/0.5% Triton X-100/5 mg per ml ovalbumin/0.5 mM PMSF/5 μg per ml CLAP/1% Trasylol) and centrifuging for 10 min at 10,000 *g* in a microfuge (Beckman Instruments, Inc.). Triton-insoluble pellets were frozen in liquid N_2 and subsequently were processed for gel electrophoresis and immunoblotting as described below. For immunoblot analysis of Triton-soluble lamins, 100 μl of incubation was added to 1 ml of PNX that did not contain ovalbumin. After centrifugation, supernatants were precipitated at 0°C with 10% TCA, extracted with 95% acetone containing 0.1 N HCl, and processed as below.

Gel Electrophoresis and Immunoblotting

For one-dimensional gels, samples were dissolved in SDS gel sample buffer, sonicated for 20 s (Branson Sonifier equipped with a microtip probe; Branson Sonic Power Co., Danbury, CT), and immediately boiled for 3 min. After alkylation with 80 mM iodoacetamide (37°C for 30 min), aliquots containing ~60 ng lamins were electrophoresed in 0.75-mm 10% polyacrylamide gels (Maizel, 1969). These were electrophoretically transferred to nitrocellulose and were labeled with guinea pig anti-lamin serum (diluted 1:200) and radioiodinated protein A as described by Burnette (1981), except that 0.5% Triton X-100 and 2% fraction V BSA (Miles Scientific Div., Naperville, IL) were present during labeling. Using an autoradiographic exposure of the immunoblot as a template, lamin-containing regions were excised from the nitrocellulose and quantitated in a Beckman gamma counter. Disassembly of the lamina was determined by comparing these values to a standard curve of serially diluted lamin standards.

For two-dimensional gel analysis, samples containing ~250 ng lamins were electrophoresed in nonequilibrium pH gradient gels (O'Farrell et al., 1977) and, subsequently, in 7.5–15% SDS gradient gels as described previously (Ottaviano and Gerace, 1985), except that sample lanes from the first dimension were equilibrated with SDS in the absence of DTT. Second-dimension gels were transferred to nitrocellulose and immunolabeled as outlined above.

Microscopy

Aliquots of cell-free disassembly reactions were centrifuged onto coverslips and processed for immunofluorescence microscopy (involving affinity-purified guinea pig anti-lamin antibodies and rhodamine-conjugated second antibody) according to Burke and Gerace (1986), except that fixation was not carried out in the presence of Triton X-100. The samples also were stained with the DNA-specific dye, Hoechst #33258 (0.5 μg/ml). Because this protocol did not preserve the structure of chromatin that condensed in vitro, parallel samples were fixed in KPM containing 2% formaldehyde, 0.1% glutaraldehyde, and 4.5 mM $MgCl_2$, and were examined by phase-contrast microscopy. (Rhodamine-conjugated antibodies could not be used with the glutaraldehyde-fixed material due to substantial autofluorescence.)

Samples were prepared for thin section electron microscopy as described previously (Burke and Gerace, 1986).

Results

Induction of a Mitotic State in Isolated CHO Cell Nuclei

We found that G2-phase CHO cell nuclei enter a prophase-like state in vitro when incubated at 33°C with a postribosomal supernatant (s140) derived from metaphase CHO cells, provided that γ-S-ATP and an ATP-regenerating system are present (see below). Initially (Fig. 1, *a* and *b*), the nuclei

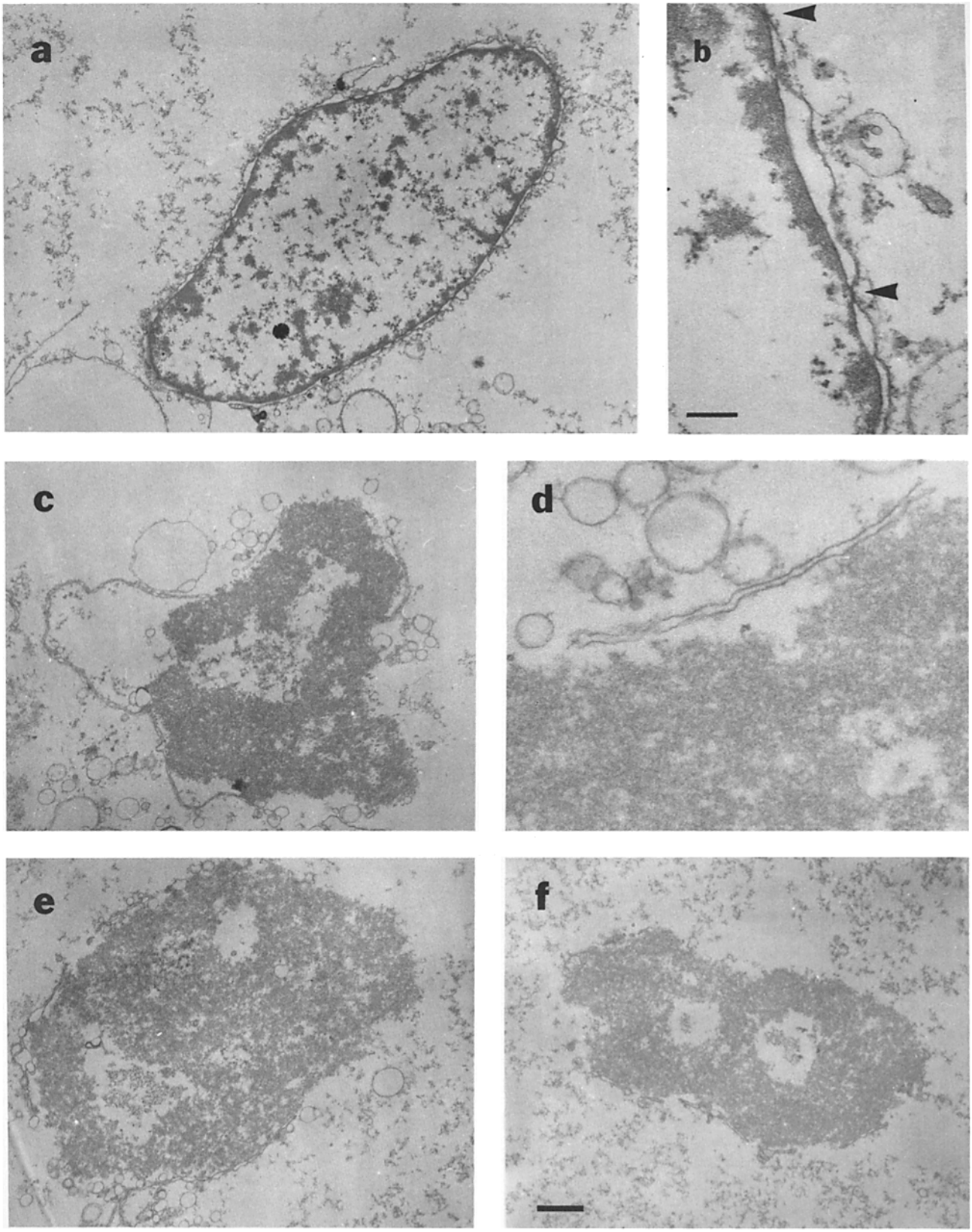

Figure 1. Analysis of cell-free nuclear disassembly by electron microscopy. Nuclei isolated from G2-phase CHO cells were incubated at 33°C with a postribosomal supernatant (s140) from metaphase cells in the presence of γ-S-ATP and an ATP-regenerating system for 0 min (*a* and *b*), 75 min (*c* and *d*), or 120 min (*e* and *f*) before samples were fixed and processed for thin section electron microscopy. Bar, (*a*, *c*, *e*, and *f*) 1 μm; (*b* and *d*) 0.2 μm.

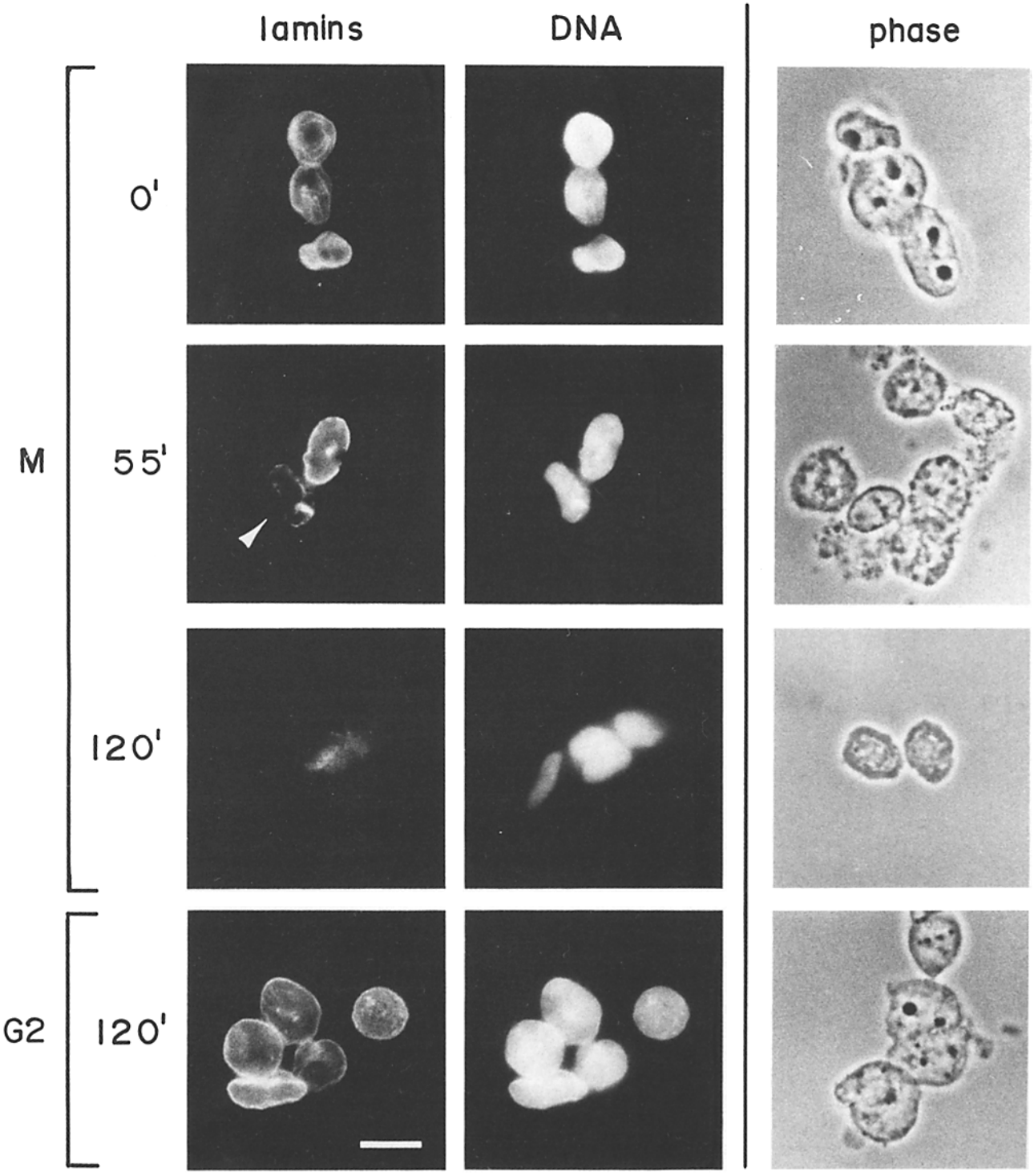

Figure 2. Analysis of cell-free nuclear disassembly by immunofluorescence and phase-contrast microscopy. Nuclei isolated from G2-phase CHO cells were incubated with s140 extracts derived from metaphase (*M*) or G2-phase (*G2*) cells as described in Fig. 1. After 0, 55, and 120 min, samples were fixed (4% formaldehyde) and labeled for immunofluorescence microscopy with affinity-purified antibodies directed against lamins A and C (*lamins*). These samples also were stained with the DNA-specific dye, Hoechst #33258 (*DNA*). Since the immunofluorescence fixation protocol did not preserve the structure of condensed chromatin, samples were fixed in parallel with 2% formaldehyde + 0.1% glutaraldehyde for phase-contrast examination (*phase*). Bar, 10 μm.

have typical interphase chromatin and NEs with pore complexes (Fig. 1 *b*, arrowheads), and nucleoli are clearly visible (Fig. 2, *phase*). After 55 min at 33°C, chromosomes are partially condensed and associated with the nuclear periphery and nucleoli have disappeared (Fig. 2, *phase*). By 75 min, most nuclei exhibit highly condensed chromatin that is partially surrounded by discontinuous double-membrane cisternae (Fig. 1 *c*). These membranes usually lack pore complexes and only periodically exhibit a direct association with chromatin (Figs. 1, *c* and *d*). By 120 min, the chromatin is

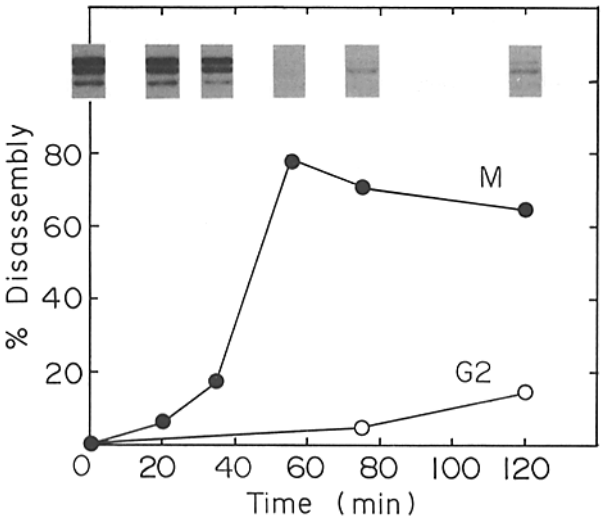

Figure 3. Time course of lamina disassembly in isolated nuclei. Aliquots of the incubations described in Fig. 2 were diluted with 10 vol of PNX buffer after various periods at 33°C and were centrifuged to pellet Triton-insoluble lamins. Quantities of lamins in the pellets were then determined by immunoblotting (see Materials and Methods). Shown at the top of the figure are autoradiographs of the immunolabeled Triton-insoluble lamins for the incubation containing metaphase sl40 (*M*). Identical quantities of disassembled lamins were measured using centrifugation for 20 min at 140,000 *g* and our routine protocol (10 min at 10,000 *g*) for incubations involving either CHO cell nuclei or an isolated rat liver lamina fraction (cf. Figs. 4–7).

largely devoid of surrounding membranes (Fig. 1, *e* and *f*) and has become "hypercondensed" and often fused into continuous masses (Fig. 2, *phase*). Presumably, the presence of γ-S-ATP, which leads to high levels of protein thiophosphorylation by kinases (Eckstein, 1975; Miller and Kennedy, 1986), promotes this extreme degree of chromatin condensation (cf. Gurley et al., 1978).

To investigate whether the lamina is disassembled in our cell-free reactions as it is in vivo during prophase (Gerace and Blobel, 1980), samples were fixed during the course of incubations, labeled with affinity-purified anti–lamin antibodies, and analyzed by means of indirect immunofluorescence microscopy (Fig. 2, *lamins*) and a DNA-specific dye (Fig. 2, *DNA*). Initially, the nuclei show a perinuclear rim of lamin staining (Fig. 2, 0 min) that is typical of interphase cells. After 55 min incubation with the metaphase sl40 fraction at 33°C, perinuclear lamin staining is discontinuous or largely absent (arrowhead) in most nuclei, although the degree of staining can vary considerably from nucleus to nucleus. By 120 min, almost no lamin staining is detectable around chromosomes. In contrast, nuclei that are incubated at 33°C with sl40 derived from G2-phase CHO cells do not undergo detectable structural changes, including lamina disassembly (Fig. 2, *G2*). Similarly, no detectable changes take place when nuclei are incubated with metaphase sl40 for 2 h at 0°C (data not shown).

Biochemical Analysis of Lamina Disassembly

For biochemical quantitation of lamina disassembly, we utilized the fact that disassembled mitotic lamins are soluble in solutions containing Triton X-100, whereas the interphase lamina is an insoluble supramolecular assembly (Gerace and Blobel, 1980; Burke and Gerace, 1986). Aliquots of cell-free reactions were added to a buffer containing 0.5% Triton X-100 and were centrifuged to pellet insoluble lamins. Subsequently, the amount of lamins remaining in pellets was determined by quantitative immunoblotting analysis. We analyzed pellet rather than supernatant fractions to measure lamina depolymerization, since lamins A and C are initially present in the metaphase cell sl40 used to induce nuclear disassembly. Disappearance of lamins from the pellet fractions during incubations is due to actual disassembly rather than proteolysis because loss from the pellets is accompanied by appearance in the supernatants (compare 0- and 120-min lanes for *M*: *γ-S-ATP + PEP* in Fig. 5).

The time course of lamina disassembly in isolated CHO cell nuclei incubated with metaphase cell sl40 was analyzed by this biochemical procedure (Fig. 3). Little disassembly occurs during the first 30 min of incubation at 33°C, while rapid depolymerization takes place during the next 30 min and reaches a plateau by 60 min. In contrast, only a slight decrease in Triton-insoluble lamins occurs in nuclei that are incubated with an sl40 extract of G2-phase cells for up to 2 h (15% disassembly). While the lamins become Triton-soluble by biochemical analysis (Fig. 3) somewhat more rapidly than they appear to dissociate from chromosomal surfaces by immunofluorescence (Fig. 2), this difference may reflect enhanced accessibility of lamins to antibody labeling after partial lamina disassembly and chromatin condensation.

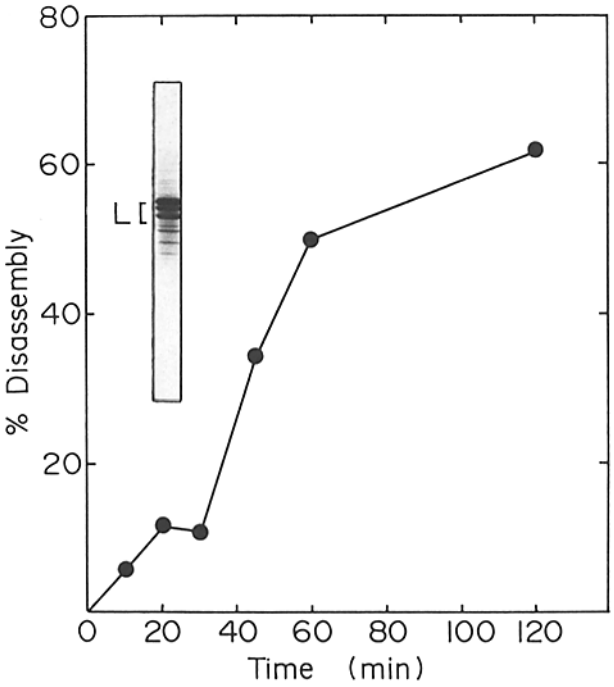

Figure 4. Time course of lamina disassembly in an isolated lamina fraction. A lamina fraction derived from rat liver nuclear envelopes was incubated at 33°C with the sl40 of metaphase CHO cells in the presence of γ-S-ATP and an ATP-regenerating system (0.025 mg/ml rat liver lamins). Periodically over the course of 2 h, aliquots of the incubation were removed for determination of Triton-insoluble lamins by immunoblotting (see Materials and Methods). Densitometric analysis of a Coomassie Blue–stained SDS polyacrylamide gel of the isolated lamina fraction (*inset*) indicates that the lamins (*L*) account for ~60% of the protein in this sample.

Mitosis-specific Disassembly of an Isolated Lamina Fraction

We examined cell-free disassembly of an isolated rat liver lamina fraction composed primarily of lamins (Fig. 4) to permit analysis of lamina depolymerization separate from other biochemical events that occur in nuclei during prophase. We found that the isolated lamina undergoes efficient disassembly with our standard incubation conditions (Figs. 4 and 5). The disassembly occurs only with metaphase cell s140, and does not take place with s140 derived from G2-phase cells (Fig. 5) or in the presence of homogenization buffer alone (Fig. 6 *b*). All detectable lamina disassembly activity in metaphase CHO cells is present in the soluble fraction of homogenates, since other subcellular fractions (a 12,000 *g* pellet and 140,000 *g* "microsomal" pellet) do not induce disassembly of the isolated rat liver lamina (data not shown). The time course of disassembly (Fig. 4) is very similar to that observed for CHO cell nuclei (Fig. 3), with most depolymerization occurring between 30 and 60 min. While we did not observe >68% disassembly of the lamina fraction during the course of our experiments (Figs. 4–7), the lack of complete disassembly apparently is due to partial aggregation of the lamina fraction during isolation, since the use of higher centrifugal forces for isolation which leads to greater macroscopic clumping of the fraction also results in substantially less disassembly (data not shown). Limiting amounts of disassembly activity are not responsible for this incomplete depolymerization, because a similar extent of depolymerization occurs over a 40-fold range of lamina concentration (Fig. 6 *a*).

Optimal disassembly of the rat liver lamina fraction requires both γ-S-ATP and an ATP-regenerating system. As shown in Fig. 5, only 38% disassembly takes place (in 2 h) when γ-S-ATP is used without phosphoenol pyruvate (PEP), while ATP + PEP results in 30% disassembly. No disassembly (above background) is detectable in the presence of the nonhydrolyzable ATP analog, AMP-PNP. The requirement for γ-S-ATP in our cell-free reactions suggests that the phosphorylation of one or more proteins is necessary for disassembly of the lamina in vitro, since γ-S-ATP leads to the stable thiophosphorylation of proteins by protein kinases (Eckstein, 1975; Miller and Kennedy, 1986). γ-S-ATP and an

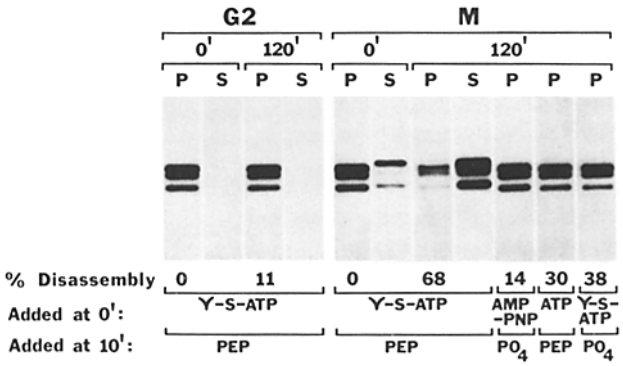

Figure 5. In vitro disassembly of the lamina fraction requires mitotic cytosol, γ-S-ATP, and an ATP-regenerating system. s140 extracts from metaphase (*M*) or G2-phase (*G2*) CHO cells were incubated at 33°C with the rat liver lamina fraction (0.17 mg/ml rat liver lamins) in the presence of 1 mM γ-S-ATP, ATP, or adenyl-5'-imidodiphosphate (*AMP-PNP*). 10 min later, the samples received additions of either 20 mM phosphoenol pyruvate + 10 U/ml pyruvate kinase (*PEP*) or 20 mM $KHPO_4$ buffer (*PO_4*). After 0 and 120 min at 33°C, aliquots of the incubations were assayed to determine quantities of Triton-soluble (*S*) and -insoluble (*P*) lamins (see Materials and Methods).

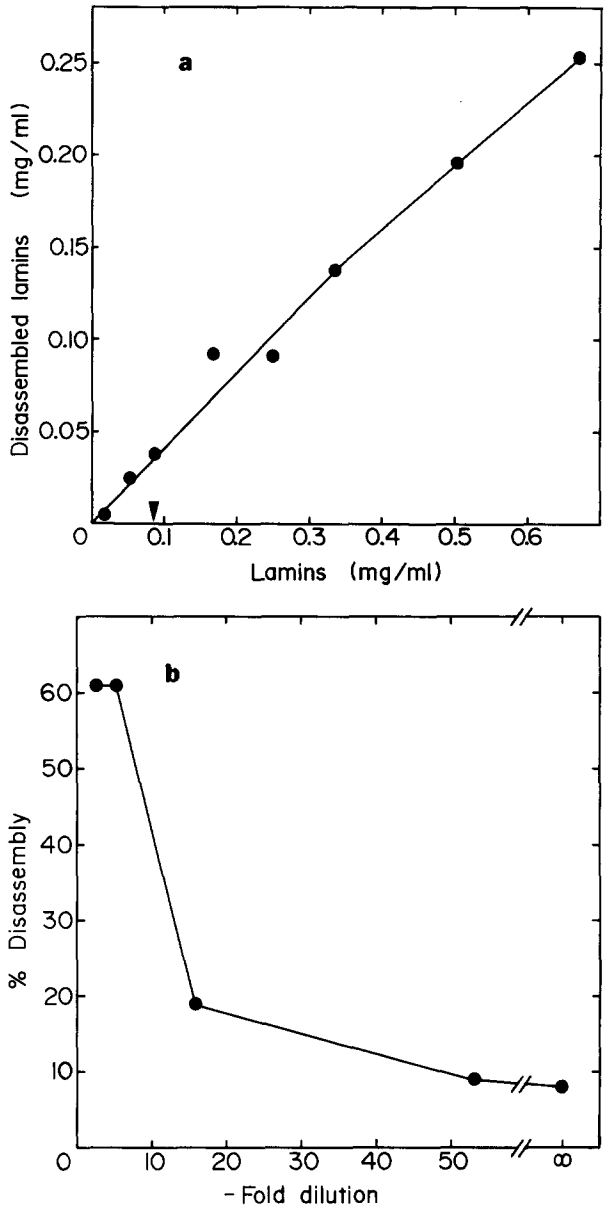

Figure 6. Dependence of cell-free lamina disassembly on the concentrations of substrate and metaphase s140. (*a*) The lamina disassembly activity of a metaphase s140 extract was assayed over a 40-fold range of substrate concentration (0.017–0.67 mg/ml rat liver lamins) as described in Fig. 4. Concentrations of rat liver and CHO cell lamins were determined by eluting lamin polypeptides (and BSA standards) from Coomassie Blue-stained SDS gels with 25% pyridine and measuring the absorbance at 605 nm (Fenner et al., 1975). The concentration of disassembled CHO cell lamins in the incubation mixture contributed by the metaphase cell s140 is indicated (*arrowhead*). (*b*) Dilutions of a metaphase s140 extract were made using homogenization buffer (complete KPM) supplemented with 1 mM ATP (infinite dilution = no s140). These were tested for their ability to disassemble the rat liver lamina fraction (0.17 mg/ml rat liver lamins) during incubation for 2 h at 33°C in the presence of γ-S-ATP and an ATP regenerating system. It was assumed that the s140 initially contained ~1 mM ATP (see Materials and Methods).

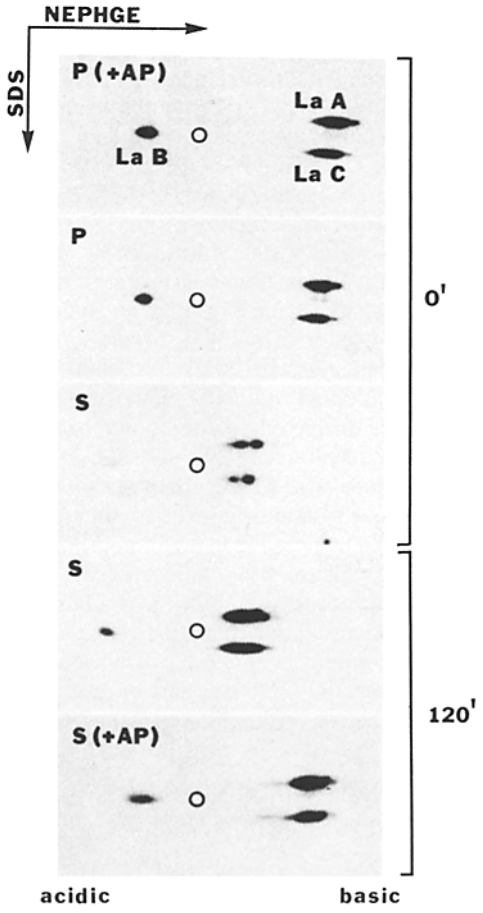

Figure 7. Two-dimensional gel analysis of in vitro lamina disassembly. The rat liver lamina fraction was incubated with s140 prepared from metaphase cells as described in Fig. 4 (0.17 mg/ml rat liver lamins). After 0 and 120 min, aliquots of the incubation were fractionated into Triton-soluble (*S*) and -insoluble (*P*) components. Portions of some samples were treated with 100 μg/ml *Escherichia coli* alkaline phosphatase (*+AP*), and samples then were analyzed by means of nonequilibrium pH gradient/SDS gel electrophoresis and immunoblotting. An internal standard (BSA [*open circles*]) was visualized by staining the immunoblots with Ponceau S.

ATP-regenerating system also were needed to obtain chromatin condensation and lamina disassembly in isolated CHO cell nuclei (data not shown).

To investigate whether cell-free disassembly of the isolated lamina fraction exhibits characteristics of an enzyme-mediated process, we analyzed the dependence of disassembly on the concentration of lamina substrate. As shown in Fig. 6 *a*, an approximately linear increase in the quantity of disassembled rat liver lamins (in 2 h) is seen over a 40-fold range of increasing lamina concentration, up to the highest concentration that is possible to test in our assay. Since this concentration is seven times greater than the level of endogenous lamins present in the metaphase s140 (Fig. 6 *a*, arrowhead), these data argue that disassembly is a catalytic rather than a stoichiometric process. In addition, we find a gradual loss of disassembly activity with increasing dilutions of the metaphase s140, leading to an undetectable level of disassembly at a 50-fold dilution (Fig. 6 *b*).

Phosphorylation of the Lamins during the Course of Cell-free Lamina Disassembly

We used two-dimensional gel electrophoresis to analyze whether metaphase-like hyperphosphorylation of the lamins is associated with disassembly of the rat liver lamina fraction in our cell-free system (Fig. 7). Initially, the rat liver lamins are largely unphosphorylated, since treatment with alkaline phosphatase only slightly shifts their electrophoretic mobility to more basic isoforms [0 min, *P* and *P(+AP)*]. The s140 used for disassembly contains small amounts of CHO cell lamins A and C that are phosphorylated at metaphase levels (0 min, *S*). After 120 min, 65% of the rat liver lamins have disassembled to a Triton-soluble form and exhibit more acidic charge isoforms that co-migrate with the mitotic CHO cell species (120 min, *S*). The acidic charge shift is entirely due to the addition of phosphate, since it is reversed by treatment with alkaline phosphatase [120 min, *S(+AP)*]. We conclude that the rat liver lamins become hyperphosphorylated in vitro to levels that are characteristic of mitotic CHO cell lamins in vivo (1.4–2.2 mol phosphate/mol lamins; Ottaviano and Gerace, 1985).

Discussion

We have devised a cell-free system in which a soluble fraction (an s140 postribosomal supernatant) from metaphase tissue culture cells induces chromatin condensation together with NE and lamina disassembly in isolated interphase nuclei. During the course of NE disassembly, the pore complexes and lamina are disassembled and nuclear membrane cisternae lose their interaction with condensed chromosomes before extensive vesiculation of the nuclear membranes takes place, as observed for prophase NE disassembly in vivo (Porter and Machado, 1960; Murray et al., 1965; also see Warren, 1985). In contrast to our disassembly system, cell-free nuclear disassembly based on amphibian egg extracts requires a postnuclear supernatant containing particulate components (Lohka and Masui, 1983; Lohka and Maller, 1985; Miake-Lye and Kirschner, 1985). In some cases, a 1–2-h preincubation of nuclei in the amphibian postnuclear supernatant is necessary to obtain subsequent disassembly. During the preincubation, sperm nuclei go through major structural changes (Lohka and Masui, 1983) and mammalian nuclei undergo a 20-fold increase in volume (Maike-Lye and Kirschner, 1985), presumably involving extensive incorporation of heterologous structural components.

Not only does a soluble metaphase CHO cell extract induce lamina disassembly in interphase nuclei; it also causes efficient depolymerization of an isolated rat liver lamina. High levels of disassembly activity are present, since a 2.5-fold diluted cell extract supports depolymerization of at least three times the concentration of lamins occurring in vivo. Thus, the components extracted from nuclei during isolation of the lamina fraction (including nuclear membranes, chromatin, and nuclear pore complexes) are not required for lamina depolymerization in our cell-free preparations. Furthermore, extraction of these components does not markedly affect the time course of lamina disassembly, since both CHO cell nuclei and the rat liver lamina show similar disassembly kinetics characterized by an initial 30-min lag phase followed by a 30-min period of rapid depolymerization. The

initial lag phase apparently is not due to limiting amounts of active disassembly components, since a similar lag was obtained over a 40-fold concentration range of lamina substrate (Fig. 6 *a*), and upon preincubation of metaphase cell extracts with γ-S-ATP and an ATP-regenerating system for 45 min before addition of substrate (data not shown). The presence of a lag phase in lamin solubilization during in vitro disassembly is consistent with the possibility that lamina depolymerization involves a cooperative process. If depolymerization is mediated by lamin hyperphosphorylation (see below), a cooperative process might entail phosphorylation of a threshold number of sites in a localized region of lamin filaments leading to a greatly increased rate of phosphorylation of additional sites in the same region.

All lamina disassembly-promoting activity is soluble in metaphase CHO cell homogenates (see Results). Although it is possible that disassembly of the rat liver lamina fraction in vitro involves activation of an endogenous lamina-associated depolymerizing factor by the CHO cell s140, we favor the possibility that the depolymerizing factor itself is soluble because the lamina fraction used for disassembly contains few proteins other than the lamins due to the stringent extraction conditions used for its preparation (see Materials and Methods).

Our results support the possibility that lamin phosphorylation mediates depolymerization of the lamina (Gerace and Blobel, 1980; Ottaviano and Gerace, 1985; Miake-Lye and Kirschner, 1985; Burke and Gerace, 1986), since in vitro disassembly of the rat liver lamina fraction is accompanied by hyperphosphorylation of the lamins to levels that are characteristic of metaphase cells in vivo (1.4–2.2 mol phosphate/mol lamins; Ottaviano and Gerace, 1985). Furthermore, lamina disassembly is strongly promoted by the presence of both γ-S-ATP and an ATP-regenerating system, which would favor protein phosphorylation. Since no detectable endogenous lamin kinase activity is associated with the isolated rat liver lamina (Gerace, L., unpublished data), and because the lamina depolymerizing activity is suggested to be soluble (see above), disassembly of the lamina fraction in our cell-free system may be accomplished by a soluble lamin kinase that is present in metaphase CHO cell extracts.

The NE increases in surface area (Maul et al., 1972; Fry, 1976) and lamins are synthesized (Gerace et al., 1984) throughout interphase in growing CHO cells, in contrast to nonproliferating rat liver cells that are used as a source of the isolated lamina fraction. A low level of phosphorylation is associated with interphase CHO cell lamins (0.3–0.5 mol phosphate/mol lamins), which is thought to be important for incorporation of newly synthesized lamins into a pre-existing lamina meshwork (Ottaviano and Gerace, 1985). An interphase lamin kinase is probably associated with the NE in these growing cells, since all detectable lamin phosphorylation occurs in assembled lamins (Ottaviano and Gerace, 1985). At present, it is not known whether interphase and mitotic lamin kinase activities in CHO cells derive from distinct enzymes, or from functionally different forms of the same enzyme. While in vitro disassembly of the rat liver lamina fraction may be mediated exclusively by a soluble lamin kinase (see above), lamina disassembly in isolated CHO cell nuclei may be accomplished by both a soluble lamin kinase present in the metaphase s140 extract and an NE-associated kinase that is activated by the extract.

In addition to having a possible role in regulating disassembly of the nuclear lamina, protein phosphorylation may be involved in the reorganization of other structural components of the cell during mitosis, since numerous cellular proteins become transiently hyperphosphorylated during this period (Gurley et al., 1978; Davis et al., 1983). Maturation-promoting factor, a protease-sensitive activity that has been purified 20–30-fold from amphibian oocytes (Masui and Markert, 1971; Wasserman and Masui, 1976; Wu and Gerhart, 1980), has been suggested to trigger entry of cells into prophase by initiating a cascade of protein phosphorylation reactions (Miake-Lye et al., 1983; Miake-Lye and Kirschner, 1985; Burke and Gerace, 1986). The observation that chromatin condensation as well as lamina disassembly in our cell-free system is promoted by conditions that favor the phosphorylated state of proteins (γ-S-ATP and an ATP-regenerating system) is consistent with this hypothesis. Since nuclear disassembly in our system is induced by soluble factors, in the future it should be possible to use straightforward biochemical fractionation for isolating molecules that regulate prophase events. Moreover, the ability to study disassembly of an isolated nuclear lamina separate from other mitotic events specifically should facilitate purification of the mitotic lamina-depolymerizing activity, and be useful for directly demonstrating the role of lamin phosphorylation in lamina disassembly.

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Note Added in Proof: The rat liver lamins disassembled in vitro sediment predominantly as an ~4.5 S species by sucrose gradient centrifugation. Thus, their sedimentation behavior is identical to that observed for lamins from metaphase CHO cells (Gerace and Blobel, 1980).

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