Lamin Dimers

PRESENCE IN THE NUCLEAR LAMINA OF SURF CLAM OOCYTES AND RELEASE DURING NUCLEAR ENVELOPE BREAKDOWN*

George N. Dessev, Christina Iovcheva-Dessev, and Robert D. Goldman

From the Department of Cell, Molecular, and Structural Biology, Northwestern University Medical School, Chicago, Illinois 60611 and the Marine Biological Laboratory, Woods Hole, Massachusetts 02543

The nuclear lamina of surf clam oocytes contains dimers of 67-kDa lamin which are stabilized by both noncovalent interactions and disulfide bonds. The latter can be reduced but re-form when the reducing agent is removed. The cysteine residues involved in these disulfide bonds are inaccessible to alkylating agents unless the protein is unfolded in urea. During nuclear envelope breakdown the lamin is released as a mixture of oligomers in which disulfide-stabilized dimers are associated noncovalently with lamin monomers. Concurrent with solubilization, both dimers and monomers are phosphorylated to a similar extent, indicating that the interactions which maintain these complexes are not destabilized by lamin phosphorylation. Our results suggest the existence of two types of interactions between the lamin molecules in the polymer, which react differently to phosphorylation during nuclear envelope breakdown.

The innermost layer of the nuclear envelope, the nuclear lamina (NL), is a polymeric structure which is built of one to three polypeptide species termed lamins (Aaronson and Blobel, 1974; Dwyer and Blobel, 1976; Gerace, 1986). The interactions between the lamins and their mode of packing into polymeric arrays have been the subject of a number of studies and theoretical considerations. Hydrodynamic measurements and electron microscopic observations have established that in solution lamins tend to form dimers (Havre and Evans, 1983; Aebi et al., 1986; Krohne et al., 1987). In vitro reconstitution studies have shown that lamins can associate into 10-nm filaments (Aebi et al., 1986) and larger paracrystalline structures (Zackroff et al., 1984; Goldman et al., 1986; Aebi et al., 1986) which both exhibit an axial periodicity. Observations on isolated nuclear envelopes of some oocytes have revealed an orthogonal lattice composed of 10-nm filaments with a crossover spacing of 52 nm (Aebi et al., 1986; Akey, 1989). Meanwhile, sequencing studies have shown that human lamina A and C exhibit a high degree of similarity in both primary and secondary structures with the intermediate filament proteins (McKeon et al., 1986; Fisher et al., 1986). Based on these results, a model has been proposed for the packing of lamins into different filamentous and paracrystalline polymorphs (Parry et al., 1987), analogous to the earlier models for the structure of intermediate filaments (Crewther et al., 1983; Parry and Fraser, 1986; Geisler et al., 1985). According to this model, two lamin polypeptides interact in parallel orientation via their central alpha-helical domains to form a dimer. These dimers further associate in an antiparallel half-staggered arrangement to build polymeric arrays. Although the model is consistent with the experimental observations to date, no direct evidence is available for the existence of lamin dimers in the NL structure.

During mitosis the lamins are extensively phosphorylated (Gerace and Blobel, 1980, Ottaviano and Gerace, 1985). It is assumed to weaken the bonds between them, resulting in depolymerization of the NL. The soluble mitotic lamins have been found to sediment as 9 S material in the case of Xenopus eggs (Benavente et al., 1985), and as 4.5-5 S material in the case of Drosophila embryos (Smith and Fisher, 1989), but the composition of these fractions has not been further analyzed. It is not known, therefore, which bonds are destabilized: the coiled-coil interactions between the two strands of a dimer or the interactions between the dimers.

The NL of the oocytes of the surf clam, Spisula solidissima, contains a single lamin species, L67 (Maul et al., 1984). Shortly after fertilization or parthenogenic activation, the oocyte undergoes nuclear envelope breakdown (NEBD), during which L67 is extensively phosphorylated and solubilized (Dessev and Goldman, 1988). This process can be reproduced in a cell-free system containing purified oocyte nuclei and cytosolic extracts from activated oocytes, in which the lamin solubilization is complete (Dessev et al., 1989). These features make the Spisula system an excellent model for studying both the NL structure and the mechanism of its disassembly during M-phase.

In this study we show that lamin dimers are present in the NL structure of surf clam oocytes, where they are stabilized by both noncovalent and disulfide bonds. After NEBD, lamin dimers are found in the soluble fraction, associated with lamin monomers in the form of heterogeneously sedimenting (6-10 S) particles. These results demonstrate that the stability of the lamin dimers is not decreased by their phosphorylation.

MATERIALS AND METHODS

Surf clams were supplied by the Department of Marine Resources at the Marine Biological Laboratory, Woods Hole, MA. Oocytes were obtained and washed as described earlier (Dessev and Goldman, 1988). Most of the methods used in the present study, including isolation of detergent treated nuclei from oocytes, immunoprecipitation, electrophoresis, and autoradiography have been described previously (Dessev and Goldman, 1988; Dessev et al., 1989). NL-enriched fraction was prepared by digestion of detergent-purified nuclei with

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
DNase I and RNase, followed by a treatment with 1.5 M NaCl (Dessev et al., 1989).

NEBD in vitro was carried out using a cell-free system consisting of a cytosolic extract from activated oocytes and purified oocyte nuclei (Dessev et al., 1989). In this system the nuclei undergo a gradual reduction in size accompanied by L67 phosphorylation and its transition from polymeric to soluble state (Dessev et al., 1989). To determine the specific radioactivities of the lamin fractions, NEBD in vitro was carried out in the presence of [γ-32P]ATP. L67 was recovered by immunoprecipitation, L67 monomers and dimers were separated by two-dimensional nonreducing/reducing SDS-PAGE, excised from the gel, and protein in the gel slices was quantitated using Coomassie Blue binding assay after elution with 2% pyridine (Fenner et al., 1975). Radioactivity was measured by Cerenkov radiation counting.

For two-dimensional nonreducing/reducing gel electrophoresis the material was dissolved in sample buffer (Laemmli, 1970) without 2-mercaptoethanol and separated in a 7.5% slab polyacrylamide gel. A 3-mm-wide gel strip containing the fractionated material was cut out, incubated in sample buffer with 5% 2-mercaptoethanol for 1 h at room temperature, placed on the top of another 7.5% slab gel, and run in the second direction. For molecular weight determination in both one- and two-dimensional gels, we used markers in the range 30-200 kDa. (Bio-Rad). The molecular mass of the lamin tetramers (270 kDa) was determined by extrapolation.

A rabbit polyclonal antibody against L67 (Pab227) (Dessev and Goldman, 1988) was used in both immunoprecipitation and immunoblotting experiments. In the latter case Pab227 was used at a dilution of 1:1000 and developed by a 1:1000 dilution of goat anti-rabbit IgG conjugated with peroxidase (Southern Biotech).

Fractionation of the soluble forms of L67 was carried out by velocity sedimentation in 5 ml of 10%-30% linear sucrose gradients containing 100 mM KCl and 20 mM Pipes, pH 7.2. Centrifugation was for 16-18 h at 5 °C at 45,000 rpm in a Beckman SW55 Ti rotor. After fractionation of the gradients, the sucrose concentration was determined by refractometry, and the sedimentation coefficients were calculated according to McEwen (1967). The calculated s values were verified by running markers (bovine serum albumin, 67 kDa, and catalase, 250 kDa, with sedimentation coefficients of 4.3 and 11.3, respectively (Siegel and Monty, 1966)) under the same conditions in separate tubes. In some experiments the protein was precipitated from each fraction by addition of dextran (Sigma, molecular weight 10,000) to 4 mg/ml, followed by two volumes of absolute ethanol (Havre and Evans, 1983). Control experiments showed no difference between samples analyzed directly and after precipitation with dextran/ethanol. After 24 h at −20 °C the precipitates were collected, dissolved in 2% SDS containing 50 mM dithiothreitol (DTT) and separated by SDS-PAGE (Laemmli, 1970) and immunoblotting with Pab227. Protein–protein cross-linking was performed using the noncleavable bifunctional reagent ethylene glycol bis-(succinimidyl succinate) (EGS). The cross-linker was added from a freshly prepared stock in dimethyl sulfoxide to a final concentration of 180-200 mg/ml, and the sample was incubated for 30 min at room temperature. The reaction was stopped by excess of glycine.

RESULTS

Presence of Lamin Dimers in the Nuclear Envelope of Surf Clam Oocytes—Whole unactivated surf clam oocytes were lysed with or without boiling in 2% SDS containing the sulfhydryl-blocking reagents iodoacetamide or N-ethylmaleimide (2.5 mM), 20 mM Pipes, pH 7.2, and fractionated by SDS-PAGE in the absence of reducing agents. Immunoblotting with Pab227, a polyclonal antibody specific for L67 (Dessev and Goldman, 1988; Dessev et al., 1989), revealed two immunoreactive fractions with molecular masses of 67 and 138 kDa (Fig. 1, lanes 1-3). Identical results were obtained if the lysis solution did not contain IAA or NEM (not shown). Similar experiments revealed the presence of a 138-kDa fraction in purified preparations of oocyte nuclei (Fig. 1, lane 4). The 138-kDa fraction can be seen also by Coomassie Blue staining in NL-enriched preparations (Fig. 1, lane 5). Two-dimensional nonreducing/reducing SDS-PAGE identified the 138-kDa material as L67 dimer stabilized by disulfide bonds (Fig. 2). The proportion of dimers in the NL-enriched fraction shown in Fig. 1, lane 5, was 48% of the total amount of lamin, as determined by Coomassie binding (Fenner et al., 1975; in different experiments it was found to vary between 35 and 50%).

Release of Lamin Dimers during NEBD—Further experiments were conducted to determine whether lamin dimers were also present in the soluble fraction after NEBD in vivo. Oocytes were activated by treatment with a solution containing 1.4 M glycerol, 20 mM phosphate buffer, pH 5.0, and 50 mM KCl (Dessev and Goldman, 1988). At different time points the cells were lysed in a solution containing 0.25 M sucrose, 0.75 mM MgCl2, 5 mM Pipes, pH 7.2, and 0.5% Nonidet P-40 (Nonidet P-40 lysis buffer) and separated into detergent-soluble and detergent-insoluble fractions by centrifugation for 10 min at 14,000 rpm in an Eppendorf microfuge. Immunoblotting analysis using Pab227 revealed that lamin dimers were present in the detergent-soluble fraction (Fig. 3A).

Lamin dimers were also released during NEBD under cell-free conditions (Dessev et al., 1989). During this process, the nuclei undergo gradual reduction in size, accompanied by phosphorylation and solubilization of L67 (Dessev et al., 1989). We found that after complete NEBD in vitro the soluble fraction contained both monomers and dimers (Fig. 3B). Both in vivo and in vitro, the lamin accumulation in the...
activated oocytes under previously established conditions for NEBD. Purified oocyte nuclei were exposed to a cytosolic extract from activated oocytes under previously established conditions for NEBD. Clam oocyte nuclei were incubated in the presence of cytosolic extract under standard conditions for NEBD, but in the absence of reducing agents. After NEBD was completed, the sample was spun in an Eppendorf microfuge for 3 min, and the supernatant was incubated for 30 min at room temperature in the presence of 30 mM DTT (lane 2) or in its absence (lane 1). Aliquots of the reduced sample were treated for 10 min at room temperature with either 75 mM IAA (lane 3) or 75 mM NEM (lane 4). Another aliquot was diluted 10 times with a solution containing 0.1 M KCl, 20 mM Pipes, pH 7.2, 5 mM MgCl₂, 10 mM EGTA, 25 mM β-glycerophosphate, and 2 mM DTT (buffer C in Dessev et al., 1989), rapidly mixed with IAA to 75 mM final concentration, followed in 2 s by 1/10 volume of 20% SDS (lane 5). All samples were made 2% in SDS, boiled for 3 min, and analyzed by SDS-PAGE and immunoblotting with Pab227. Prior to electrophoresis, the sample in lane 5 was concentrated using an Amicon Centriprep 10 concentrator. In another experiment, NEBD in vitro was carried out in the presence of 30 mM DTT. After centrifugation for 3 min the supernatant was analyzed directly (lane 6), after treatment with 75 mM IAA (lane 7), and after treatment with 75 mM NEM for 10 min (lane 8). All samples were made 2% in SDS, boiled for 3 min, fractionated by SDS-PAGE in the absence of reducing agents, transferred to nitrocellulose, and reacted with Pab227. 1x, lamin monomers; 2x, lamin dimers.

In a number of experiments we noticed that the ratio dimer/monomer appeared to be released slightly faster than monomers. The failure of IAA and NEM to prevent disulfide bond formation suggested that the cysteine residues involved in these conditions was rapid and appeared unaffected by the concentration of L67 (Fig. 4, lane 4). These results were compatible with a zero-order kinetics of the reaction and suggested that the existing dimers did not dissociate upon reduction; i.e., they were stabilized also by disulfide-independent interactions. Therefore, it was unlikely that the monomers in the soluble fraction were formed by dimer dissociation.

In the absence of reducing agents, NEBD was carried out in vitro (Fig. 4, lane 3) and for a very short period of time (2 s). The re-formation of disulfide bonds under these conditions was rapid and appeared unaffected by the concentration of L67 (Fig. 4, lane 5). These results were compatible with a zero-order kinetics of the reaction and suggested that the existing dimers did not dissociate upon reduction; i.e., they were stabilized also by disulfide-independent interactions. Therefore, it was unlikely that the monomers in the soluble fraction were formed by dimer dissociation.

In a number of experiments we noticed that the ratio dimer/monomer appeared to be higher in the soluble fraction than in the polymer, even when NEBD was carried out in the presence of DTT (compare Fig. 4, lanes 7 and 8 with Fig. 1, lane 4; also Fig. 1, lanes 1–3 with Fig. 3A). These observations suggested that some lamin dimerization might occur during or after NEBD. It should be pointed out, however, that results based on immunoblotting are not strictly quantitative.

The failure of IAA and NEM to prevent disulfide bond formation suggested that the cysteine residues involved in forming these bonds were protected by protein folding. To test this possibility, we carried out the reduction/alkylation cycle in the presence of 8 M urea. Under these conditions no disulfide bonds were formed (Fig. 5) suggesting that the inaccessibility of the cysteines was due to the secondary or tertiary structures of the complex. Below, the lamin dimers which are capable of spontaneous formation of disulfide bonds in the presence of alkylating agents are referred to as “perfect” dimers.

Oxidation Leads to Formation of Lamin Trimers and Tetramers but Does Not Convert All Monomers into Oligomers—In all experiments described so far, L67 monomers were present

FIG. 3. Release of lamin dimers during NEBD. A, in vivo. Clam oocytes were activated by resuspending them in 15 mM phosphate buffer, pH 8.0, containing 1.4 M glycerol and 50 mM KCl at room temperature (Dessev and Goldman, 1988). Samples were taken 0, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13, and 15 min after activation, corresponding to lanes 1–12, respectively; the cells were recovered by a brief centrifugation and lysed in 10 volumes of Nonidet P-40 lysis buffer at 0 °C. The lysates were centrifuged for 10 min in an Eppendorf microfuge in the cold; the supernatants were made 2% in SDS and fractionated by SDS-PAGE in the absence of reducing agents. The lamin monomers; 2x, lamin dimers. 1x, lamin monomers; 2x, lamin dimers.

Soluble fractions was accompanied by a corresponding decrease in the insoluble fractions (not shown). These results demonstrate that the existence of disulfide bonds between two lamin monomers in a dimer does not impede NEBD. It should be noted that both in vivo and in vitro, dimers appear to be released slightly faster than monomers.

Properties of the Lamin Dimers—In an experiment similar to that shown in Fig. 3B, NEBD was carried out in vitro in the absence of reducing reagents, yielding a soluble fraction which contained both monomers and dimers (Fig. 4, lane 1). Treatment of this material with DTT (30 mM, 30 min, room temperature) led to disappearance of the 13% kDa fraction (Fig. 4, lane 2). The reduction of the disulfide bonds required at least 10 mM DTT; below this concentration some dimers persisted (not shown). Surprisingly, when DTT was neutralized by an excess of IAA (Fig. 4, lane 2) or NEM (Fig. 4, lane 4) before boiling in SDS, the dimers were restored in about the same proportion. Similar results were obtained when NEBD was carried out in the presence of DTT and then the soluble fraction was boiled in SDS either without (Fig. 4, lane 6) or with addition of an excess of alkylating reagents (Fig. 4, lanes 7 and 8). These results suggested that either the dimers dissociated upon reduction with subsequent re-formation in the presence of IAA, or the dimers persisted under reducing conditions. In order to determine which possibility was true, we carried out the alkylation step on a sample diluted 10 times compared with the control (Fig. 4, lane 3) and for a very short period of time (2 s). The re-formation of disulfide bonds under these conditions was rapid and appeared unaffected by the concentration of L67 (Fig. 4, lane 5). These results were compatible with a zero-order kinetics of the reaction and suggested that the existing dimers did not dissociate upon reduction; i.e., they were stabilized also by disulfide-independent interactions. Therefore, it was unlikely that the monomers in the soluble fraction were formed by dimer dissociation.

In the absence of reducing agents, NEBD was carried out in vitro (Fig. 4, lane 3) and for a very short period of time (2 s). The re-formation of disulfide bonds under these conditions was rapid and appeared unaffected by the concentration of L67 (Fig. 4, lane 5). These results were compatible with a zero-order kinetics of the reaction and suggested that the existing dimers did not dissociate upon reduction; i.e., they were stabilized also by disulfide-independent interactions. Therefore, it was unlikely that the monomers in the soluble fraction were formed by dimer dissociation.

In the absence of reducing agents, NEBD was carried out in vitro (Fig. 4, lane 3) and for a very short period of time (2 s). The re-formation of disulfide bonds under these conditions was rapid and appeared unaffected by the concentration of L67 (Fig. 4, lane 5). These results were compatible with a zero-order kinetics of the reaction and suggested that the existing dimers did not dissociate upon reduction; i.e., they were stabilized also by disulfide-independent interactions. Therefore, it was unlikely that the monomers in the soluble fraction were formed by dimer dissociation.

In a number of experiments we noticed that the ratio dimer/monomer appeared to be higher in the soluble fraction than in the polymer, even when NEBD was carried out in the presence of DTT (compare Fig. 4, lanes 7 and 8 with Fig. 1, lane 4; also Fig. 1, lanes 1–3 with Fig. 3A). These observations suggested that some lamin dimerization might occur during or after NEBD. It should be pointed out, however, that results based on immunoblotting are not strictly quantitative.

The failure of IAA and NEM to prevent disulfide bond formation suggested that the cysteine residues involved in forming these bonds were protected by protein folding. To test this possibility, we carried out the reduction/alkylation cycle in the presence of 8 M urea. Under these conditions no disulfide bonds were formed (Fig. 5) suggesting that the inaccessibility of the cysteines was due to the secondary or tertiary structures of the complex. Below, the lamin dimers which are capable of spontaneous formation of disulfide bonds in the presence of alkylating agents are referred to as “perfect” dimers.

Oxidation Leads to Formation of Lamin Trimers and Tetramers but Does Not Convert All Monomers into Oligomers—In all experiments described so far, L67 monomers were present
During NEBD L67 Is Released in the Form of Oligomers Containing Perfect Dimers—In all experiments described thus far, the protein samples were fractionated by SDS-PAGE. Under these conditions we would have failed to detect eventually present higher L67 oligomers maintained by noncovalent interactions. To explore this possibility, we analyzed the material solubilized during NEBD in vitro using velocity sedimentation in sucrose gradients. NEBD was carried out either in the presence of DTT (20 mM in the reaction mixture and 10 mM in the sucrose gradient) or in its absence. Identical results were obtained in both cases. The gradient fractions were treated with IAA in excess to let the disulfide bonds in the perfect dimers reform in the case of reducing gradients and subjected to SDS-PAGE followed by immunoblotting. The lamin-containing material was found to sediment as a broad zone (Fig. 7). The electrophoretic analysis of this material revealed the presence of perfect dimers and monomers with small amounts of trimers (about 200 kDa) and tetratiemers (about 270 kDa) in the peak fractions, but also material giving rise to perfect dimers only (without monomers detectable under our conditions), sedimenting as a slower and a faster shoulder of the main peak (Fig. 7). The average sedimentation rate of the zone was higher than that reported for lamin dimers (4–5 S) by Havre and Evans (1983) and Aebi et al. (1986). From the composition of the L67-containing material and its sedimentation behavior, it appeared likely that the soluble fraction after NEBD contained mostly a mixture of lamin dimers, trimers, and tetratiemers, sedimenting as three overlapping zones: dimers in the slower shoulder (4–5 S), trimers in the central region (6–9 S), and tetratiemers in the faster shoulder (10–11 S). No free monomers, which should be expected to sediment at or slower than 5 S (Havre and Evans (1983; Aebi et al. (1986)) were found in these experiments. Still, our data should be interpreted with caution since (a) in this, as well as in other cases (Benavente et al., 1985; Smith and Fisher, 1989), the sedimentation behavior of the lamin-containing particles might be affected by interactions with other proteins present in the gradients, and (b) some of these complexes (trimers and tetratiemers) may have been formed in the soluble fraction after disassembly of the NL.

In order to determine by an independent method whether higher lamin oligomers were present in the soluble fraction, we used protein-protein cross-linking with EGS carried out in the presence of 30 mM DTT. EGS creates covalent bonds between two aliphatic aminogroups at a distance of about 1.2 nm. Apart from lamin dimers, these experiments revealed trimers and tetratiemers as major reduction-resistant products of cross-linking (Fig. 8), in agreement with the sedimentation results. The presence of monomers probably reflects the fact that the cross-linking efficiency is less than 100%.

Lamin Phosphorylation Does Not Affect Dimer Stability—We have shown previously that during NEBD, L67 acquires about 3 mol of phosphate/mol of protein on the average (Dessev et al., 1989). The analysis of the soluble fraction after NEBD both in vivo and in vitro (Figs. 3) suggested that protein phosphorylation did not lead to dissociation of the lamin dimers or larger soluble oligomers. However, since unpaired lamin molecules were always present in this fraction together with perfect dimers, it was possible that the observed increase in the phosphate level might be due to modification of one of these forms only. In order to elucidate this point,
with EGS. Soluble fraction was prepared after NEBD in the presence of 30 mM DTT and treated for 20 min at room temperature with 200 µg/ml EGS added from a 10 mg/ml stock in dimethyl sulfoxide. The excess of cross-linker was neutralized by glycine added in excess, the sample was centrifuged for 3 min in an Eppendorf centrifuge. L67 monomers and dimers were recovered from the pellet (A) and the supernatant (B) by immunoprecipitation using Pab227. The immunoreducing SDS-PAGE and autoradiographed, followed by determination of their specific radioactivities as described under "Materials and Methods." 1x, lamin monomers; 2x, lamin dimers.

**FIG. 8. Cross-linking of the lamins in the soluble fraction with EGS.** Soluble fraction was prepared after NEBD in the presence of 30 mM DTT and treated for 20 min at room temperature with 200 µg/ml EGS added from a 10 mg/ml stock in dimethyl sulfoxide. The excess of cross-linker was neutralized by glycine added in excess, the sample was dissolved by boiling in sample buffer containing 5% 2-mercaptoethanol and analyzed by SDS-PAGE and immunoblotting with Pab227. The position of the lamin monomers, dimers, trimers, and tetramers (1x, 2x, 3x, and 4x, respectively) and those of the molecular weight markers are indicated.

**FIG. 9. Phosphorylation of L67 monomer and dimer during NEBD in vitro.** NEBD was carried out in a standard reaction mixture (Dessev et al., 1989) containing 135 µCi/ml of [γ-32P]ATP and no reducing agents. After the nuclei reached about 50% of their initial diameter, corresponding to about 65% lamin solubilization, the sample was centrifuged for 8 min in an Eppendorf centrifuge. L67 monomers and dimers were recovered from the pellet (A) and the supernatant (B) by immunoprecipitation using Pab227. The immunoprecipitates were fractionated by two-dimensional nonreducing/reducing SDS-PAGE and autoradiographed, followed by determination of their specific radioactivities as described under "Materials and Methods." 1x, lamin monomers; 2x, lamin dimers.

We measured the 32P incorporation into the lamin monomers and dimers in both soluble and insoluble fraction after partial NEBD in vitro. Monomers and dimers were separated in two-dimensional nonreducing/reducing gels (Fig. 9). The specific radioactivities of these two fractions were found to be similar (2735 and 3737 cpm/A600 for the soluble monomers and dimers respectively, shown in Fig. 9B). This difference is not significant, taking into account the low accuracy of the Coomassie binding assay (Fenner et al., 1975). We conclude that L67 molecules are phosphorylated to a similar extent regardless of whether they are associated as perfect dimers or unpaired. These results also demonstrate that phosphorylation during NEBD has no detectable effect on the stability of the lamin dimers found in the soluble fraction under the present conditions.

**DISCUSSION**

Our experiments provide direct evidence for the presence of lamin dimers in the NL polymer of Spisula oocytes. It appears that the structure also contains unpaired lamin molecules. The lamin dimers are stabilized by both noncovalent and disulfide bonds. The two lamin polypeptides are complexed in such a way that juxtaposed cysteines should be located within a distance of one amino acid residue, which is suggestive of parallel orientation (Parry et al., 1987; Steinert and Roop, 1988). The spontaneous formation of disulfide bonds between these cysteines, their inaccessibility to alkylating agents, and the high concentration of DTT required for their reduction suggest that they are located in the α-helical domains of the lamin molecules and are protected by the secondary/tertiary structure of the complex. If this structure is unfolded in urea, the protection is abolished, and the sulfhydryl groups become susceptible to alkylation. Reduction of the disulfide bonds appears to be reversible and does not cause dimer dissociation, suggesting that the two strands are held together also by strong noncovalent interactions. These interactions do not appear to be affected during NL disassembly.

Disulfide-cross-linked lamin A and B homo-oligomers have been described earlier in mammalian cells (Lam and Kasper, 1979). Maul et al. (1984), at variance with our results (Fig. 6), have obtained lamin trimers as a major product of oxidative crosslinking of clam oocyte nuclear envelope. Later, Kaufmann et al. (1983) have suggested that these oligomers may be artifacts, formed during the isolation of nuclear envelopes under oxidizing conditions. Disulfide bonds have been shown to form in dimers of bacterially expressed recombinant Xenopus lamin L1 (Krohne et al., 1987).

Do disulfide bonds play a role in stabilizing the NL structure? Some of the lamins which have been sequenced do not have cysteines in their central α-helical regions (Fisher et al., 1986; McKeon et al., 1986; Stick, 1988) and should not be able to form disulfide-stabilized dimers. Furthermore, our results show that the presence of disulfide bonds does not affect NEBD and the process of lamin solubilization. The possibility should be considered, therefore, that the disulfide bonds have no special biological function and are present only in those cases where the primary structure of the lamins favors their formation.

We can imagine the structural integrity of the NL polymer as dependent on at least two types of interactions: type I, mostly hydrophobic interactions between the central α-helical domains of the lamin polypeptides, leading to formation of two-stranded coiled coils, and type II, interactions between lamin dimers, probably involving the nonhelical terminal domains of the molecules. Type I interactions can be "reinforced" by disulfide bonds, as happens to be the case with the surf clam oocytes (see also Krohne et al., 1987) probably depending on the oxidative potential of the environment, but they are not affected by protein phosphorylation. Therefore, assuming a mechanism of NEBD based on lamin phosphorylation (Gerace and Blobel, 1980; Ottaviano and Gerace, 1985; Dessev and Goldman, 1988), the NL disassembly appears to involve phosphorylation-induced weakening of type II interactions. The location of the phosphorylation sites in the lamin molecules has not yet been determined. However, an assumption by analogy with the intermediate filament proteins (McKeon et al., 1986; Fisher et al., 1986) that the phosphates are in the N- and/or C-terminal domains of the lamin molecules (Steinert and Roop, 1988) would be compatible with the involvement of these domains in type II interactions (Parry et al., 1987).
Lamin Dimers in Surf Clam Oocytes

Does the existence of unpaired lamin molecules together with dimers have any functional significance? The NL of the surf clam oocytes is closely associated with the nuclear membrane, nuclear pore complexes, and chromosomes (Maul, 1980). Perhaps such a diversity of interactions could be achieved on the basis of structural, as opposed to compositional, heterogeneity in the NL polymer. For example, whereas dimers may serve as a backbone of the lamin polymer, the monomers may be involved in interactions with the other structural elements of the nuclear envelope. Another possibility is that the observed heterogeneity may be due to modification of a fraction of the lamin molecules. Isoprenylation of C-terminal cysteines has been suggested to play a role in the attachment of the NL to the nuclear membrane (Vorburger et al., 1989; Krohne et al., 1989; Farnsworth et al., 1989; Kitten et al., 1989; Beck et al., 1989). Isoprenylated cysteines would be unable to form disulfide bonds and the corresponding lamin polypeptides would appear as monomers under our conditions. Finally, the presence of unpaired monomers may be explained by the process of NL growth during oogenesis, which may not allow all new lamin molecules to form perfect dimers upon their insertion into the pre-existing polymer lattice. These possibilities are currently under investigation in our laboratory.

Acknowledgments We thank Dr. Alvin Telser for providing laboratory space and equipment, Dr. Robert Palazzo of the Marine Biological Laboratory for a sample of oocyte lysate, and Drs. Alvin Telser, Russ Kohnken, and Ying-Hou Chou for critical reading of the manuscript.

REFERENCES