Spontaneous Assembly of Pore Complex–containing Membranes ("Annulate Lamellae") in *Xenopus* Egg Extract in the Absence of Chromatin

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Abstract. Extract prepared from activated *Xenopus* eggs is capable of reconstituting nuclei from added DNA or chromatin. We have incubated such extract in the absence of DNA and found that numerous flattened membrane cisternae containing densely spaced pore complexes (annulate lamellae) formed de novo. By electron and immunofluorescence microscopy employing a pore complex–specific antibody we followed their appearance in the extract. Annulate lamellae were first detectable at a 30-min incubation in the form of short cisternae which already contained a high pore density. At 90–120 min they were abundantly present and formed large multilamellar stacks. The kinetics of annulate lamellae assembly were identical to that of nuclear envelope formation after addition of DNA to the extract. However, in the presence of DNA or chromatin, i.e., under conditions promoting the assembly of nuclear envelopes, annulate lamellae formation was considerably reduced and, at sufficiently high chromatin concentrations, completely inhibited. Incubation of the extract with antibodies to lamin L_{III} did not interfere with annulate lamellae assembly, whereas in the presence of DNA formation of nuclear envelopes around chromatin was inhibited. Our data show that nuclear membrane vesicles are able to fuse spontaneously into membrane cisternae and to assemble pore complexes independently of interactions with chromatin and a lamina. We propose that nuclear envelope precursor material will assemble into a nuclear envelope when chromatin is available for binding the membrane vesicles, and into annulate lamellae when chromatin is absent or its binding sites are saturated.

MITOSIS of higher eukaryotic cells is accompanied by profound rearrangements and molecular changes of the nuclear envelope constituents that comprise the double-layered nuclear membrane, the lamina lining the inner nuclear membrane, and the pore complexes. At the onset of mitosis the nuclear envelope breaks down and its disassembled component parts redistribute throughout the cytoplasm (reviewed by Gerace and Burke, 1988). How they are targeted at the end of mitosis to the surface of the telophase chromosomes of the daughter cells and in which order they reassemble into native nuclear envelopes is at present poorly understood. This holds in particular for the assembly pathway of the nuclear pore complexes and their mode of interaction with the nascent nuclear membranes.

Cell-free systems derived from activated amphibian eggs (Lohka and Masui, 1983; Newmeyer et al., 1986; Newport, 1987; for reviews see Lohka and Maller, 1987; Lohka, 1988), *Drosophila melanogaster* embryos (Ulitzur and Gruenbaum, 1989; Berrios and Avilion, 1990), and mitotic somatic cells (Burke and Gerace, 1986; Nakagawa et al., 1989; Burke, 1990) have been used to study the assembly pathway of nuclear envelopes in vitro. Extracts prepared from *Xenopus* eggs have the advantage to contain large amounts of all molecular components required for de novo formation of nuclear envelopes, such as depolymerized lamin L_{III} (the only lamina protein species present in these cells; Benavente et al., 1985; Stick and Hausen, 1985), membrane vesicles competent for nuclear membrane formation (Wilson and Newport, 1988), and soluble pore complex proteins, some of which occur in form of discrete macromolecular assemblies (Dabauvalle et al., 1990). In addition, egg homogenates also contain a pool of prefabricated material required for chromatin assembly and hence are capable to reconstitute nuclei not only from added chromatin but also from protein-free DNA by a sequential process involving a number of intermediate steps (Newmeyer et al., 1986; Blow and Laskey, 1986; Newport, 1987).

By studying nuclear envelope assembly in vitro, two models have been proposed how pore complexes might be formed (reviewed by Lohka, 1988). According to the "vesicle precursor model," membrane vesicles first bind to the surface of chromatin and then fuse laterally into extended flat cisternae. Subsequently, pores are formed by a localized fusion of the inner and outer nuclear membranes (Lohka and Masui, 1984; Lohka, 1988). The pore-specific integral membrane glycoprotein gp210 might be involved in the induction of such local membrane breakage-fusion events in concert with soluble pore complex components (Wozniak et al., 1989; Greber et al., 1990). In contrast, the "prepore model" implies that the surface of decondensing chromatin

acts as a nucleation site where assembly of pore complexes takes place, which then, in turn, act as receptors for nuclear membrane vesicle binding (Sheehan et al., 1988). In spite of the differences in the proposed sequence of events of nuclear envelope formation, both models consider chromatin as an essential element for pore complex formation.

We have recently noted that *Xenopus* egg extract incubated in the presence of lambda DNA contained, besides the reconstituted nuclei, stacks of annulate lamellae (Dabauvalle et al., 1990). Annulate lamellae, most commonly observed in the cytoplasm of germ cells and rapidly dividing somatic cells such as embryonic or tumor cells, are flattened membrane cisternae, arranged either singly or in stacks of parallel sheets, which contain densely spaced pore complexes (for review see Kessel, 1989). Contrary to other authors who have also observed annulate lamellae in *Rana* or *Xenopus* egg extract after incubation with demembranated sperm nuclei or plasmid DNA (Lohka and Maller, 1987; Blow and Sleeman, 1990), we never found them exclusively in association with chromatin surfaces or newly formed nuclei. We were intrigued by the possibility that the occurrence of annulate lamellae in the egg extract might indicate an assembly pathway of pore complexes independent of chromatin.

Materials and Methods

Antibodies

PI1 (IgM) is a mouse mAb which has been previously described (Chaly et al., 1984). It recognizes a major *N*-acetylglucosamine (GlcNAc)-containing pore complex glycoprotein of M_r 68,000 from *Xenopus* oocytes (Dabauvalle et al., 1988) and mammalian cells (Benavente et al., 1989a). IgMs were purified from mouse ascites fluid by hydroxylapatite chromatography and kindly provided by Dr. David L. Brown (Department of Biology, University of Ottawa, Canada). S49H2 (IgG) is a murine mAb directed against *Xenopus laevis* lamin L_{III}. Ascites fluid was a gift of Dr. Georg Krohne (German Cancer Research Center, Heidelberg, FRG).

Egg Extract

Extracts were prepared from dejellied *Xenopus* eggs 20 min after parthenogenetic activation by electric shock (Karsenti et al., 1984) essentially as described by Newport (1987). After addition of an ATP regenerating system (2 mM ATP, 20 mM phosphocreatine, 50 µg/ml creatine kinase), the homogenate was incubated at 22°C. Aliquots were removed at different time intervals (up to 2 h) and examined by immunofluorescence or EM.

To promote nuclear reconstitution, bacteriophage lambda DNA (Gibco-BRL; Eggenstein, FRG) was added to the egg extract as outlined by Newport (1987). Alternatively, demembranated *Xenopus* sperm nuclei (prepared according to Blow and Laskey, 1986) were added to the extract at concentrations ranging between 600 and 60,000 nuclei per microliter.

Manipulation of the Egg Extract

10 µl of antilamin antibody S49H2 (adjusted to a concentration of 10 mg/ml in PBS) were added to 100 µl of extract just before incubation with lambda DNA. After 90 or 120 min, aliquots of the extract were processed for EM. Control extracts were incubated with nonimmune mouse IgG at the same concentration.

Separation of the extract into a soluble supernatant fraction and a membrane-enriched pellet was achieved by centrifugation at 100,000 *g* in an SW 50 rotor (Beckman Instruments, Inc., Palo Alto, CA) for 1 h. Both fractions were tested separately for their ability to assemble annulate lamellae by immunofluorescence and EM as described above. Extract was reconstituted by mixing both fractions at a ratio of 1:1.

Immunofluorescence Microscopy

Frozen sections (5 µm thick) of *Xenopus laevis* ovary and eggs were air

dried, fixed in −20°C acetone for 10 min, and air dried again. After incubation with PI1 or S49H2 antibodies for 15 min followed by several wash steps in PBS, bound antibodies were visualized by goat anti-mouse IgG/IgM antibodies conjugated to Texas-Red (Dianova, Hamburg, FRG; diluted 1:20).

Extract was fixed by dilution with 4 vol of 3% formaldehyde in PBS (freshly prepared from paraformaldehyde). After centrifugation onto microscope slides using a cytocentrifuge (15 min at 1,000 rpm in a Cytospin 2; Shandon, Frankfurt, FRG), specimens were fixed in −20°C acetone for 10 min and processed for immunofluorescence microscopy as described above. Photographs were taken with a Zeiss Axiophot (Carl Zeiss, Oberkochen, FRG) equipped with epifluorescence optics.

EM

Small pieces from a *Xenopus* ovary were fixed for 4 h in cold 2.5% glutaraldehyde buffered with 50 mM sodium cacodylate to pH 7.4. After several washes in cold cacodylate buffer, they were postfixed in 2% OsO₄ for 2 h, washed in distilled water, and dehydrated through a series of graded ethanol solutions. After embedding in Epon, ultrathin sections were cut and stained according to standard protocols.

Samples of the extract (50 µl each) were fixed by fourfold dilution with cold 2.5% glutaraldehyde in 0.2 M cacodylate buffer (pH 7.4) for 30 min. The pellet obtained by centrifugation for 10 min at 3,000 *g* was resuspended in a drop of liquid 2% low melting agarose (Gibco-BRL) prepared in 0.2 M cacodylate buffer at 30°C. After solidifying the agarose by chilling, the samples were postfixed for 5 min in glutaraldehyde followed by 2% OsO₄ (15 min) and processed for EM as described above. Micrographs were taken with a Zeiss EM 10 electron microscope.

Results

For the purpose of the present study it was essential to establish that the annulate lamellae observed in *Xenopus* egg extract after the incubation period originated from a spontaneous assembly process and did not preexist as maternally derived structures. In view of the abundance of annulate lamellae in mature *Xenopus* oocytes (Balinsky and Devis, 1963; Steinert et al., 1974; for further refs. see Kessel, 1989), it is not unreasonable to assume that they might also exist in eggs and homogenates therefrom as suggested by Newport (1987). However, by careful inspection of a large number of electron microscopic sections of several different extract preparations fixed after an incubation time of 0–5 min, we were unable to detect annulate lamellae. Obviously, conclusive evidence for their absence would require serial sectioning of complete extract samples. To circumvent such an extremely time-consuming approach we looked for conditions that would allow visualization of annulate lamellae by immunofluorescence microscopy.

Annulate Lamellae of Xenopus Oocytes Can Be Visualized by Immunofluorescence Microscopy

It is well known that annulate lamellae occur in the cytoplasm of *Xenopus* oocytes (Balinsky and Devis, 1963; Steinert et al., 1974; for further refs. see Kessel, 1989). In electron microscopic sections of *Xenopus* oocytes, annulate lamellae commonly appear in form of stacked parallel arrays of pore complex–bearing flat membrane cisternae, often in curvilinear or semicircular arrangements (Fig. 1 *a*). Pore complexes are usually densely spaced. In fact, it seems to be a characteristic feature of annulate lamellae from oocytes of *Xenopus* and amphibians in general that their pore frequency is markedly higher as compared to that of the nuclear envelope of the same cell (for quantitative data see Scheer and Franke, 1969; Scheer, 1972).

When cryosections of *Xenopus* oocytes were incubated
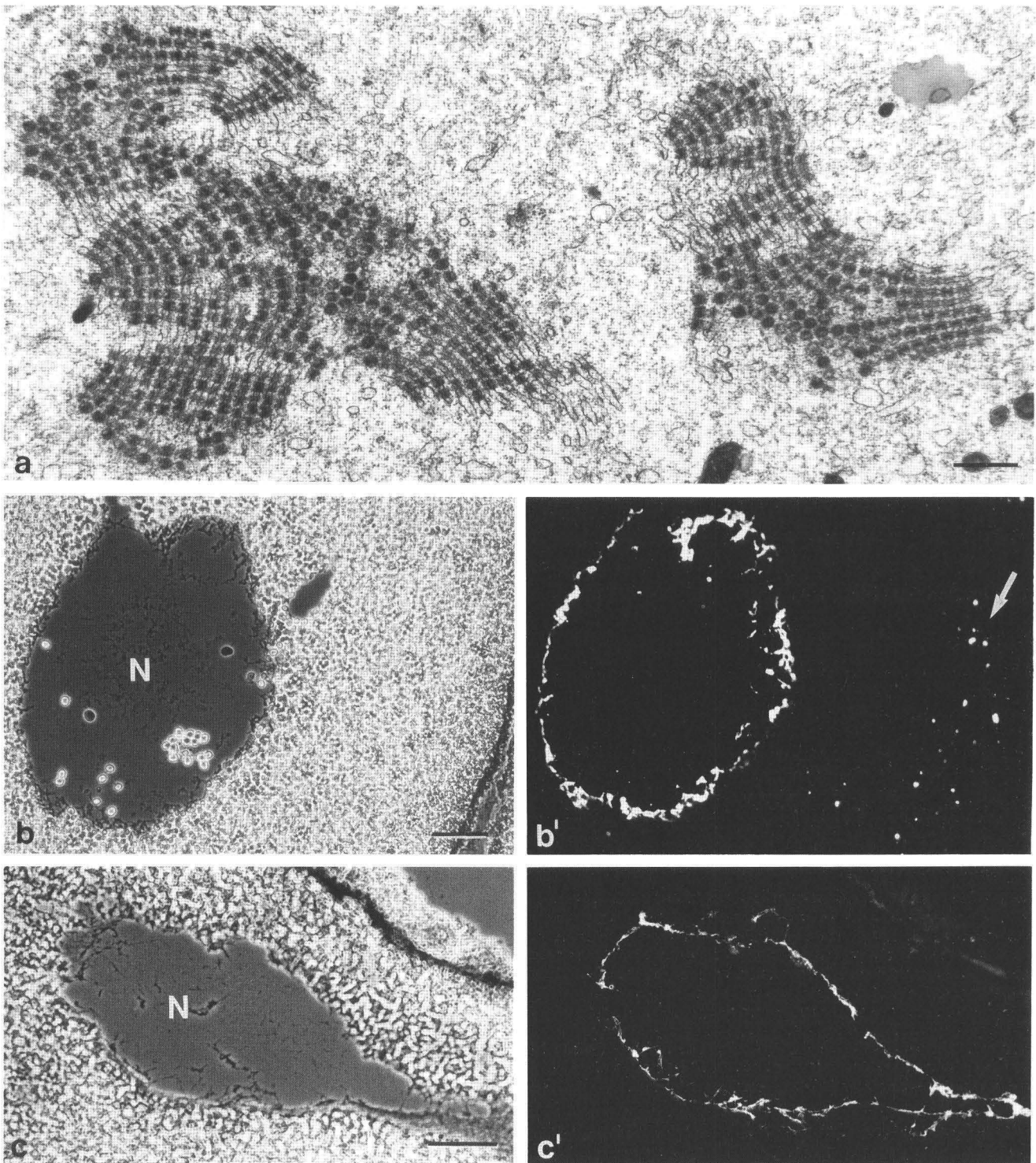
Figure 1. Annulate lamellae in *Xenopus laevis* oocytes. The electron micrograph of an ultrathin section (*a*) shows stacks of annulate lamellae in the ooplasm. Note the high pore density. Immunofluorescence microscopy using the pore-specific antibody PI1 reveals the distribution of annulate lamellae in a cryosectioned *Xenopus* oocyte (*b'*). Antibody PI1 stains the nuclear envelope (*N*, nucleus) as well as numerous annulate lamellae that are located preferentially in the periphery of the oocyte (*arrow* in *b'*; shown here is a full-grown oocyte). In contrast, antilamin antibody S49H2 stains exclusively the nuclear periphery (*c'*). The corresponding phase-contrast images are shown in *b* and *c*. Bars: (*a*) 0.5 μm; (*b* and *c*) 50 μm.

with mAb PI1 which is directed against a major nuclear pore complex glycoprotein of M_r 68,000 (p68; Dabauvalle et al., 1988), the nuclear envelope revealed a strong fluorescence (Fig. 1 *b'*). In addition, numerous fluorescent dots were seen in the cytoplasm, preferentially in its peripheral zone. Based on the specificity of the antibody and the well-documented structural identity of pore complexes of the nuclear envelope and annulate lamellae (Scheer and Franke, 1969; reviewed
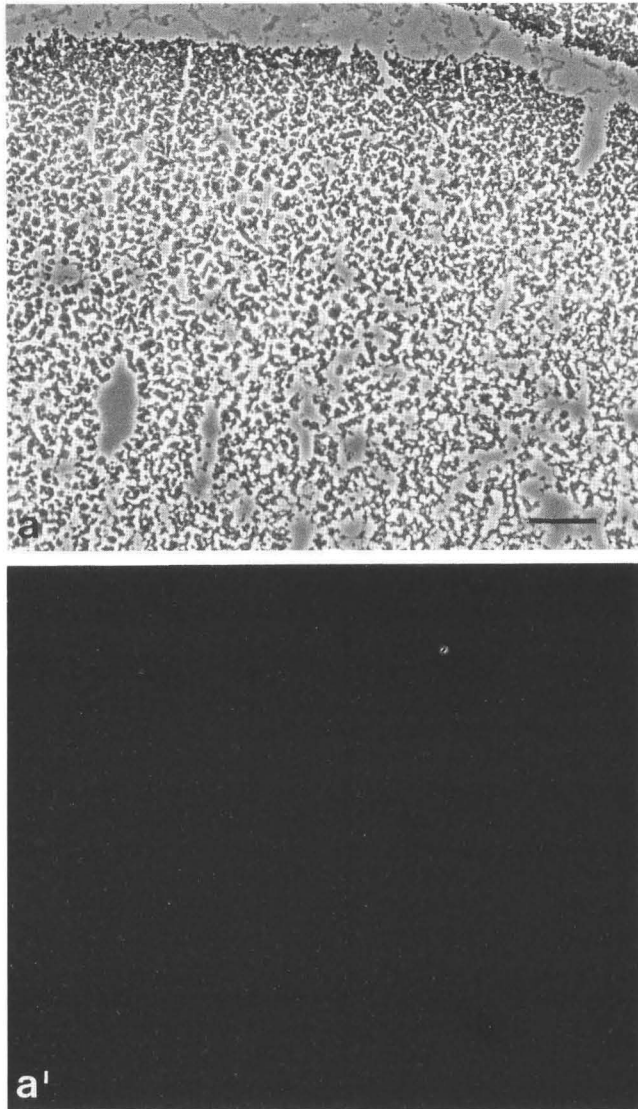
Figure 2. Absence of annulate lamellae in *Xenopus* eggs. A frozen section was incubated with antibody PI1 followed by Texas red-labeled secondary antibodies and viewed with epifluorescence (*a'*) and phase-contrast (*a*) optics. Bar, 50 μm.

by Kessel, 1989), we conclude that the punctate cytoplasmic fluorescence reflects the presence of annulate lamellae (see also Klymkowsky and Maynell, 1989). In contrast, antibody S49H2 directed against lamin L$_{III}$ decorated exclusively the nuclear periphery (Fig. 1 *c'*), indicating that a distinct lamina layer is absent from the membranes of the annulate lamellae.

Annulate Lamellae Are Absent from Xenopus Eggs and Freshly Prepared Egg Extract

Frozen sections were prepared from *Xenopus* eggs directly after oviposition and from activated eggs 20 min after their release from metaphase arrest by electrical shock. Immunofluorescence microscopy with antibody PI1 failed to detect the dot-like fluorescence pattern characteristic of oocytes (Fig. 2 *a'*).

When extract was analyzed at early time points of incubation (0.5–5 min) by EM, annulate lamellae were never seen (Fig. 3 *a*). This holds true not only for the conspicuous stacked form of annulate lamellae but also for individual membrane cisternae containing pore complexes. Furthermore, despite extensive search we could not detect structures that resembled either free pore complexes (see Fig. 8 *d* in Scheer et al., 1988) or pore complexes inserted solitarily in membranes. In essence, our electron microscopical analysis showed that the extract contained large amounts of membrane vesicles, membranous stacks of the Golgi apparatus, coated vesicles, mitochondria, and ribosomes but lacked pore complexes. When the same extract was examined by immunofluorescence microscopy employing the pore-specific antibody PI1, no positive signal could be detected after an incubation time of 0.5–5 min (Fig. 4 *a'*). We conclude that annulate lamellae neither exist in intact *Xenopus* eggs nor in the initial egg extract.

In Vitro Assembly of Annulate Lamellae Does Not Require Addition of DNA to the Extract

Egg extract was incubated without exogenous DNA and examined at increasing time intervals by EM. A few annulate lamellae, usually in form of single or paired membrane cisternae, were first encountered 30 min after incubation (Fig. 3 *b*). Initially the annulate lamellae profiles were relatively short and appeared to grow by lateral fusion and flattening of membrane vesicles similar to what has been described for nuclear envelope formation around sperm chromatin (Lohka and Masui, 1984; Lohka and Maller, 1987). A further common feature between nascent annulate lamellae and nuclear envelopes was the presence of a coat of electron-dense material covering their cytoplasmic surfaces (Fig. 3 *b*; see Lohka and Masui, 1984). The initial step in the formation of a pore complex appeared to be a localized fusion of both membranes of a newly formed cisterna followed by structural rearrangements of the coat on both sides of the pore margin into distinct subunits (for a discussion of possible modes of pore complex formation see Franke, 1974). Furthermore, it is interesting to note that the cisternal space of the nascent annulate lamellae contained a network

Figure 3. Time course of assembly of annulate lamellae in *Xenopus* egg extract incubated in the absence of DNA as seen in electron microscopic sections. The starting extract (5-min incubation) is rich in membrane vesicles, mitochondria, ribosomes, and coated vesicles (some are denoted by *small arrows*), but devoid of pore-containing structures (*a*). A subpopulation of the membrane vesicles is filled with fine filaments arranged in a loose network (*thick arrows* in *a*). After an incubation time of 30 min, a few annulate lamellae with densely spaced pore complexes are recognized (*b*). The architecture of an early formed pore complex is depicted at higher magnification in the insert of *b* (annulus subunits are denoted by *arrows*). The cisternal space of the nascent annulate lamellae is filled with filamentous material (*b*). Size and number of annulate lamellae profiles increase gradually over a time period of ∼2 h (*c*, 60 min; *d–f*, 90 min). At 90 min the extract contains numerous large stacks of annulate lamellae as shown in transverse (*d* and *e*) and tangential sections (*f*). Bars: (*a–f*) 0.5 μm; (*insert* in *b*) 0.1 μm.
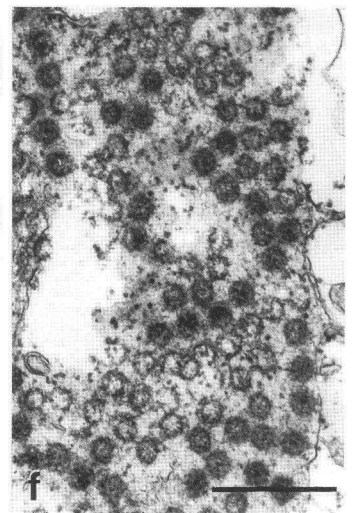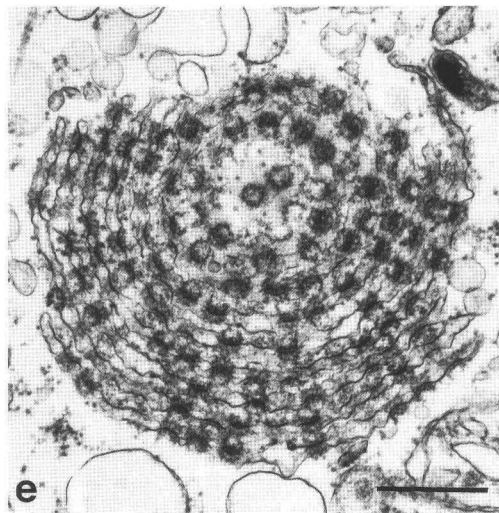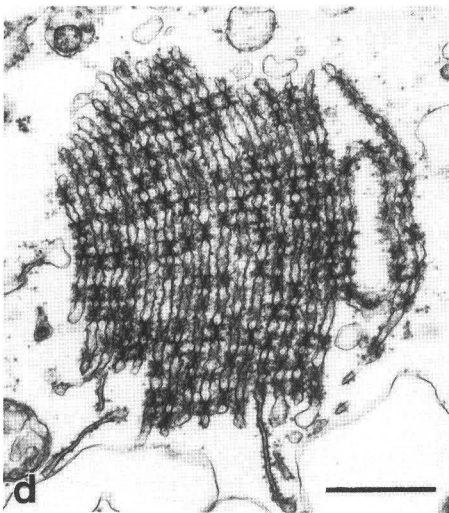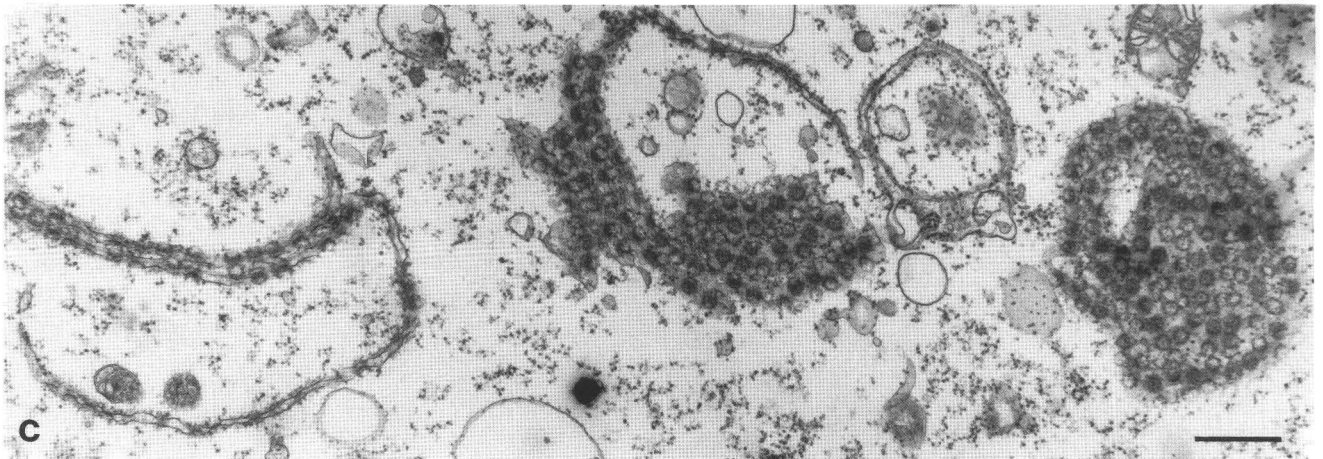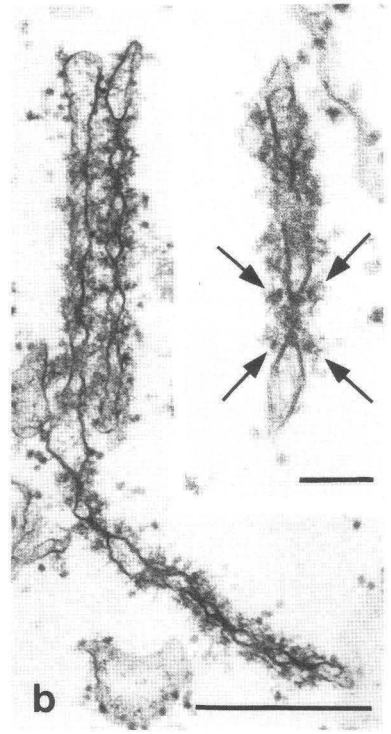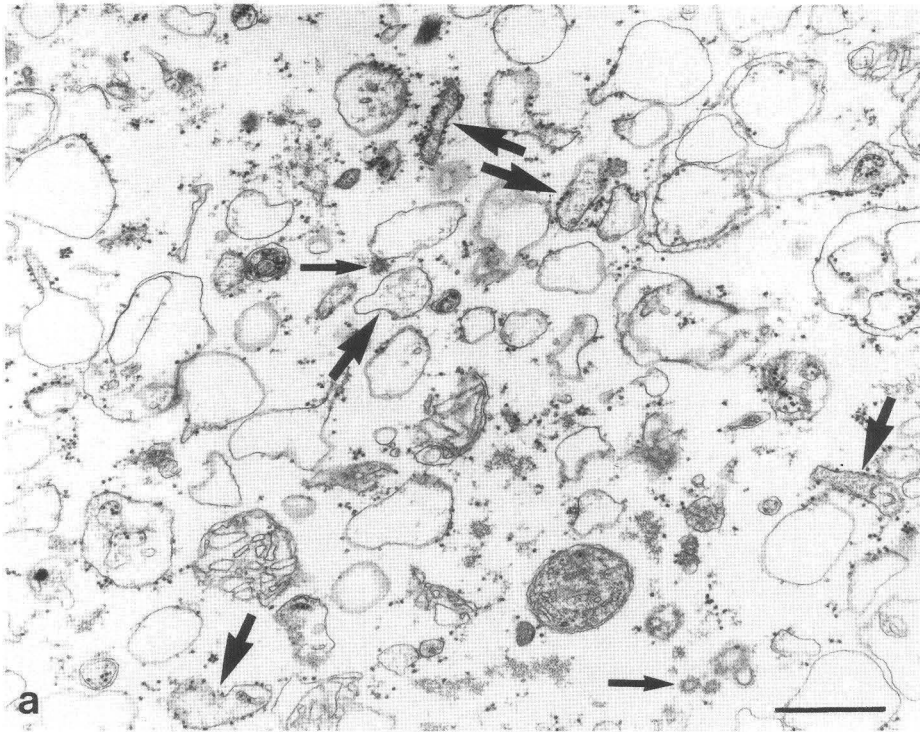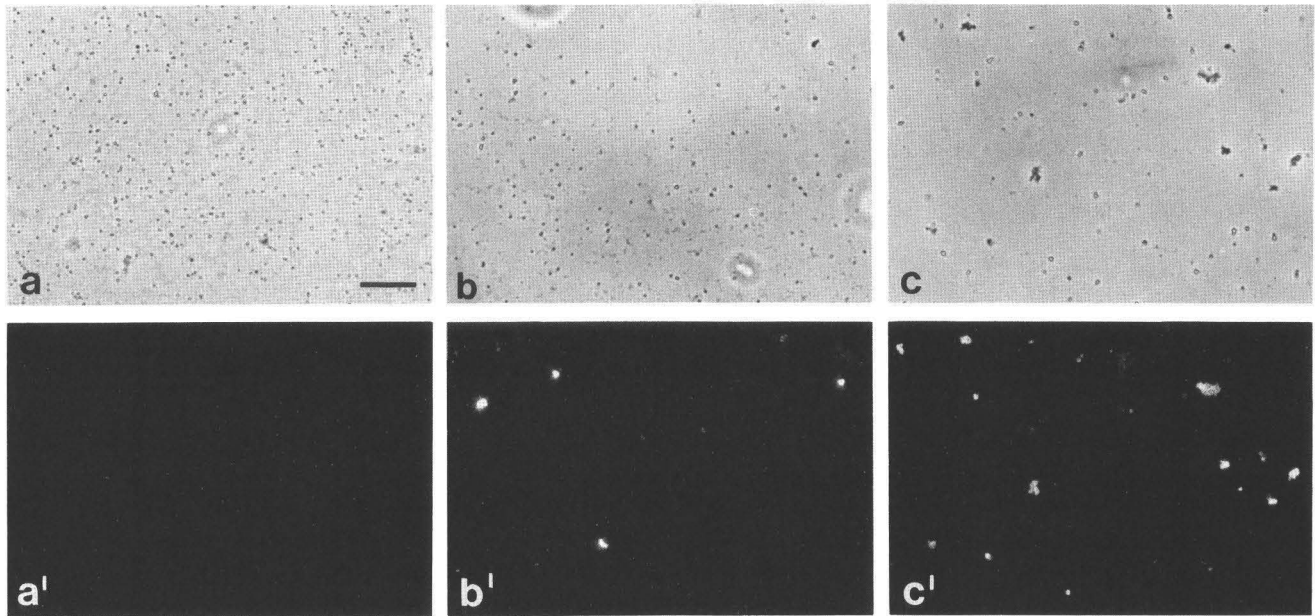
Figure 4. Time course of annulate lamellae formation in egg extract incubated without DNA as revealed by immunofluorescence microscopy using the pore-specific antibody PI1 (*a'*, 0 min; *b'*, 30 min; *c'*, 60-min incubation). The corresponding phase-contrast images are shown in *a–c*. Bar, 20 μm.

of fine filaments (Fig. 3 *b*). Since membrane vesicles with a similarly structured content occurred in the initial egg extract (Fig. 3 *a*), we suppose that this specific vesicle subpopulation represents the major membrane source of annulate lamellae.

After an incubation time of 60 min the number and size of annulate lamellae increased considerably (Fig. 3 *c*). At 90 min, large stacks of annulate lamellae consisting of 20–30 parallel cisternae were abundantly present, frequently in circular or semicircular arrangements (Fig. 3, *d* and *e*). The coat adhering to the surface of the nascent annulate lamellae gradually disappeared as a function of the incubation time and was absent from the surface of "mature" annulate lamellae (Fig. 3, *d* and *e*). Sections tangential to an annulate lamella revealed the characteristic pore complex architecture including the central element as well as the high density of pores per membrane area unit (Fig. 3 *f*).

Immunofluorescence microscopy using the pore-specific antibody PI1 confirmed the results obtained by EM. As illustrated in Fig. 4, annulate lamellae were undetectable in the extract at the beginning of incubation (*a'*) but became visible at 30 min in form of a few strongly fluorescent dot-like structures (*b'*) and were abundant at 60 min (*c'*). It is remarkable that most annulate lamellae stacks grew to sizes that allowed their identification by phase-contrast microscopy (Fig. 4 *c*). Antibodies to lamin L$_{III}$ used in parallel experiments gave negative results (not shown).

Both the Soluble and Vesicular Fraction Is Required for Annulate Lamellae Assembly

We have fractionated the egg extract into a soluble and vesicular fraction by centrifugation at 100,000 *g* for 1 h and examined the ability of both fractions to support annulate lamellae formation by electron and immunofluorescence microscopy. Neither the soluble nor the vesicular fraction alone were able to promote assembly of annulate lamellae. However, when

both fractions were recombined, annulate lamellae identical to those formed in the unfractionated extract were observed (data not shown). Nuclear envelope assembly also requires the presence of both fractions (Lohka and Masui, 1984; Newport, 1987; Sheehan et al., 1988).

Time Course of Pore Complex Formation in Annulate Lamellae and Nuclear Envelopes

We next asked whether pore complexes appeared with similar kinetics in annulate lamellae and nuclear envelopes. Egg extract was incubated with lambda DNA and examined at different incubation times by EM. At 30 min, chromatin masses were partly surrounded by nuclear envelope fragments of variable lengths that already contained pore complexes (Fig. 5 *a*). After a 90-min incubation the amount of nuclear envelopes had increased considerably and the nuclei were now surrounded by continuous nuclear envelopes (Fig. 5 *b*). Some annulate lamellae in the form of single or stacked membrane cisternae were also present, although in much lesser quantities as compared to DNA-free extract. We conclude that annulate lamellae assemble with the same kinetics as nuclear envelopes.

Assembly of Annulate Lamellae Is Inhibited by the Presence of Chromatin

Our electron microscopical analyses indicated that fewer annulate lamellae were formed in the presence of chromatin, i.e., under conditions promoting reconstitution of nuclear envelopes. To clarify the quantitative relationship between both pore complex-bearing structures, we have incubated egg extract with increasing concentrations of chromatin for 2 h, followed by immunofluorescence microscopy with the pore-specific antibody PI1. To distinguish pore complexes in the nuclear envelopes and in annulate lamellae, we have used demembranated sperm nuclei as a chromatin source. Although they undergo extensive structural rearrangements dur-
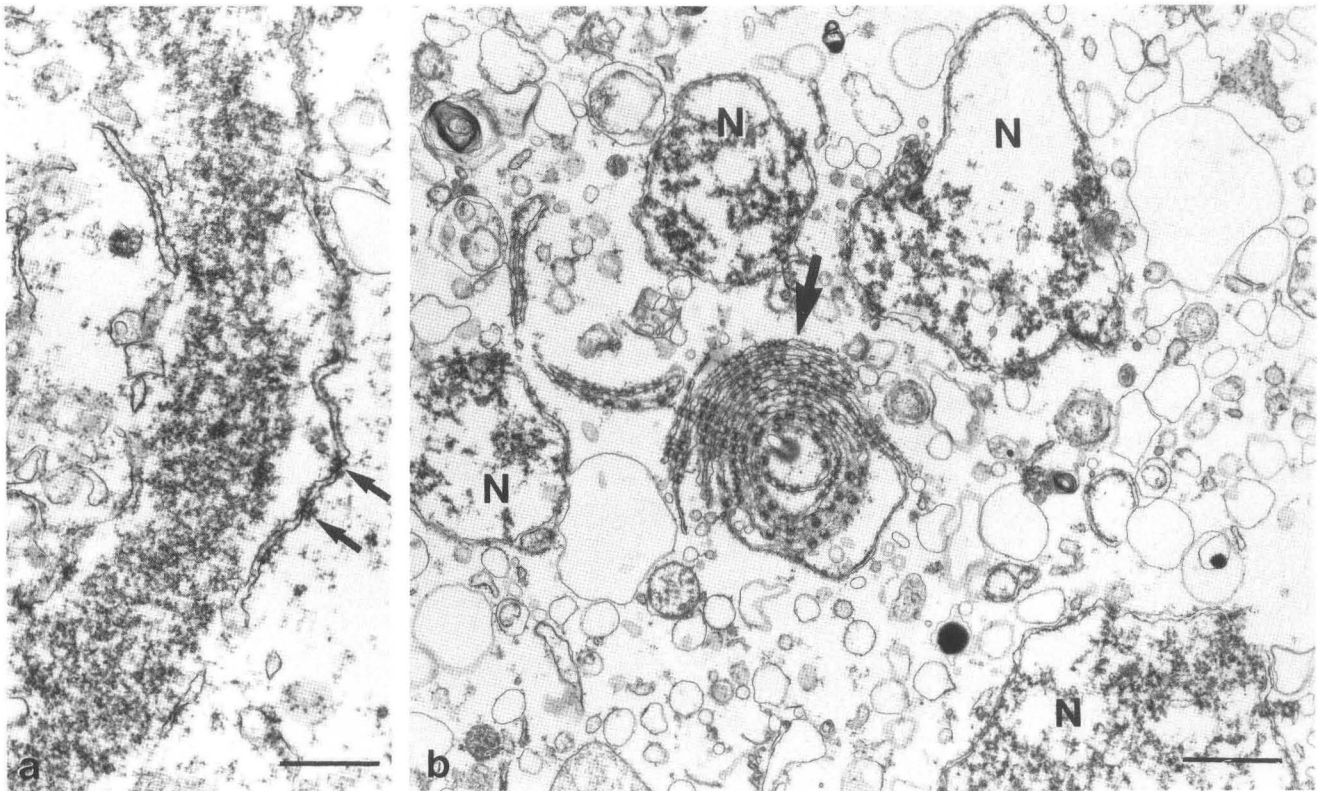
Figure 5. Electron micrographs of nuclei at different stages of reconstitution. Egg extract was incubated with lambda DNA and fixed after 30 min (*a*) and 90 min (*b*). Some pore complexes are clearly visible in the nuclear envelope fragments covering the chromatin after a 30-min incubation (*arrows* in *a*). At 90 min the nuclei (*N*) are enclosed by continuous envelopes with numerous pore complexes (*b*). An annulate lamellae stack is indicated by the arrow. Bars: (*a*) 0.5 μm; (*b*) 1 μm.

ing their transformation into interphase nuclei (Lohka and Masui, 1984; Lohka and Maller, 1987), they remain visible as distinct entities. Thus, sperm chromatin is readily identified by Hoechst staining and can be clearly distinguished from the much smaller annulate lamellae.

When we incubated 600 demembranated sperm nuclei per microliter extract, the sperm chromatin acquired a nuclear envelope with pore complexes as judged from the distinct peripheral labeling with antibody PI1, often in a finely punctate pattern (Fig. 6 *a*). In addition, a large number of annulate lamellae were visible by their specific reaction with the antibody (Fig. 6 *a*). In contrast, when we added 30,000 demembranated sperm nuclei per microliter extract, assembly of annulate lamellae was completely inhibited (Fig. 6 *b*).

Is Lamina Assembly Required for Annulate Lamellae Formation?

By immunodepletion of specific lamins from mitotic CHO cell extract, Burke and Gerace (1986) could directly demonstrate that lamina assembly around chromosomes is essential for nuclear envelope formation. We obtained essentially identical results when we added antibodies against lamin L_{III} to the *Xenopus* egg extract at concentrations that efficiently inhibited lamina assembly. Incubation of lambda DNA in this extract allowed formation of chromatin aggregates, but abolished their ability to act as nucleation sites for nuclear envelope assembly (Fig. 7 *a*). Most chromatin aggregates were devoid of any membranous structures or had

only a few nuclear membrane fragments, occasionally with pore complexes, attached to their surface (Fig. 7 *a*). In marked contrast, annulate lamellae formed normally in the presence of the antilamin antibodies (Fig. 7 *b*).

Discussion

From previous studies involving *Rana pipiens* egg extract it has been concluded that in vitro assembly of nuclear envelopes and the structurally related annulate lamellae required the interaction of chromatin, membrane vesicles, and soluble components (Lohka and Masui, 1984; Lohka and Maller, 1987; Lohka, 1988). Therefore, our results came quite as a surprise that nuclear envelope-like membrane cisternae containing numerous pore complexes are spontaneously assembled upon incubation of *Xenopus* egg extract in the absence of exogenous DNA or chromatin templates. Since the endogenous chromatin is removed during preparation of the egg extract by centrifugation (Newport, 1987), we conclude that pore complex–containing membranes are able to assemble in this cell-free system independently of any interaction with chromatin.

The annulate lamellae described in the present communication originated from a spontaneous self-assembly process during incubation of egg extract and did not preexist at time zero. In addition to EM we have applied immunofluorescence microscopy to detect pore complex–containing structures. To this end we have selected the mAb PI1 which has been shown to react with a nuclear pore complex glycopro-
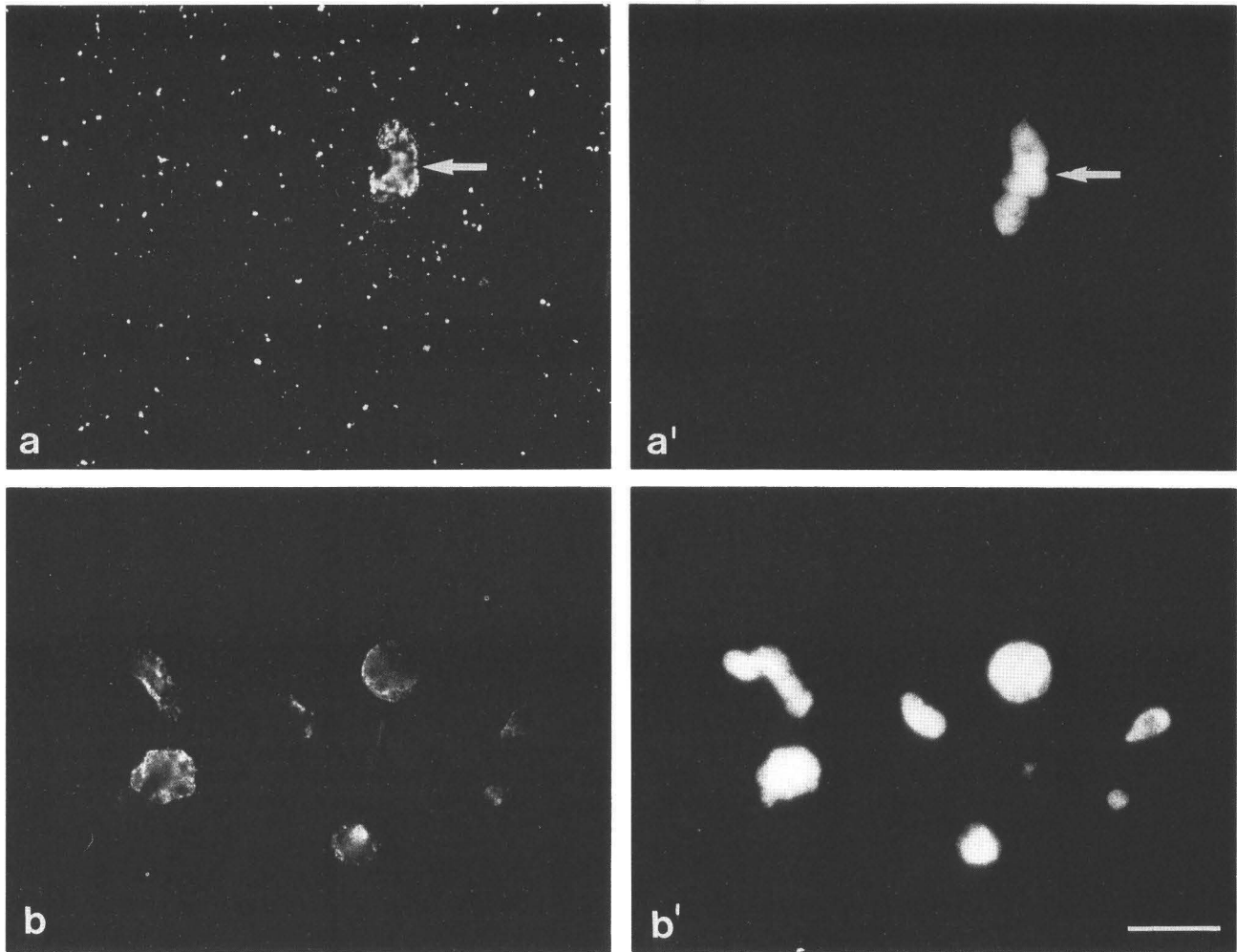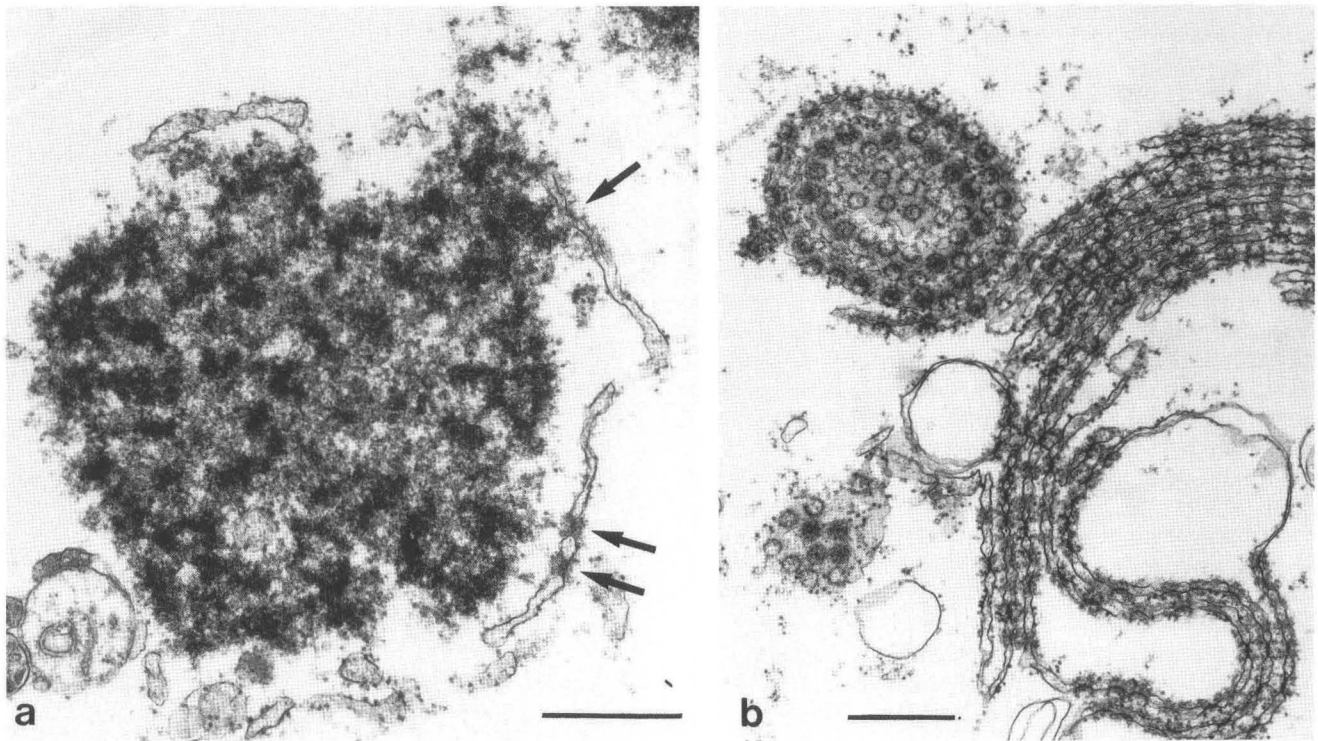
Figure 6. Inhibition of annulate formation by addition of chromatin in form of demembranated *Xenopus* sperm nuclei. Egg extract was incubated for 2 h in the presence of 600 (*a*) and 30,000 (*b*) demembranated sperm nuclei per microliter extract and processed for immunofluorescence microscopy using the pore-specific antibody PI1 (*a* and *b*). The corresponding Hoechst fluorescence is shown in *a'* and *b'*. Annulate lamellae form only in presence of low chromatin concentrations as indicated by the numerous fluorescent dots (*a*). The decondensed sperm chromatin has acquired a nuclear envelope with pore complexes as judged from the rim staining (*a, arrow* and *b*). Bar, 20 µm.

tein (p68, a member of the family of the O-linked *N*-acetylglucosamine containing nucleoporins; Dabauvalle et al., 1988). Here we demonstrate that this antibody is a useful marker for pore complexes in general since it labels, on frozen sections of *Xenopus* oocytes, not only the nuclear envelope but also the cytoplasmic annulate lamellae system (similar results were recently obtained with another monoclonal antibody directed against nucleoporins; Klymkowsky and Maynell, 1989; Allen, 1990). While annulate lamellae were abundantly present in the oocytes (see Fig. 1 *b'* and for cytological details Balinsky and Devis, 1963; Steinert et al., 1974), they were undetectable in intact eggs and freshly prepared egg extract, both by immunofluorescence and EM. The absence of annulate lamellae in *Xenopus* eggs is the result of their disintegration concomitantly with the breakdown of the nuclear envelope on completion of maturation of the oocytes (Balinsky and Devis, 1963; Steinert et al., 1974; Klymkowski and Maynell, 1989). Disappearance of annulate lamellae during oocyte maturation seems to be a general phenomenon since this process has also been ob-

served in other amphibian species (e.g., Kessel and Subtelny, 1981; Imoh et al., 1982).

Pore complex-containing membrane cisternae appeared first after a 30-min incubation of the extract as shown by electron and immunofluorescence microscopy. Their number and size increased with continued incubation until, at 90–120 min, large stacks of annulate lamellae composed of numerous membranous cisternae were abundantly present in the extract. Frequently, these stacks reached sizes that allowed their visualization by phase-contrast microscopy. It is notable that the annulate lamellae assembled with the same kinetics as nuclear envelopes which formed when the extract was supplemented with exogenous DNA. A dynamic relationship between both membrane systems has also been observed in cycling cells where annulate lamellae are disassembled and reassembled during mitosis in synchrony with the nuclear envelope (e.g., Gulyas, 1972; Stafstrom and Staehelin, 1984*a*).

Addition of chromatin in the form of demembranated sperm nuclei to the extract in order to allow reconstitution

Figure 7. Antilamin antibodies inhibit formation of nuclear envelopes but not of annulate lamellae. Egg extract was incubated with lambda DNA in the presence of antibody S49H2 for 2 h and then prepared for EM. Only a few nuclear envelope fragments, some with pore complexes (*arrows*), are associated with the chromatin surface (*a*). In contrast, stacks of annulate lamellae have formed normally (*b*). Bars, 0.5 μm.

of nuclear envelopes, reduced and, at sufficiently high chromatin concentrations, completely inhibited annulate lamellae formation. Thus, annulate lamellae are formed from a pool of precursor material that can be depleted by adding chromatin, probably through binding of nuclear membrane vesicles to the chromatin surface. Our evidence suggests that the extract has the capacity to assemble a certain amount of pore complex–containing membranes in the form of nuclear envelopes and/or annulate lamellae, while the relative proportion between both types of membranes is dependent upon the chromatin surface area available for interaction with the nascent membranes. According to this view, annulate lamellae and nuclear envelopes are equivalent structures that are indistinguishable from each other except the close apposition of the latter to chromatin. In support of this, both membrane systems appear to be derived from a morphologically distinct subpopulation of membrane vesicles containing a network of fine filaments. Similar vesicles with an electron-opaque content contribute also in *Rana* egg extract to nuclear envelope growth (Lohka and Masui, 1984). Our evidence suggests that these vesicles which might be related to the "nucleus-specific" vesicles described by Wilson and Newport (1988) have the ability to fuse spontaneously into flattened membrane cisternae and to acquire pore complexes independently of any interaction with chromatin. Whether they contain the pore-specific membrane-spanning glycoprotein gp210 (Wozniak et al., 1989; Greber et al., 1990) and how they interact with soluble, nonmembranous pore complex components are intriguing questions.

Are nuclear lamins involved in the assembly process of an-

nulate lamellae? *Xenopus* eggs contain only one lamina protein species (L_{III}) in a completely disassembled form and free of membranes (Benavente et al., 1985). It is well established that nuclei reconstituted in *Xenopus* egg extract acquire a distinct lamina layer which is readily identified by immunofluorescence microscopy (Newport, 1987; Dabauvalle et al., 1990). In contrast, we were unable to detect a lamina structure associated with the in vitro formed annulate lamellae using the same approach. The absence of a lamina seems to be a characteristic feature of annulate lamellae also in intact cells since they are stained neither in *Xenopus* oocytes (see Fig. 1 *c'* and Klymkowsky and Maynell, 1989) nor mouse L-cells (Chen and Merisko, 1988) by antilamin antibodies. To evaluate directly whether lamins are essential for annulate lamellae assembly, we have added antibodies against lamin L_{III} to the extract at concentrations that inhibited assembly of a lamina around chromatin (under these conditions the lamins formed numerous free paracrystalline arrays of filaments; unpublished observations). After incubation with lambda DNA assembly of nuclear envelopes around the reconstituted chromatin was prevented while annulate lamellae formed normally.

Considered together, our data indicate that a lamina is not required for fusion of nuclear membrane vesicles into membrane cisternae and assembly of pore complexes, i.e., formation of annulate lamellae. A lamina is, however, essential for targeting the nuclear membrane vesicles to chromatin. In an earlier study based on a mitotic cell-free system, Burke and Gerace (1986) have concluded that nuclear envelope reformation at telophase is dependent on the assembly of a lamina

structure around the chromosomes. Our present results confirm this notion and, beyond that, allow us to define more precisely which step of the nuclear envelope assembly pathway is obligatory coupled to the presence of a lamina structure.

Although annulate lamellae are of widespread occurrence, little is known about their function and mode of origin (see Kessel, 1989). Based on our results we think that in any cell an excess of nuclear envelope precursor material will lead to the formation of annulate lamellae (similar conclusions were drawn recently from a detailed analysis of early *Drosophila* embryos; Stafstrom and Staehelin, 1984b). What could be the function of overproduction of nuclear envelope precursor material? In case of the *Xenopus* oocytes it is obviously to produce a stockpile of nuclear membrane and pore complex material to be used for rapid assembly of nuclei during early embryonic development. Whether annulate lamellae contribute directly to the growth of nuclear envelopes in the sense that whole membrane cisternae with pore complexes become inserted into preexisting nuclear envelopes is not known. However, since it is quite likely that annulate lamellae generally disintegrate during each mitotic event in step with the nuclear envelope, as this has been observed during rabbit and *Drosophila* embryogenesis (Gulyas, 1972; Stafstrom and Staehelin, 1984a), their disassembled components may provide readily available material for assembly of nuclear envelopes. When considering somatic cells it is noticeable that annulate lamellae occur most commonly in rapidly dividing cells (reviewed by Kessel, 1989). Overproduction of nuclear envelope precursor material would be a simple and efficient strategy of such cells to ensure that their postmitotic chromosomes become rapidly and completely enclosed by a nuclear envelope containing functional pore complexes, a prerequisite to enter the G1-phase (Benavente et al., 1989a,b). Thus, we propose that the occurrence of annulate lamellae in interphasic cells is merely the consequence of a pool of nuclear envelope precursor material too large to become entirely exhausted by integration into the reforming nuclear envelopes.

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