Acta Biochimica Polonica

Vol. 42 No. 2/1995

133–144

QUARTERLY

The paper was presented at the "First International Seminar on Nuclear Matrix"

Evidence for the direct involvement of lamins in the assembly of a replication competent nucleus*

Hazel Jenkins[a], William G.F. Whitfield[a], Martin W. Goldberg[b], Terry D. Allen[b] and Christopher J. Hutchison[a]

[a]*Department of Biological Sciences, The University of Dundee, Dundee, DD1 4HN, Scotland;*
[b]*CRC Department of Structural Cell Biology, Paterson Institute for Cancer Research, Christie Hospital NHS Trust, Wilmslow Road, Manchester M20 9BX, U.K.*

Key words: lamins, DNA replication, FEISEM, *Xenopus* egg extract

Monoclonal antibodies linked to paramagnetic immunobeads (Dynabeads) have been used to investigate the distribution of lamin B_3 in fractions of *Xenopus* egg extracts. Lamin B_3 behaved as if it were completely soluble and did not co-precipitate with membrane fractions. Sperm pronuclei assembled in lamin depleted egg extracts were compared to pronuclei assembled in mock depleted extracts by field emission in-lens electron scanning microscopy (FEISEM). This technique revealed that the surface structures of the nuclear envelopes, including nuclear pores, appeared to be identical, indicating that lamin depletion does not affect nuclear envelope assembly. One-dimensional and two-dimensional gel electrophoresis was used to analyze soluble proteins co-precipitated with lamin B_3 on Dynabeads. Our results indicate that two major species (molecular mass: 105 kDa and 57 kDa) specifically co-precipitate with lamin B_3 as well as several minor species. At least three proteins which co-precipitate with lamin B_3 were identified as nuclear matrix proteins. Lamin B_3 was separated from these proteins and re-inoculated into lamin depleted extracts. This resulted in partial rescue of both lamina assembly and DNA replication. These results imply that lamin B_3 is directly involved in the assembly of structures required for the initiation of DNA replication.

The nuclear lamina is the major structural component, which remains associated with nuclear envelopes following extraction with non-ionic detergents and salt [1–3]. It is composed of intermediate filament proteins of diameter 10 nm that, at least in amphibian oocytes, form a regular basket-weave pattern over the entire surface of interphase nuclei [4–6]. Two classes of lamins have been identified in most embryonic cells, termed A-type and B-type. These differ both in their primary sequence [7, 8] and in their behaviour at mitosis. Of the two lamin species, B-type lamins are expressed constitutively in most embryonic [9–12] and somatic cells [9, 13], while expression of A-type lamins is highly regulated during development, suggesting that lamin status may be important for cell differentiation [10, 14, 15]. Recently, interest

*This work was supported by grants from the Cancer Research Campaign.

[1]Abbreviations: BSA, bovine serum albumin; FEISEM, field emission in-lens scanning electron microscopy; LSS, cell-free extract of *Xenopus* eggs; MP1, membrane pellet fraction 1; MP2, residual membrane pellet fraction; PBS, phosphate buffered saline; USS, cytosol fraction of *Xenopus* eggs extract.

in the lamins has increased due to their identification as a major cellular substrate of the universal cell cycle control protein kinase complex $p34^{cdc2}$ [16–18]. As a result of reversible phosphorylation by $p34^{cdc2}$-kinase, in higher vertebrates, the lamina disassembles at mitosis and reassembles at telophase [16, 18–21]. Following lamina disassembly, A-type lamins are dispersed throughout the cytoplasm probably as dimers, tetramers and oligomers, whereas B-type lamins remain associated with small vesicles that are presumed to be the remnants of the nuclear envelope [22, 23].

Because of their position in interphase nuclei, their behaviour at mitosis and the changes in lamin composition during embryogenesis in some species, lamins have been ascribed putative roles in nuclear envelope reassembly at telophase [24–27], chromatin organization [28, 29] and in maintaining the structural integrity of the nuclear envelope [30]. However, with the exception of their role in nuclear envelope reassembly there is little experimental evidence to confirm or reject these hypotheses.

In cleavage embryos of the amphibian *Xenopus laevis*, only a single lamin species, termed lamin L_{III}, has been identified [15]. The primary amino-acid sequence of this lamin identifies it as a B-type lamin [31] but it differs from B-type lamins in one important respect: it is freely soluble in both meiotic and mitotic cytoplasms of the egg and early embryo and is not associated with membrane vesicles [32, 33]. Because of its unusual characteristics lamin L_{III} has been renamed lamin B_3 [7]. Cell-free extracts of *Xenopus* eggs, prepared by centrifugation, support nuclear assembly and DNA replication *in vitro* [34–36]. Upon addition of a suitable DNA template such as demembranated sperm heads, pronuclei are assembled which then act as independent units of DNA replication [37]. The initiation of DNA replication in *in vitro* assembled nuclei is dependent upon the assembly of a nuclear envelope and nuclear transport [38, 39, 40]. Replication forks are assembled in discrete foci within these nuclei, each focus containing up to 300 replication origins [41, 42]. Thus it is implied that a high degree of structural organization is required in order to initiate DNA replication. Direct evidence for the involvement of nuclear structures in DNA replication is derived from immunodepletion experiments in which monoclonal or polyclonal anti-lamin antibodies have been used to either physically or functionally deplete extracts of lamin B_3 [32, 43, 44]. Lamin depleted extracts are capable of nuclear assembly [32] and these nuclei, even though lacking a detectable lamina, are capable of nuclear transport [43]. Nevertheless, "lamina deficient nuclei" do not initiate DNA replication and do not accumulate replication proteins such as proliferating cell nuclear antigen (PCNA) at sites which resemble replication foci [43, 44]. Thus it appears that filamentous structures are essential for the formation of replication origins.

Despite this evidence we were concerned that the failure of lamina deficient nuclei to replicate DNA was an indirect consequence of lamin depletion. In particular, a recent report has indicated that novel lamin species are present in *Xenopus* egg extracts which behave as integral membrane proteins [45]. Therefore removal of these lamins from the extracts may deplete specific membrane fractions and may result in co-precipitation of other proteins which are essential for DNA replication. To exclude this possibility we have used one-dimensional and two-dimensional gel electrophoresis to investigate the protein complement of immunoprecipitates from soluble and insoluble fractions of egg extracts. We report that, when paramagnetic beads are used for immunodepletion, lamin B_3 does not co-precipitate with membrane vesicles. However, a characteristic fraction of soluble proteins does co-precipitate with lamin B_3 and at least three of these proteins are nuclear matrix proteins. Separation of lamin B_3 from these proteins has been achieved and this purified lamin fraction is capable of rescuing lamina assembly and DNA replication in lamin depleted egg extracts.

MATERIALS AND METHODS

Preparation of cell-free extracts. Cell-free extracts of *Xenopus* eggs (LSS) were prepared as described by Blow & Laskey [36]. The extracts were supplemented with glycerol (5%, v/v) and flash frozen in liquid nitrogen. Fractionation of egg extracts was performed as follows: 4 ml of LSS was subjected to ultracentrifugation at 200000 × *g* for 90 min at 4°C in a Beckman TLS55 rotor. This separated the extract into three layers. A gel containing ribosomes at the

bottom of the tube was discarded. A membrane pellet was collected just above this layer, suspended in 4 ml MEB (500 mM KCl, 20 mM β-glycerophosphate, 2 mM $MgCl_2$, 1 mM EGTA, 10 mM Hepes, pH 7.5, 0.5 M sucrose, 1 mM ATP, 5 μg/ml cytochalasin B, 10 μM GTPγS, 10 μg/ml aprotinin). The suspension was then subjected to centrifugation at 200000 × *g* for a further 60 min and a washed membrane pellet was collected and suspended in 400 μl of MEB. This fraction was termed MP1. The supernatant layer was collected above the membrane pellet. This layer was either subjected to centrifugation for a further 60 min at 200000 × *g* to produce USS or was diluted by the addition of 4 volumes of MEB and then subjected to centrifugation for 90 min at 200000 × *g*. A second membrane pellet was collected at the end of this step and suspended in 400 μl of MEB. This fraction was referred to as MP2.

Immunoprecipitation. Immunoprecipitation was carried out essentially as described by Jenkins *et al.* [44]. The anti-lamin monoclonal antibody L6 5D5 was linked to paramagnetic immunobeads (Dynabeads) by overnight incubation. Dynabeads (50 μl) were then mixed with 75 μl of LSS, USS, MP1 or MP2 for 40 min at room temperature. The Dynabeads were then recovered using a magnetic particle collector and suspended in phosphate buffered saline (PBS, pH 7.4) containing 1 mg/ml BSA. The Dynabeads were again recovered and then resuspended in PBS/BSA. This procedure was repeated three times but at the final step BSA was omitted. After final recovery, the Dynabead was suspended in 6 M urea (50 mM Tris/HCl, pH 8.0, 5 mM dithiothreitol]. To obtain partially purified lamin B_3, Dynabead pellets recovered from USS were washed with high salt PBS (containing 0.5 M KCl) prior to suspension in 6 M urea.

Gel electrophoresis. One-dimensional and two-dimensional gel electrophoresis and immunoblotting were performed as described by Jenkins *et al.* [44].

Field emission in-lens electron scanning microscopy (FEISEM). Samples were prepared for FEISEM essentially as described by Goldberg & Allen [46]. Pronuclei were recovered from extracts by centrifugation through 30% sucrose cushions at 3000 × *g* and collected on etched silicon chips. The nuclei were fixed in 2% glutaraldehyde, 0.2% tannic acid in 0.1 M Hepes (pH 7.4) and then in 0.1% OsO_4 and 1% uranyl acetate for 10 min each. The samples were then dehydrated repeatedly with ethanol and finally dried from CO_2 *via* Arklone (ICI). The dried samples were then sputter coated with tantalum and viewed with a Topcon DS 130F field emission electron microscope at 30 kV accelerating voltage.

Fluorescence microscopy. Indirect immunofluorescence microscopy was performed according to Blow & Laskey [36].

Rescue experiments. Lamin depleted extract was prepared according to the method of Jenkins *et al.* [44]. The depleted extract (200 μl) was then supplemented with 10 μl of partially purified lamin B_3 (4 μg protein) and 10 μl of glycerol. The mixture was then flash frozen in liquid nitrogen for future use.

DNA replication assays. DNA replication was assayed by [^{32}P]dCTP incorporation as described by Blow & Laskey [36].

RESULTS

Removal of lamin B3 from egg extracts does not deplete membrane fractions and does not affect nuclear envelope assembly

To confirm and extend our earlier findings [44] we wished to re-investigate the association of lamin B_3 with nuclear envelope precursor fractions. Egg extracts were fractionated into cytosol (USS), washed membrane pellets (MP1) and residual membrane pellets (MP2). Fractions equivalent to 75 μl of LSS were incubated with either L6 5D5 or the anti-nuclear envelope antibody 1G4, linked to 50 μl of Dynabeads. After recovery, the Dynabeads were washed and then boiled in SDS sample buffer. The samples were resolved on 8% SDS/PAGE, transferred to nitrocellulose and blotted with L6 8A7 antibodies. More than 75% of total cytoplasmic lamin B_3 was precipitated after incubation of L6 5D5 Dynabeads in LSS (Fig. 1, lane 1). A similar quantity of lamin B_3 was precipitated after incubation of L6 5D5 Dynabeads in USS (Fig. 1, lane 4). In contrast, no lamin B_3 was recovered after incubation of L6 5D5 Dynabeads with either MP1 (Fig. 1, lane 2) or MP2 (Fig. 1, lane 3). As expected, lamin B_3 did not co-precipitate with 1G4 Dynabeads after incubation with LSS (lane 5), USS (lane 8), MP1 (lane 6) or MP2 (lane 7). From these data we conclude that lamin B_3

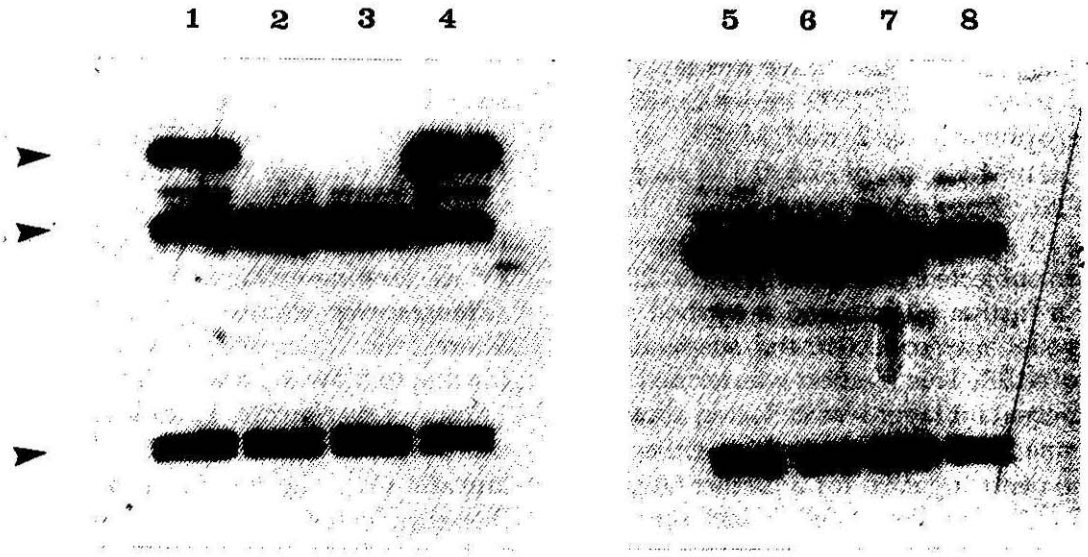

Fig. 1. Western blot analysis of immunoprecipitates from fractions of Xenopus egg extracts.
Xenopus egg extracts were fractionated into LSS (lanes 1 and 5), USS (lanes 4 and 8), MP1 (lanes 2 and 6) and MP2 fractions (lanes 3 and 7). Each fraction, 75 μl, was mixed with 50 μl of either L6 5D5 Dynabead (lanes 1–4) or 50 μl of 1G4 Dynabead (lanes 5–8; a monoclonal antibody reagent which detects an 84 kDa protein associated with nuclear envelope precursor fractions). After recovery of the Dynabead and washing the samples were resolved on 8% SDS/PAGE, transferred to nitrocellulose and blotted with L6 8A7 anti-lamin antibodies. Development was with ECL. The band migrating at 55 kDa and the doublet migrating at 22–23 kDa is IgG heavy and light chains.

is not recovered from insoluble fractions of egg cytoplasm and does not co-precipitate with nuclear envelope precursor fractions when these are removed from LSS. Thus it seems unlikely that immunodepletion, with L6 5D5 Dynabeads, removes essential membrane precursor fractions from *Xenopus* egg extracts.

To confirm that lamin B_3 depletion of LSS with L6 5D5 Dynabeads does not affect nuclear envelope assembly we went on to perform FIE-SEM on sperm pronuclei assembled in these extracts. This technique has previously been used to describe the fine structure of the oocyte germinal vesicle envelopes [45]. Here, surface views of pronuclei recovered from depleted and mock depleted LSS were obtained. Whole pronuclei are compared in Fig. 2 (panel C, D are control pronucleus and panel A, B are pronuclei recovered from a lamin depleted extract). Control pronuclei were more rounded and larger than pronuclei recovered from lamin depleted extracts. The size and morphology of pronuclei recovered from lamin depleted extracts is typical of pronuclei at a pre-initiation stage in undepleted extracts (Hutchison *et al.* [47]). Since larger pronuclei were never recovered from lamin depleted extracts, we infer that as in previous studies lamin depletion prevents nuclear envelope growth. The morphology and density of nuclear pores in each pronucleus is very similar (Fig. 2, panels C and D). Indeed, high power views reveal a similar sub-unit arrangement of outer pore granules in each nucleus and in each instance spokes can be seen within some of the pores (Fig. 3). These results support our previous findings that nuclear transport is not impaired in pronuclei assembled in lamin depleted extracts [43]. Furthermore, the data imply a high degree of structural integrity in the nuclear envelope of pronuclei assembled in lamin depleted extracts.

Several nuclear matrix proteins co-precipitate with lamin B3 on L6 5D5 Dynabeads

Since pronuclei assembled in lamin depleted extracts appear to assemble a complete nuclear envelope, we went on to investigate proteins which specifically co-precipitated with lamin B_3. LSS, 75 μl (Fig. 4, lanes 1 and 3) or USS, 75 μl (Fig. 4, lanes 2 and 4) were incubated with 50 μl of L6 5D5 Dynabead (Fig. 4, lanes 3 and 4) or 50 μl of Dynabead conjugated to an irrelevant mouse IgG (Fig. 4, lanes 1 and 2). Each Dynabead pellet was recovered, washed extensively and then boiled in SDS sample buffer. The samples were resolved on 8% SDS/PAGE and stained with Coomassie brilliant blue. Three major bands appeared in each of lanes 3 and 4 which were absent from lanes 1 and 2 (large arrow heads). The slowest migrating band had

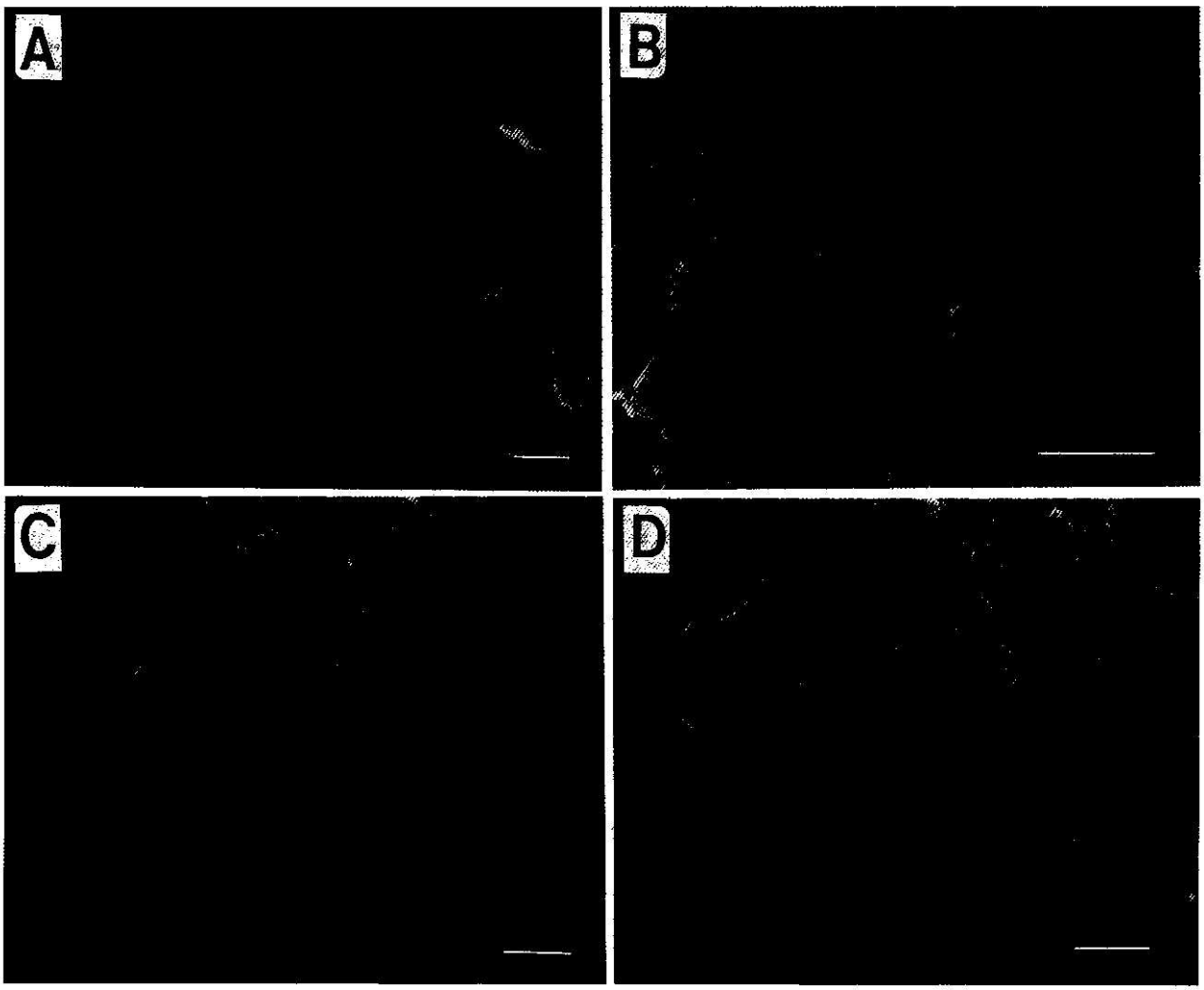

Fig. 2. Field emission in-lens scanning microscopy of pronuclei recovered from lamin depleted (A and B) and control extracts (C and D).

Pronuclei were isolated from extracts by centrifugation through sucrose cushions and fixed onto silicon chips. The nuclei were then viewed using FEISEM. Scale bars are 1 μm. A and C show whole nuclei. B and D show close up views of the same nuclei to illustrate nuclear pore density.

a molecular mass of 105 kDa. The middle band had a molecular mass of 82 kDa and was identified by immunoblotting as lamin B_3 (not shown). The fastest migrating band had a molecular mass of 57 kDa. Other minor bands in the molecular mass range 80–106 kDa were also evident in lanes 3 and 4 but were again absent from lanes 1 and 2. The protein profiles of immunoprecipitates from LSS was reproducibly identical to profiles obtained from USS, again indicating that additional (membrane bound) material did not co-precipitate with L6 5D5 Dynabeads after incubation in LSS.

Since several protein species specifically co-precipitated with lamin B_3 we wished to compare these proteins to nuclear matrix proteins. Nuclear matrix fractions were prepared as described previously [44]. L6 5D5 Dynabeads (50 μl) were incubated with 75 μl of USS, recovered, washed extensively and then suspended in 6 M urea. Each sample was resolved on isoelectric focusing (IEF) tube gels followed by 8% SDS/PAGE. The gels were then either silver stained (Fig. 5, panels A and B) or samples were transferred to nitrocellulose and immunoblotted with L6 8A7 antibody (panel C). Lamin B_3 recovered from Dynabeads migrated as three spots each with a molecular mass of 82 kDa (panels B and C, small arrow head). One species migrated at a neutral pH while the other two species migrated at a slightly basic pH. Identical species were recovered in nuclear matrix fractions (panel A, small arrow head). The large spot migrating with a molecular mass of 45 kDa and at a neutral pH in both panels A and B is actin. The doublet migrating at 84 kDa

Fig. 3. High power view of the structure of nuclear pores in a lamina deficient nucleus.
Note that in some pores spokes are clearly visible. Scale bar = 50 nm.

and at a slightly acidic pH and the spot migrating at 105 kDa and at a neutral pH (both indicated with large arrow heads in panels A and B) are the only species which co-precipitate with lamin B_3 on Dynabeads and which can with certainty be identified as nuclear matrix proteins.

Immunoisolated lamin B_3 rescues lamina assembly and DNA replication in lamin-depleted LSS

Since several protein species co-precipitated with lamin B_3 on Dynabeads we wished to investigate the influence of these proteins on nuclear assembly and DNA replication. To do this we partially purified immunoisolated lamin B_3 and then tested the capacity of this fraction to rescue DNA replication in lamin depleted LSS. L6 5D5 antibody was cross-linked to Dynabeads with dimethyl pimelimidate. L6 5D5 Dynabead, 1 ml, was then used to remove lamin B_3 from 1.5 ml of USS. After recovery the Dynabead was washed with 1ml of PBS followed by 1 ml of high salt PBS. The lamin was then eluted from the Dynabead with 100 μl of 6 M urea. 80 μg of the material was recovered and this was analyzed by two-dimensional gel electrophoresis and silver staining. The fraction was found to contain two major protein species, lamin B_3 and actin (Fig. 5, panel D). Next the fraction was dialysed in 500 mM KCl buffer and then used to supplement lamin depleted LSS. Pronuclear assembly in mock-depleted LSS, lamin depleted LSS and lamin depleted LSS which had been supplemented with partially purified lamin B_3 was investigated by indirect immunofluorescence. Pronuclei assembled in mock-depleted LSS were typically 15–20 μm in diameter, had chromatin arranged as distinct fibrils and possessed

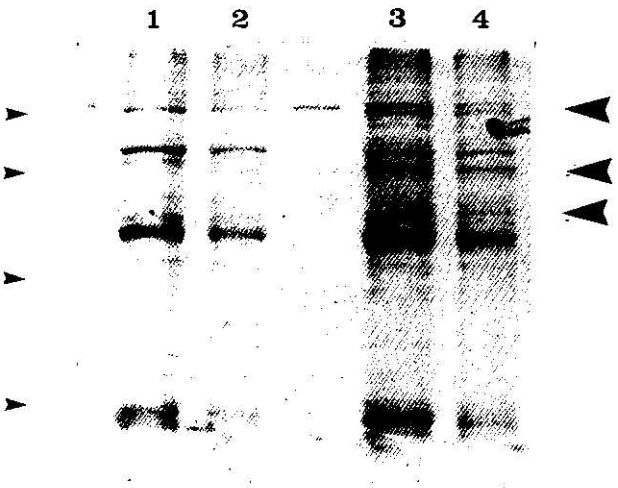

Fig. 4. SDS/PAGE analysis of proteins which co-precipitate with lamin B_3 on Dynabeads.
LSS, 75 µl, (lanes 1 and 3) or USS (lanes 2 and 4) were incubated with 50 µl of either L6 5D5 Dynabeads (lanes 3 and 4) or an irrelevant mouse IgG linked to Dynabeads (lanes 1 and 2). Dynabeads were recovered, washed and solubilised in SDS sample buffer. Samples were resolved on 8% SDS/PAGE and stained with Coomassie brilliant blue. Molecular mass markers (small arrow heads) are 106 kDa, 80 kDa, 49.5 kDa and 32.5 kDa.

an extensive lamina (Fig. 6A and B). In contrast, pronuclei assembled in lamin depleted extracts were only 7–10 µm in diameter, had a uniform distribution of chromatin and did not possess a lamina (Fig. 6C and D). However, when supplemented with partially purified lamin B_3, large pronuclei (diameter 15–20 µm) with fibrous chromatin and an extensive lamina (Fig. 6E and F) were assembled in lamin depleted extracts.

To test whether DNA replication had occurred in the extracts, the extracts were supplemented with [^{32}P]dCTP. Pronuclei assembled in the extracts were labelled for a 3 h period, recovered and solubilized in SDS. After digestion with proteinase K, the samples were resolved on a 0.8% agarose gel. The gels were dried under vacuum and autoradiographed. Quantification was achieved by densitometric analysis of the autoradiographs and the results expressed as a

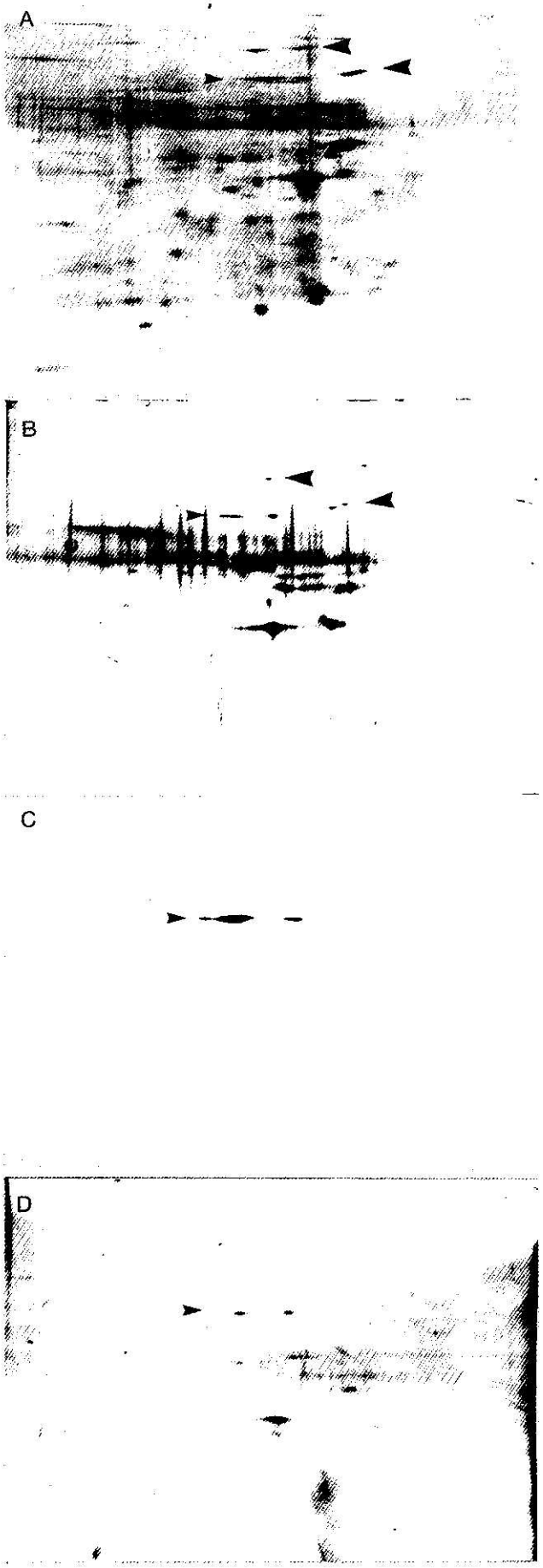

Fig. 5. Two-dimensional gel electrophoresis of the eluates from Dynabeads.
USS, 75 µl, was lamin depleted with 50 µl of L6 5D5 Dynabead. Fractions were either washed and suspended in sample buffer (B and C) or washed sequentially with PBS, followed by high salt PBS before suspension in sample buffer (D). They were either silver stained (A, B and D) or Western blotted and probed with L6 8A7 antibodies (C). Panel A shows a nuclear matrix fraction prepared from 2×10^5 sperm pronuclei.

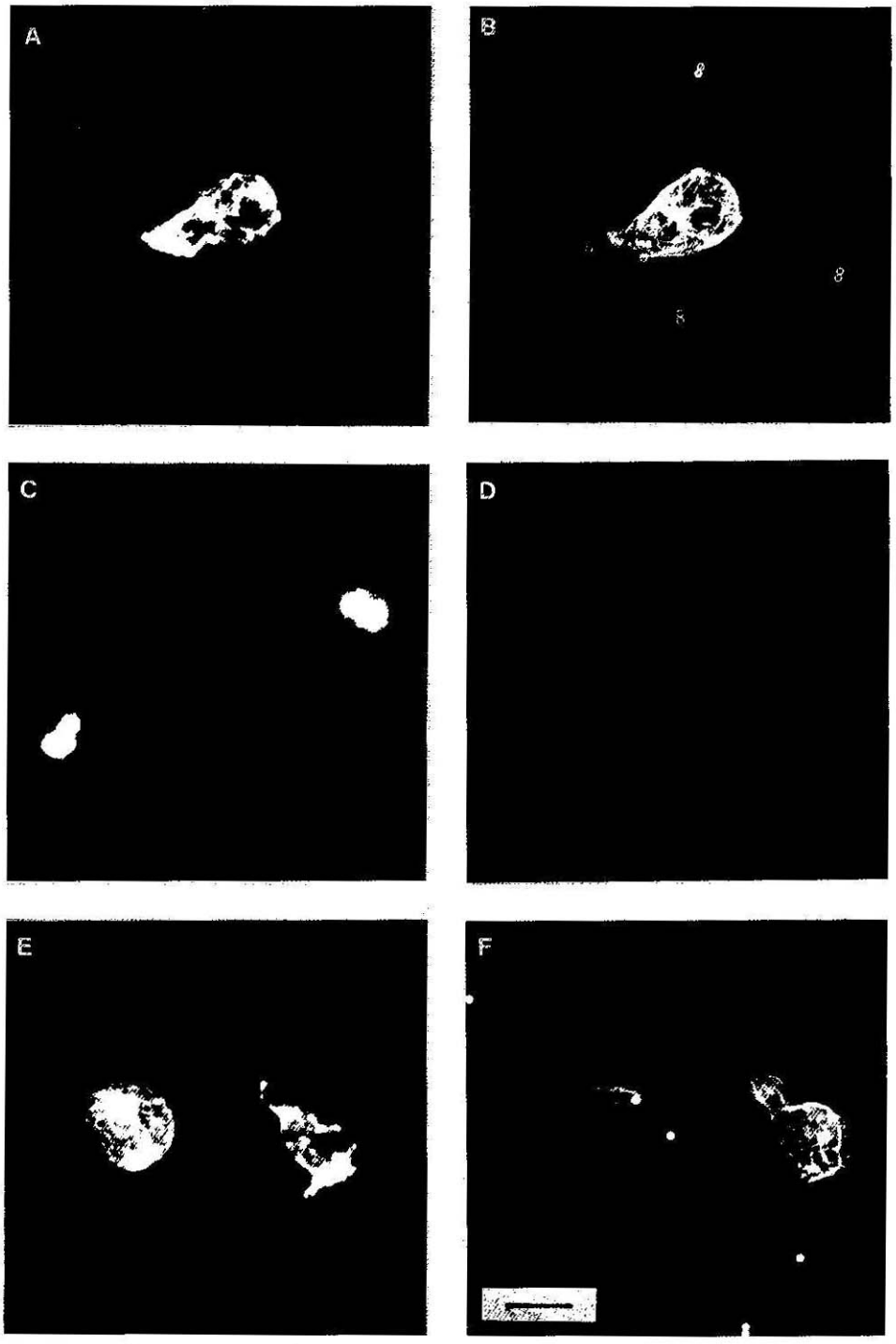

Fig. 6. Indirect immunofluorescence microscopy of lamina assembly.
Sperm pronuclei were assembled in mock-depleted extracts (A and B) lamin depleted extracts (C and D) or lamin depleted extracts supplemented with partially purified lamin B_3 (E and F). Pronuclei were fixed with ethylene glycol-bis-succinic acid (EGS) and recovered onto glass coverslips. The nuclei were co-stained with DAPI (to reveal the distribution of chromatin; A, C and E) and L6 5D5 antibody followed by FITC goat anti-mouse Ig (see [47]) (to reveal the distribution of lamins; B, D and F). Scale bar = 10 μm.

percentage of the controls (undepleted extracts). Incorporation of [^{32}P]dCTP into DNA in mock depleted extracts was only 80% of control levels. However, in lamin depleted extracts incorporation of [^{32}P]dCTP was < 5% of control levels. In contrast, after supplementing with partially purified lamin B_3, the capacity of lamin depleted extracts to replicate DNA increased to 56% of control levels (Fig. 7).

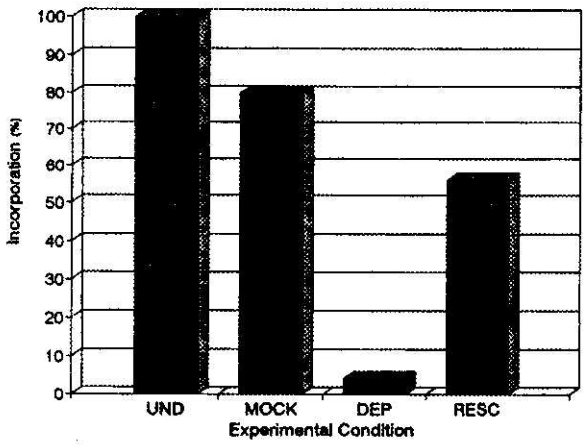

Fig. 7. DNA replication in lamin depleted extracts. Undepleted extract (UND), mock-depleted extracts (MOCK), lamin depleted extracts (DEP) or lamin depleted extracts supplemented with partially purified lamin B_3 (RESC) were inoculated with demembranated sperm heads and incubated with [^{32}P]dCTP. After 3 h samples were prepared for agarose gel electrophoresis. The gels were then washed and dried before autoradiography. Densitometric analysis of autoradiographs was performed with a BioImager. The level of DNA replication in each depleted extract was expressed as a percentage of the undepleted extract.

DISCUSSION

The data presented above indicate that in *Xenopus* egg extracts, lamin B_3 behaves as a cytosolic protein which does not associate with nuclear envelope precursors. This data is consistent with some reports [15, 33] but contradicts one other report of Lourim & Krohne [45]. Since we have used identical monoclonal antibody reagents to the ones used by Stick and co-workers [15, 33] it is hardly surprising that our result concur. However, the differences between our results and those reported by Lourim and Krohne [45] cannot be explained by different antibody reagents since L6 5D5 will detect and precipitate membrane associated lamin B_3 [33]. Furthermore, immunodepletion of extracts with L6 5D5 removes all detectable lamin [44]. Despite this, FEISEM indicates that the morphology of the nuclear envelope and nuclear pores is identical in control and lamina deficient sperm pronuclei. Therefore, it seems likely that lamin depletion does not affect nuclear envelope assembly in egg extracts.

Consistent with this view, the range of proteins which co-precipitate with lamins on Dynabeads is identical in LSS and USS fractions. Furthermore, at least three of these proteins can be identified as nuclear matrix proteins. Previous work has revealed that all three proteins are accumulated in lamina deficient nuclei and are insoluble [43]. In addition, actin is also present in nuclear matrices prepared from lamina deficient nuclei. The consistent association of the majority of nuclear matrix proteins with lamina deficient nuclei indicates that lamin depletion is responsible for the failure of these nuclei to replicate DNA. This view is strongly supported by the observation that a partially purified fraction containing lamin B_3 and actin rescues lamina assembly and DNA replication in lamin depleted extracts.

Why do lamina deficient nuclei fail to replicate DNA? Previous studies have indicated that the majority of replication foci in *in vitro* assembled nuclei are not associated with the envelope [41]. However, more recent work suggests that groups of replication foci are organized around individual chromosomes [42]. Since each chromosome is linked to the nuclear envelope through the lamina, there is an indirect link between replication foci and lamins.

The association of lamin B_3 with three nuclear matrix proteins in immunoprecipitates suggests an alternative explanation for the inability of lamina deficient nuclei to replicate DNA. Perhaps these protein-protein interactions reflect an association between two different filament forming proteins. Resinless section E. M. has indicated that in nucleoskeleton preparations from HeLa cells, nucleoplasmic filaments abut the lamina [48]. If the lamina is required for correct assembly of nucleoplasmic filaments, then in the absence of a lamina the nucleoplasmic filaments may be disorganized or absent. Nucleoplasmic filaments provide direct support for replication factories [48]. Thus failure to assemble nucleoplasmic filaments would probably prevent the formation of replication factories.

The authors are grateful to Dr. Riemer Stick for the supply of monoclonal antibodies L6 5D5 and L6 8A7.

REFERENCES

1. Aaronson, R.P. & Blobel, G. (1974) On the attachment of the nuclear pore complex. *J. Cell Biol.* **62**, 746–754.
2. Dwyer, N. & Blobel, G. (1976) A modified procedure for the isolation of a pore complex-lamina fraction from rat liver nuclei. *J. Cell Biol.* **70**, 581–591.
3. Fawcett, D.W. (1966) On the occurrence of fibrous lamina on the inner aspect of the nuclear envelope in certain cells of vertebrates. *Am. J. Anat.* **119**, 129–146.
4. Aebi, U., Cohn, J., Buhle, L. & Gerace, L. (1986) The nuclear lamina is a meshwork of intermediate-type filament. *Nature (London)* **323**, 560–564.
5. Fisher, D.Z., Chaudhary, N. & Blobel, G. (1986) cDNA sequencing of nuclear lamins A and C reveals primary and secondary structural homology to intermediate filament. *Proc. Natl. Acad. Sci. U.S.A.* **83**, 6450–6454.
6. McKeon, F.D., Kirschner, M.W. & Caput, D. (1986) Homologies in both primary and secondary structure between nuclear envelope and intermediate filament proteins. *Nature (London)* **319**, 463–468.
7. Doring, V. & Stick, R. (1990) Gene structure for nuclear lamin L_{iii} of *Xenopus laevis*. A model for the evolution of IF proteins from a lamin-like ancestor. *EMBO J.* **9**, 4073–4080.
8. Weber, K., Plessmann, U., Dodemont, H. & Kossmagk-Stephan, K. (1988) Amino acid sequences and homopolymer-forming ability of the intermediate filament proteins from an invertebrate epithelium. *EMBO J.* **7**, 2995–3001.
9. Lehner, C.F., Kurer, V., Eppenberger, H.M. & Nigg, E.A. (1987) Differential expression of nuclear lamin proteins during chicken development. *J. Cell Biol.* **105**, 577–587.
10. Rober, R.-A., Weber, K. & Osborn, M. (1989) Differential timing of nuclear lamin A/C expression in the various organs of the mouse embryo and the young animal. A developmental study. *Development* **105**, 385–378.
11. Stewart, C. & Burke, B. (1987) Teratocarcinoma stem cells and early mouse embryos contain only a single major lamin polypeptide closely resembling lamin B. *Cell* **51**, 383–392.
12. Wolin, S.L., Krohne, G. & Kirschner, M.W. (1987) A new lamin in *Xenopus* somatic tissues displays strong homology to human lamin A. *EMBO J.* **6**, 3809–3818.
13. Vorburger, K., Lehner, C.F., Kitten, G., Eppenberger, H.M. & Nigg, E.A. (1989) A second higher vertebrate B-type lamin: cDNA sequence determination and *in vitro* processing of chicken lamin B2. *J. Mol. Biol.* **208**, 405–415.
14. Benevente, R., Krohne, G. & Franke, W.W. (1985) Cell type-specific expression of nuclear lamina proteins during development of Xenopus laevis. *Cell* **41**, 177–190.
15. Stick, R. & Hausen, P. (1985) Changes in the nuclear lamina composition during the early development of Xenopus laevis. *Cell* **41**, 191–200.
16. Peter, M., Nakagawa, J., Doree, M., Labbe, J.C. & Nigg, E.A. (1990) *In vitro* disassembly of the nuclear lamina and M-phase-specific phosphorylation of lamins by cdc2 kinase. *Cell* **61**, 591–602.
17. Luscher, B., Brizuela, L., Beach, D. & Eiseman, R.N. (1991) A role for the $p34^{cdc2}$ kinase and phosphatases in the regulation of phosphorylation and disassembly of lamin B_2 during the cell cycle. *EMBO J.* **10**, 865–875.
18. Dessev, G., Iorchera-Dessev, C., Bischoff, J.R., Beach, D. & Goldman, R. (1991) A complex containing $p34^{cdc2}$ and cyclin B phosphorylates the nuclear lamina and disassembles nuclei of clam oocytes *in vitro*. *J. Cell Biol.* **112**, 523–533.
19. Gerace, L. & Blobel, G. (1980) The nuclear envelope lamina is reversibly depolymerised during mitosis. *Cell* **19**, 277–287.
20. Ottaviano, Y. & Gerace, L. (1985) Phosphorylation of the nuclear lamins during interphase and mitosis. *J. Cell Biol.* **101**, 518–523.
21. Miake-Lye, R. & Kirschner, M.W. (1985) Induction of early mitotic events in a cell-free system. *Cell* **41**, 165–175.
22. Stick, R., Angres, B., Lehner, C.F. & Nigg, E.A. (1988) The fates of chicken nuclear lamin proteins during mitosis. Evidence for a reversible redistribution of lamin B_2 between inner nuclear membrane and elements of the endoplasmic reticulum. *J. Cell Biol.* **107**, 397–406.
23. Meier, J. & Georgatos, S. (1994) Type B lamins remain associated with the integral nuclear envelope protein p58 during mitosis; implications for nuclear assembly. *EMBO J.* **13**, 1888–1897.
24. Benevent, R. & Krohne, G. (1986) Involvement of nuclear lamina in post-mitotic reorganisation of chromatin as demonstrated by microinjection of lamin antibodies. *J. Cell Biol.* **103**, 1847–1854.
25. Burke, B. & Gerace, L. (1986) A cell-free system to study reassembly of the nuclear envelope at the end of mitosis. *Cell* **44**, 639–652.

26. Glass, J.R. & Gerace, L. (1990) Lamins A and C bind and assemble at the surface of mitotic chromosomes. *J. Cell Biol.* **111**, 1047–1057.
27. Burke, B. (1990) On the cell-free association of lamins A and C with metaphase chromosomes. *Exp. Cell Res.* **186**, 169–176.
28. Nigg, E.A. (1989) The nuclear envelope. *Curr. Opin. Cell Biol.* **1**, 435–440.
29. Paddy, M.R., Belmont, A.S., Saunweber, H., Agard, D.A. & Sedat, J.W. (1990) Interphase nuclear lamins form a discontinuous network that interacts with only a fraction of the chromatin in the nuclear periphery. *Cell* **62**, 89–106.
30. Whytock, S. & Stewart, M. (1988) Preparation of shadowed nuclear envelopes from *Xenopus* oocyte germinal vesicles for electron microscopy. *J. Microsc.* **151**, 115–126.
31. Stick, R. (1988) cDNA cloning of the developmentally regulated lamin L_{III} of *Xenopus laevis*. *EMBO J.* **7**, 3189–3197.
32. Newport, J.W., Wilson, K.L. & Dunphy, W.G. (1990) A lamin-independent pathway for nuclear envelope assembly. *J. Cell Biol.* **111**, 2247–2259.
33. Firmbach-Kraft, I. & Stick, R. (1993) The role of Caax-dependent modifications in membrane association of *Xenopus* nuclear lamin B_3 during meiosis and the fate of B_3 in transfected mitotic cells. *J. Cell Biol.* **123**,1661–1670.
34. Döring, V. & Stick, R. (1990) Gene structure for nuclear lamin LIII of *Xenopus laevis;* a model for the evolution of IF proteins from a lamin-like ancestor. *EMBO J.* **9**, 4073–4081.
35. Lohka, M.J. & Maller, J.L. (1985) Induction of nuclear envelope breakdown, chromosome condensation and spindle formation in cell-free extracts. *J. Cell Biol.* **101**, 518–523.
36. Blow, J.J. & Laskey, R.A. (1986) Initiation of DNA replication in nuclei and purified DNA by a cell-free extract of *Xenopus* eggs. *Cell* **47**, 577–587.
37. Hutchison, C.J., Cox, R., Drepaul, R.-S., Gomperts, M. & Ford, C.C. (1987) Period DNA synthesis in cell-free extracts of *Xenopus* eggs. *EMBO J.* **6**, 2003–2010.
38. Blow, J.J. & Watson, J.V. (1987) Nuclei act as independent and integrated units of replication in a *Xenopus* cell-free DNA replication system. *EMBO J.* **6**, 1997–2002.
39. Sheehan, M.A., Mills, A.D., Sleeman, A.M., Laskey, R.A. & Blow, J.J. (1988) Steps in the assembly of replication-competent nuclei in a cell-free system from *Xenopus* eggs. *J. Cell Biol.* **106**, 1–12.
40. Cox, L.S. (1992) DNA replication in cell-free extracts from *Xenopus* eggs is prevented by disrupting nuclear envelope function. *J. Cell Sci.* **101**, 43–53.
41. Mills, A.D., Blow, J.J., White, J.G., Amos, W.B., Wilcock, D. & Laskey, R.A. (1989) Replication occurs at discrete foci spaced throughout nuclei replicating *in vitro*. *J. Cell Sci.* **94**, 471–477.
42. Hutchison, C.J. (1995) Local and global changes in the morphology and distribution of replication centres in rapidly expanding nuclei. *Chromosome Res.* **3**, 16–26.
43. Meier, J., Campbell, K.H.S., Ford, C.C., Stick, R. & Hutchison, C.J. (1991) The role of lamin L_{III} in nuclear assembly and DNA replication, in cell-free extracts of *Xenopus* eggs. *J. Cell Sci.* **98**, 271–279.
44. Jenkins, H.E., Holleman, T., Lyon, C., Lane, E.B., Stick, R. & Hutchison, C.J. (1993) Nuclei which lack a lamina accumulate karyophilic proteins and assmeble a nuclear matrix. *J. Cell Sci.* **106**, 275–285.
45. Lourim, D. & Krohne, G. (1993) Membrane-associated lamins in *Xenopus* egg extracts: identification of two vesicle populations. *J. Cell Biol.* **123**, 501–512.
46. Goldberg, M.W. & Allen, T.D. (1992) High resolution scanning electron microscopy of the nuclear envelope; demonstration of a new, regular fibrous lattice attached to the baskets of the nucleoplasmic face of the nuclear pores. *J. Cell Biol.* **119**, 1429–1440.
47. Hutchison, C.J., Cox, R. & Ford, C.C. (1988) The control of DNA replication in a cell-free extract that recapitulates a basic cell cycle *in vitro*. *Development* **103**, 553–566.
48. Hozak, P., Hassen, A.B., Jackson, P.A. & Cook, P.R. (1993) Visualisation of a replication factories attached to a nucleoskeleton. *Cell* **73**, 361–373.