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Heat-Induced Morphological and Biochemical Changes in the Nuclear Lamina From Ehrlich Ascites Tumor Cells In Vivo

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Abstract Membrane-depleted nuclei from Ehrlich ascites tumor (EAT) cells isolated at low ionic strength in the presence of EDTA exhibit highly decondensed chromatin fibers and a loss of morphologically identifiable nucleoli. Treatment of these nuclei with nucleases and 2 M NaCl followed by low-speed centrifugation permitted the facile isolation of the nuclear lamina layer. Under the same conditions, but after heat-shock treatment of the living cells, the chromatin appears in a more condensed state, the nucleoli are well-defined, and the nuclear lamina layer was destabilized in concert with the appearance of an internal nuclear matrix and nucleolar skeleton. Furthermore, we also found both an increase in the protein mass as well as the appearance of a relatively large number of new proteins in this fraction, which are phosphorylated. The major proteins of the nuclear lamina, the lamins, and the residual vimentin remained insoluble. These heat-shock-induced changes were also accompanied by a dephosphorylation of lamins A and C but not of lamin B.

Key words: heat-shock, nuclear lamina, dephosphorylation, EAT, EDTA

Elevated temperatures induce the so-called heat-shock response in practically all living cells [Lindquist, 1986; Welch et al., 1991]. This response results in an increase of the expression of a group of proteins (heat-shock proteins) and leads to marked changes in the morphology of the cell [Welch and Suhan, 1985]. Recent studies have revealed that a number of "molecular chaperones" are members of the heat-shock protein family [reviewed in Welch, 1991; Ellis and van der Vies, 1991]. Heat-shock also induces the rapid appearance of another set of proteins designated as the "prompt heat-shock proteins" which are associated with the nuclear matrix-intermediate filament complex [Reiter and Penman, 1983; Ornelles and Penman, 1990].

During the past 15 years, a great number of publications have shown that the interphase nucleus contains an insoluble protein skeleton which is operationally defined as a nuclear matrix. In general, this nuclear structure is composed of three distinct domains: the peripheral lamina, the internal nuclear matrix, and the nucleolar skeleton. It is believed that most of the nuclear functions occur in close association with the nuclear matrix and that the chromatin is organized in discrete loops bordered by nuclear matrix attachment regions [for recent reviews see Verheijen et al., 1988; Gasser et al., 1989; Berezney, 1991; van Driel et al., 1991]. However, some skepticism persists concerning both the structure and function of the nuclear skeleton. For example, some authors have demonstrated that even apparently minor changes in the isolation protocol can result in incompatible conclusions [Kaufmann et al., 1986; Cook, 1988; de Jong et al., 1990].

A number of reports have demonstrated changes in the composition and structural stability of the nuclear matrix either by exposure of isolated nuclei to physiological or heat-shock temperatures, or in response to heat-shock conditions in vivo. In general, these incubations lead to an association of some nuclear proteins with the nuclear matrix [p62$^{c\text{-}myc}$: Evan and Hancock, 1985; p53: Littlewood et al., 1987; polyoma large T antigen: Humphrey and Pigiet, 1987; gp 188 and topoisomerase II: McConnell et al., 1987;

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RNA polymerase: Fisher et al., 1989; α-DNA polymerase: Martelli et al., 1990]. Recently, Martelli et al. [1991] have shown an apparent morphological stabilization of the internal nuclear matrix and the nucleolar skeleton following heat-shock. The temperature-induced stabilisation of the nuclear matrix is also a prerequisite in some experimental protocols for determining the matrix attachment regions of eucaryotic genes [reviewed in Gasser et al., 1989].

The nuclear lamina is the best characterized part of the interphase nuclear skeleton. It is the most stable component of the nuclear matrix and for all practical purposes its structure is the least dependent on the experimental protocol employed for its isolation. A highly purified nuclear lamina fraction from Ehrlich ascites tumor (EAT) cells with a well-preserved nuclear shape can be isolated from decondensed, membrane-depleted nuclei after treatment with DNase II and 2M NaCl [Krachmarov et al., 1986]. Here, we present evidence that, after heat-shock, the nuclear lamina is destabilized in concert with a stabilization of the internal nuclear matrix and the nucleolar skeleton. These changes are accompanied also with a dephosphorylation of lamins A and C but not of lamin B.

MATERIALS AND METHODS

Isolation of Nuclear Lamina

EAT cells were grown in suspension culture to a density of 5×10^5 cells/ml as described previously [Egberts et al., 1976]. For induction of the heat-shock response, samples were incubated for 2 h in a 42°C water bath with gentle rocking. Both control (37°C) and heat-shocked (42°C) cells were used for isolation of the nuclear lamina fraction following the same protocol [Krachmarov et al., 1986]. Cells were collected at 1,000*g* for 5 min and washed twice with 20 volumes of 0.25 M sucrose, 5 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride (PMSF), adjusted to pH 8 with NaOH before use. This and all subsequent operations were carried out at 0–4°C and the volumes refer to the original volume of the collected cells. After centrifugation at 600*g* for 5 min, the cells were washed with 20 volumes of 0.25 M sucrose, 0.1 mM EDTA, and 0.1 mM PMSF, pH 8.0, resuspended in 4 volumes of the same solution, and mixed with 4 volumes of 0.5% Nonidet NP 40. The sample was layered on a cushion of 20 volumes of 0.25 M sucrose, 0.1 mM EDTA, pH 7.0, and centrifuged for 15 min at 3,500*g*. The decondensed, membrane-depleted nuclei sedimented into a semitransparent pellet.

For the isolation of the nuclear lamina fraction, the nuclei were suspended in 0.25 M sucrose, 2 mM Tris-HCl, and 0.2 mM EDTA, pH 7.0, at a concentration of 5 A_{260} units/ml and digested for 1 h at 25°C with 20 units DNase II per A_{260} unit plus 10 μg/ml pancreatic RNase. After nuclease treatment, the samples were mixed with an equal volume of 4 M NaCl, 20 mM Tris-HCl, and 2 mM EDTA, pH 8.0, and layered on a cushion of 5 volumes of 0.5 M sucrose, 2 M NaCl, 10 mM Tris-HCl, and 1 mM EDTA, pH 8.0. The nuclear lamina was collected by centrifugation of lamin-containing fractions for 30 min at 3,500*g*.

Alkaline phosphatase from E. coli was used for dephosphorylation of the lamina fraction. About 200 μg of proteins were dispersed by brief sonication in 1 ml of 10 mM Tris-HCl, pH 7.6, 10 mM KCl, and 3 mM $MgCl_2$ and incubated for 4 h at 37°C with 200 μg alkaline phosphatase.

Radioactive Labeling and Analysis

For in vivo [^{32}P]orthophosphate labeling, logarithmically growing EAT cells were pelleted, resuspended in phosphate-free minimum essential medium to a density of 5×10^6 cells/ml, and labeled for 2 h at 37°C or 42°C, respectively, with 1 mCi/ml $^{32}P_i$. Nuclear lamina fractions were isolated as described above and samples were electrophoresed on SDS-containing polyacrylamide gels. The radioactivity was located by autoradiography and was quantified by excising the Coomassie blue stained bands and Cerenkov counting. The approximate quantity of protein in the bands was determined from Coomassie blue binding using bovine serum albumin as a standard [Fenner et al., 1975].

Electron Microscopy

For transmission electron microscopy, samples were fixed in 2% glutaraldehyde in 50 mM cacodylate buffer, pH 7.4, for 1 h at 4°C. After three washes with the buffer, the particles were post-fixed with 2% OsO_4, dehydrated in a graded ethanol series and propylene oxide, and embedded in Epon resin. Sections were stained with uranyl acetate and Reynolds lead citrate. For immunolabeling, samples were fixed for 1 h in 4% paraformaldehyde in PBS. After three washes with PBS, the material was preincubated for 30 min in PBS, containing 1% FCS and 1% BSA, and incubated in the same buffer with the mono-

clonal anti-lamins antibody (dilution 1:1) [Burke et al., 1983] for 1 h, at room temperature. The samples were washed three times with PBS, 1% FCS, and 1% BSA, containing 0.1% Tween-20 and incubated for 4 h with 10 nm gold-conjugated goat antimouse IgM, at a dilution of 1:20. Three washes with PBS containing 0.1% Tween-20 were followed by fixation for 1 h with 4% glutaraldehyde and the material was processed for embedding in Epon. For spreading, samples were layered on a cushion of 0.1 M sucrose containing 2 M NaCl and 1% paraformaldehyde and centrifuged in a microcentrifugation chamber for 5 min at 3,500*g* onto freshly glow-discharged, carbon-coated grids. The specimens were stained in 1% phosphotungstic acid in 70% ethanol and rinsed in absolute ethanol. Electron microscopy was performed on a JEM 100B instrument at an accelerating voltage of 80 kV.

Polyacrylamide Gel Electrophoresis and Immunoblotting

Nine to fifteen percent gradient SDS-polyacrylamide gel electrophoresis was carried out as described [Egberts et al., 1976]. Isoelectric focusing followed by SDS-polyacrylamide gel electrophoresis in the second dimension was performed as described by Bravo [1984]. Proteins were electrophoretically transferred onto nitrocellulose sheets [Kyhse-Andersen, 1984] and nuclear lamina proteins and vimentin were identified by their reaction with the monoclonal anti-lamin antibody [Burke et al., 1983] or with the monoclonal anti-IF antibody [Pruss et al., 1981]. Antibody-antigen complexes were visualized with alkaline phosphatase-conjugated secondary antibodies.

RESULTS

Heat-Induced Morphological Changes in the Structural Stability of the Nuclear Skeleton

The method employed for the isolation of the purified nuclear lamina fraction includes the preparation of highly decondensed nuclei (chromatin structures) as a first step. Treatment with hypotonic solutions containing EDTA and removal of the nuclear membranes with nonionic detergents lead to the production of clean nuclei with a preserved nuclear lamina layer that surrounds the extensively decondensed chromatin without morphologically distinct nucleoli or blocks of heterochromatin (Fig. 1A) [see also Hancock, 1974; Krachmarov et al., 1986]. The effect of heat-shock (following incubation in vivo at 42°C) on the morphology of these nuclei is evident in Figure 1B. There are well-expressed nucleoli and electron-dense nuclear structures (arrows). As a whole, the chromatin is in a more condensed form relative to that obtained from cells grown at the "normal" temperature of 37°C. Morphologically, the nuclear lamina seems unaffected by the heat-shock treatment. Apparently, the heat-shock induces morphological changes which reflect primarily on the stability of the nucleoli and the condensation state of chromatin fibers under hypotonic conditions and in the presence of EDTA. It should be noted that the vitality of the cells was not affected by incubation for 2 h at 42°C.

For the isolation of the nuclear lamina, the chromatin was extensively digested with DNase II and RNase and extracted with 2 M NaCl. Omitting the RNase had no effect on the morphology and protein composition of the lamina fraction. Ultrathin sections of this fraction revealed a typical nuclear lamina in the control cells (Fig. 2A) [Krachmarov et al., 1986]. No internal nuclear matrix or nucleolar skeleton were seen. The same procedure applied to the in vivo heat-shocked cells yielded the structures shown in Figure 2B. Surprisingly, substantial morphological changes occurred within the nuclear lamina fraction. The nuclear lamina was not well-defined and an extensive internal nuclear matrix and nucleolar skeleton could be seen. More detailed analysis by higher magnification and immunogold labeling with the monoclonal anti-lamin antibody [Burke et al., 1983] is shown in Figure 2C. The nuclear lamina layer is altered but its peripheral nuclear localization remains. (These observations are confirmed by the biochemical evidence described.) Alterations were also visible when the preparations for electron microscopy were spread (Fig. 3) rather than sectioned. The "normal" nuclear lamina of cells grown at 37°C appeared as a flattened sphere of fibrous material with a preserved nuclear shape [Simard et al., 1986; Krachmarov et al., 1986], but the incubation of the cells at 42°C clearly disrupted this order and the structures collapsed. Nonetheless, both electron microscopy techniques indicate that the nuclear lamina fraction consists of discrete structures which derive from single nuclei and are not just amorphous aggregates of insoluble proteins.

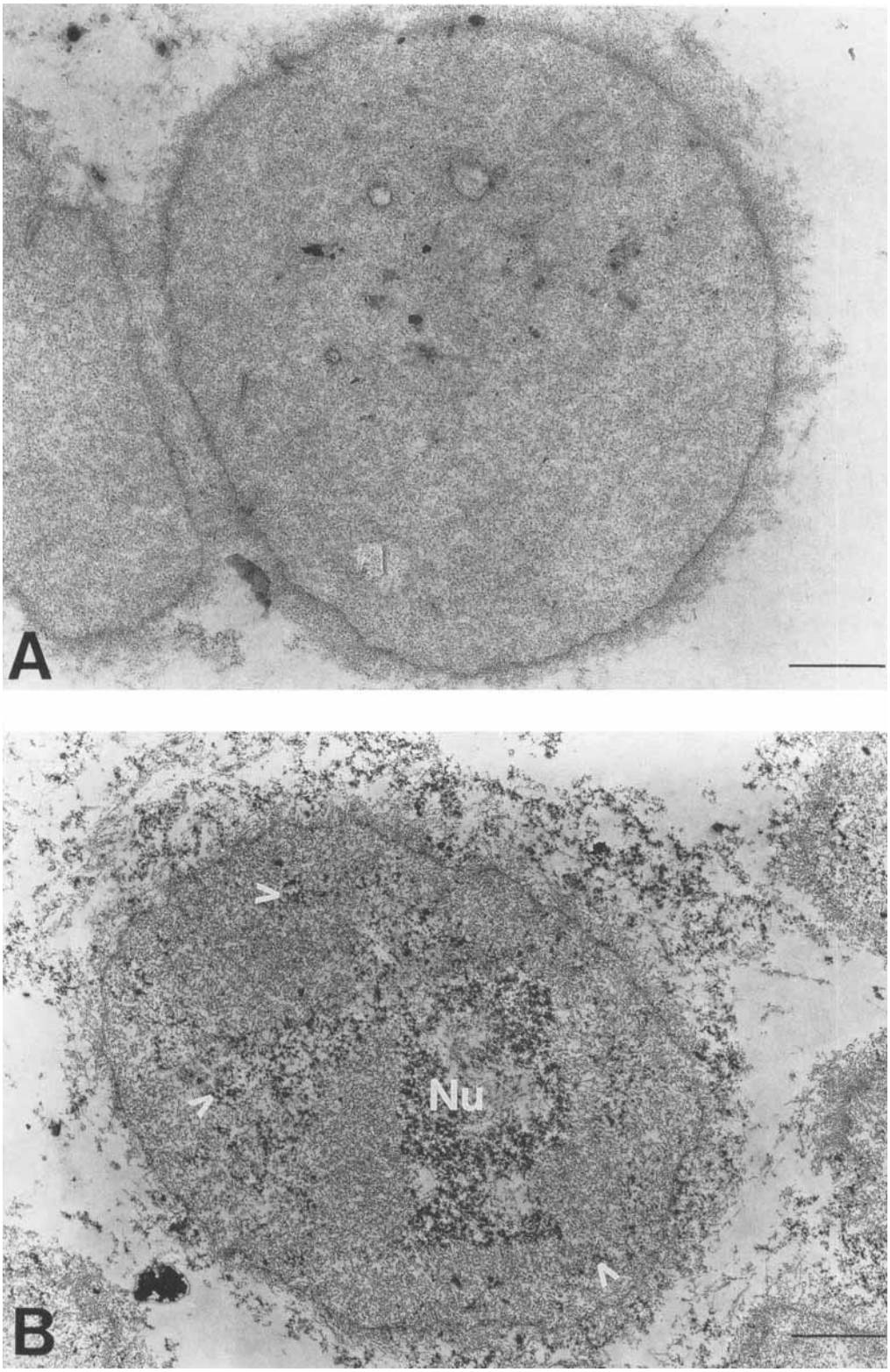
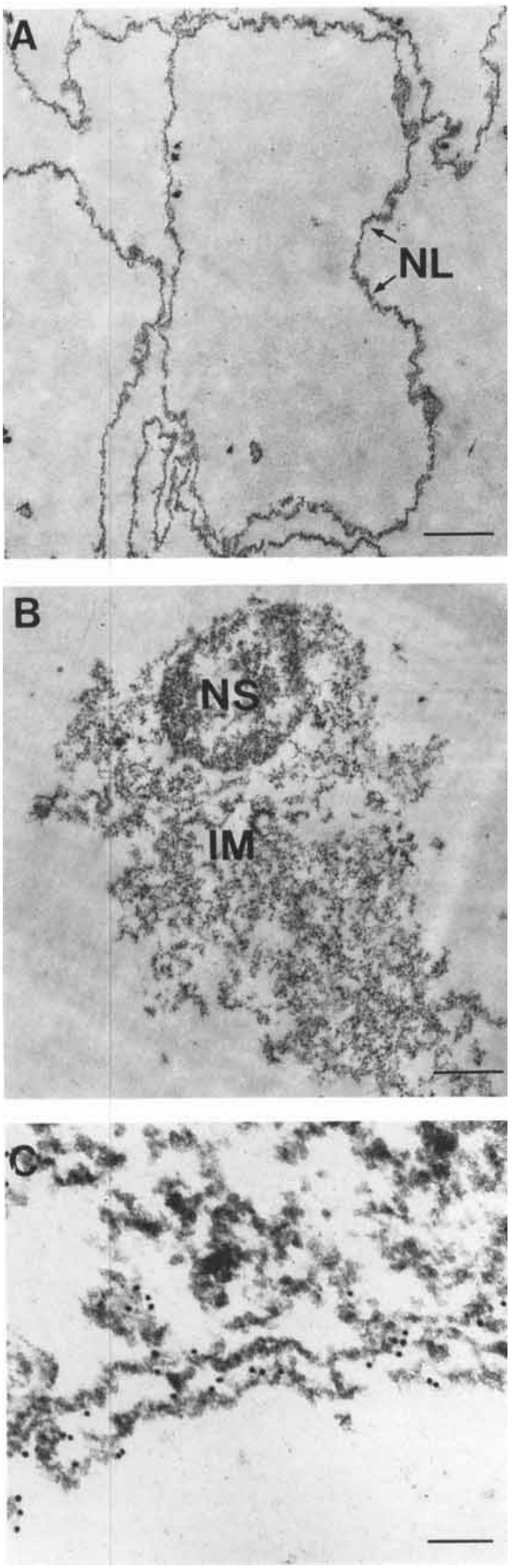


Fig 1 Ultrathin sections of membrane depleted nuclei from EAT cells isolated in hypotonic solutions containing EDTA from **A** cells grown at 37°C **B** cells incubated for 2 h at 42°C prior to isolation The nucleolus (Nu) and unknown electron dense structures (arrowheads) are indicated Bar denotes 1 μm



Heat-Induced Biochemical Changes in the Nuclear Lamina Fraction

The nuclear lamina fraction from EAT cells isolated according to the described procedure represents about 3% of total nuclear protein as estimated by radioactive labeling of proteins or employing the method of Lowry [Krachmarov et al., 1986]. It is composed mainly of nuclear lamins and a small amount of associated vimentin. Under the same isolation conditions, about 20% of the total nuclear protein obtained from heat-shocked cells was sedimented in the nuclear lamina fraction. In SDS-polyacrylamide gels, the nuclear lamina preparations from cells subjected to heat-shock possess a set of new proteins with molecular weights higher and lower than those of nuclear lamins (Fig. 4). This protein pattern is comparable to the composition of complex nuclear matrix preparations obtained by many other authors.

In order to follow the fate of the nuclear lamina proteins after in vivo heat-shock, we performed immunoblotting with the monoclonal anti-lamin antibody [Burke et al., 1983]. It was also interesting to find out whether these proteins became soluble after nuclease and high salt treatment. Figure 4B shows clearly that the lamins from control (37°C) and heat-shocked cells remained in the insoluble fraction.

Our morphological observations revealed dramatic changes in the nuclear lamina fraction after heat-shock. However, one-dimensional SDS gel electrophoresis and immunoblot analysis did not reveal any differences in the major nuclear lamina proteins. Thus, more detailed analysis of these proteins seemed necessary since it is well documented that lamina proteins are subject to posttranslational modifications, especially phosphorylation/dephosphorylation during the cell cycle [reviewed in Dessev, 1991; Nigg, 1992]. Two-dimensional gel electrophoresis showed the typical distribution of nuclear lamins from control EAT and other cells in the pH interval

Fig. 2. Ultrathin sections of nuclear skeletal fractions isolated from EAT nuclei from (**A**) cells grown at 37°C and (**B** and **C**) from in vivo heat-shocked cells (2 h at 42°C). The nuclear lamina (NL), the internal nuclear matrix (IM), and the nucleolar skeleton (NS) are indicated. For immunogold labeling, samples were probed with the monoclonal anti-lamins antibody [Burke et al., 1983] and visualized with secondary 10 nm gold-conjugated antimouse IgM. Bar denotes 0.5 μm (A and B) and 0.13 μm (C).

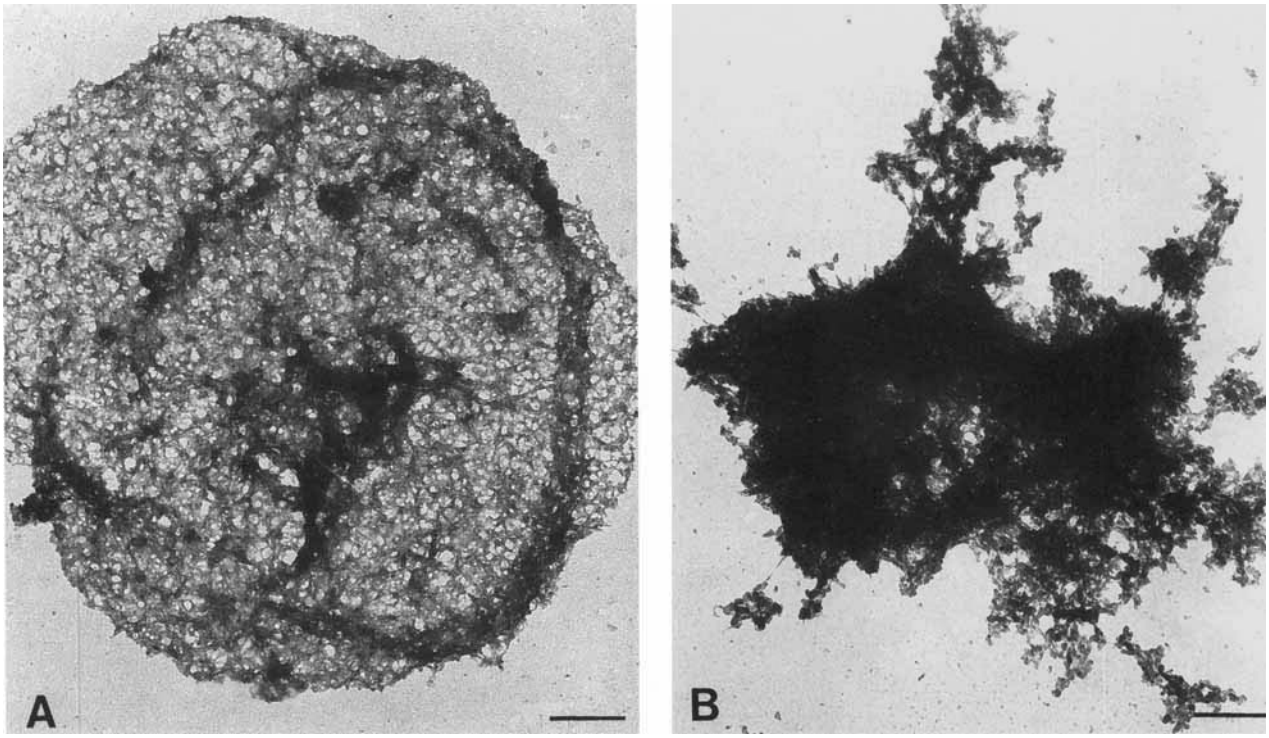


Fig. 3. Electron micrographs of the same material as in Figure 2 prepared according to the spreading technique (see Materials and Methods) **A:** The surface of the nuclear lamina isolated from "normally" grown EAT cells (at 37°C) **B:** The surface of the structures from the insoluble nuclear skeletal fraction obtained from heat-shocked cells (2 h at 42°C) Bar denotes 0 5 μm

between 5.5 and 7.0, with a great heterogeneity of lamins A and C (Fig. 5A) [Traub et al., 1988; Höger et al., 1990; Kasahara et al., 1991]. Analysis of the positions of the nuclear lamins from heat-shocked cells (Fig. 5B) demonstrated that lamin B retains its isoelectric point but lamins A and C change their positions toward higher pH values. In agreement with data published by Ornelles and Penman [1990], a number of heat-shock-induced insoluble proteins also appear in this pH region. These shifts in the positions of the nuclear lamins were confirmed on immunoblots with the monoclonal anti-IF antibody [Pruss et al., 1981], which is directed against an epitope common to almost all intermediate filament proteins, including lamins and vimentin (Fig. 6). (In addition, the same immunoblotting results were obtained with Burke's monoclonal anti-lamins antibody; data not shown.) In the two-dimensional gels and immunoblots obtained with material from heated cells, proteins with the molecular mass of vimentin and its cleavage products [Nelson and Traub, 1983] and lamin B_2 [Höger et al., 1990; Kasahara et al., 1991] but with higher isoelectric points are also visible. Their origin and chemical characterization will be described elsewhere (Krachmarov and Traub, in preparation).

In order to investigate whether the changes in the isoelectric point were due to dephosphorylation, we treated the proteins with alkaline phosphatase. Changes similar to those seen after heat-shock were obtained after phosphatase treatment of the nuclear lamina fraction of control cells (Fig. 6), which suggests that at least some dephosphorylation of lamins A and C results from heat-shock treatment.

Finally, we also performed in vivo labeling of nuclear lamins with $^{32}P_i$. Autoradiography after SDS-polyacrylamide gel electrophoresis confirms the partial dephosphorylation of lamins A and C (Fig. 4C). Moreover, most of the insoluble proteins of the lamina fraction after heat-shock are phosphorylated. A quantitative analysis of the level of lamins dephosphorylation induced by heat-shock is presented in Table I. These data once again manifest specific dephosphorylation of lamins A and C in vivo.

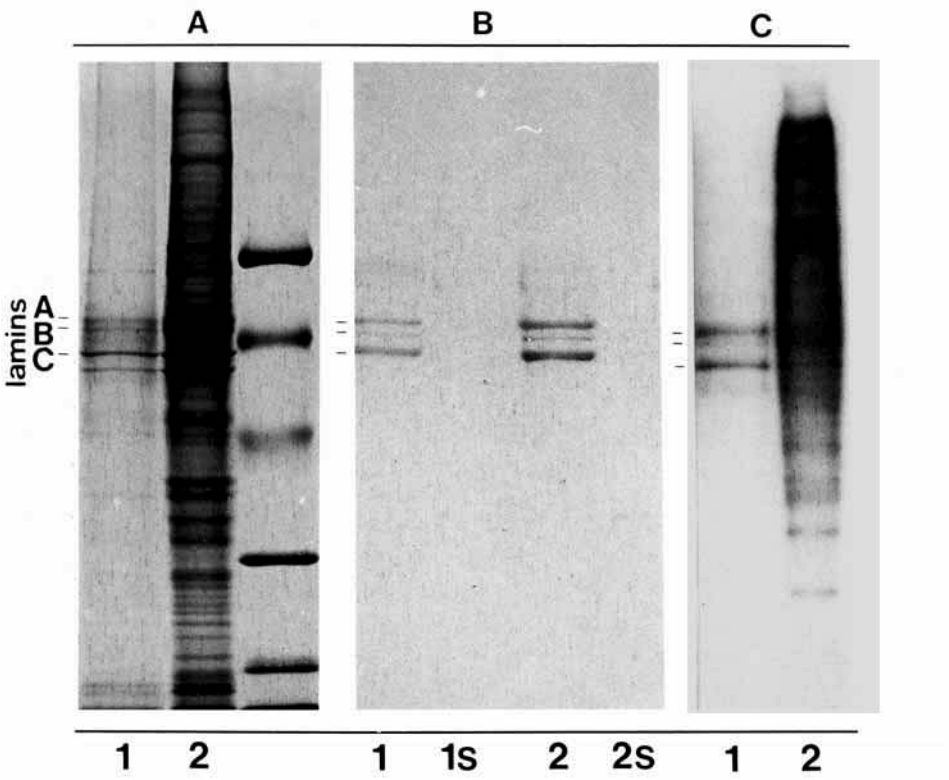


Fig. 4. **A:** Electrophoresis in a 9-15% gradient SDS-polyacrylamide gel of the nuclear skeletal fractions isolated from EAT cells. Samples applied were: the EAT nuclear lamina layer from control cells (lane 1) and the insoluble nuclear proteins from heat-shocked cells (2 h at 42°C) (lane 2). Each lane contains the protein equivalent from approximately 10^7 cells. The gel was stained with Coomassie Brilliant Blue G-250. [Lane M represents the protein standard from Pharmacia-LKB (LMW kit) with M_r values from top to the bottom of 94, 68, 43, 30, 20, and 14 kDa]. **B:** Identification of nuclear lamina proteins in the insoluble fractions after 2 M NaCl treatment and in their corresponding supernatants. Proteins were separated on a polyacrylamide gel, electroblotted onto a nitrocellulose sheet and reacted with the monoclonal anti-lamins antibody [Burke et al., 1983]. Antibody-antigen complexes were visualized with alkaline phosphatase-conjugated antimouse antibody. Lane 1 represents the nuclear lamina fraction isolated from cells grown at 37°C and lane 1S is its corresponding supernatant. Lane 2 is the insoluble nuclear skeletal fraction from heat-shocked cells (2 h at 42°C) and lane 2S is its corresponding supernatant. **C:** Autoradiograms of in vivo $^{32}P_i$-labeled nuclear skeletal proteins from control cells (lane 1) and from heat-shocked cells (lane 2) after SDS-polyacrylamide gel electrophoresis. [For comparison, see (A) in this figure.]

DISCUSSION

The cell nucleus is without doubt a target of the influence of elevated temperatures on living cells. In the present study, we have shown that in vivo heat-shock on EAT cells leads to severe changes in the morphology and biochemistry of the nuclear skeleton.

A number of morphological changes in the cell nucleus after in vivo heat-shock have been described previously, such as condensation of chromatin [Pekkala et al., 1984], appearance of actin filaments throughout the nucleus, and considerable alterations in the integrity of the nucleoli [Welch and Suhan, 1985]. Here we show that membrane-depleted nuclei from EAT cells isolated in hypotonic solutions containing EDTA exhibit a highly decondensed state of the chromatin fibers but that, after heat-shock, this condition is reversed and the chromatin remains relatively condensed (Fig. 1). Since it is well known that bivalent cations play a role in the condensation of chromatin, we took care to remove them in our procedure. Thus, we can conclude that heat-shock-induced condensation is at least in part due to changes in the stability of the internal nuclear matrix and the residual nucleolar skeleton (Fig. 2). Furthermore, RNase treatment does not disturb the nucleolar skeleton in heat-shocked cells, as has been reported for mammalian cells grown at the "normal" temperature of 37°C [reviewed in Verheijen et al., 1988]. This stabilization of the nucleolus may be regarded as an additional feature of the heat-shock response of the nucleolus, which has been

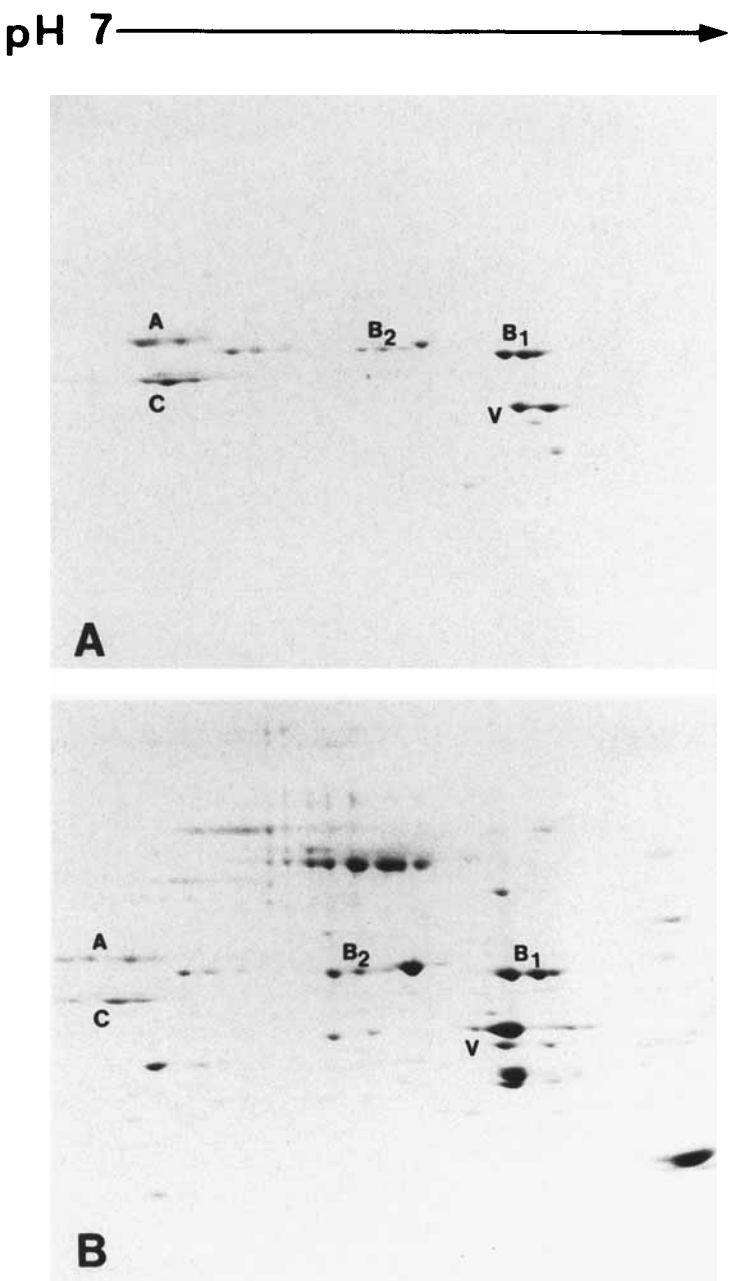


Fig. 5. Two-dimensional isoelectrofocusing/SDS-polyacrylamide gel electrophoresis of the polypeptides from the nuclear skeletal fraction obtained from EAT cells (**A**) grown at 37°C and (**B**) incubated for 2 h at 42°C. Gels were stained with Coomassie Brilliant Blue G-250. The positions of lamins (A, B_1, B_2, and C) and residual vimentin (V) are indicated based on their known isoelectric points and molecular masses.

documented in a series of reports [reviewed in Welch et al., 1991].

The major factors which can influence the stability of the internal nuclear matrix and the nucleolar skeleton during the isolation procedure are bivalent cations, disulfide bridges, RNase treatment, and temperature. All of these parameters can apparently change, albeit to different extents, the expression and protein composition of the final preparations. Most of the papers concerning heat-induced changes in the nuclear matrix demonstrate the incorporation of some proteins after incubation of isolated nuclei at physiological (37°C) or heat-shock (42°C) temperatures (see Introductory paragraphs). The first systematic, morphological work which showed a temperature effect on the structural stability of the nuclear matrix was

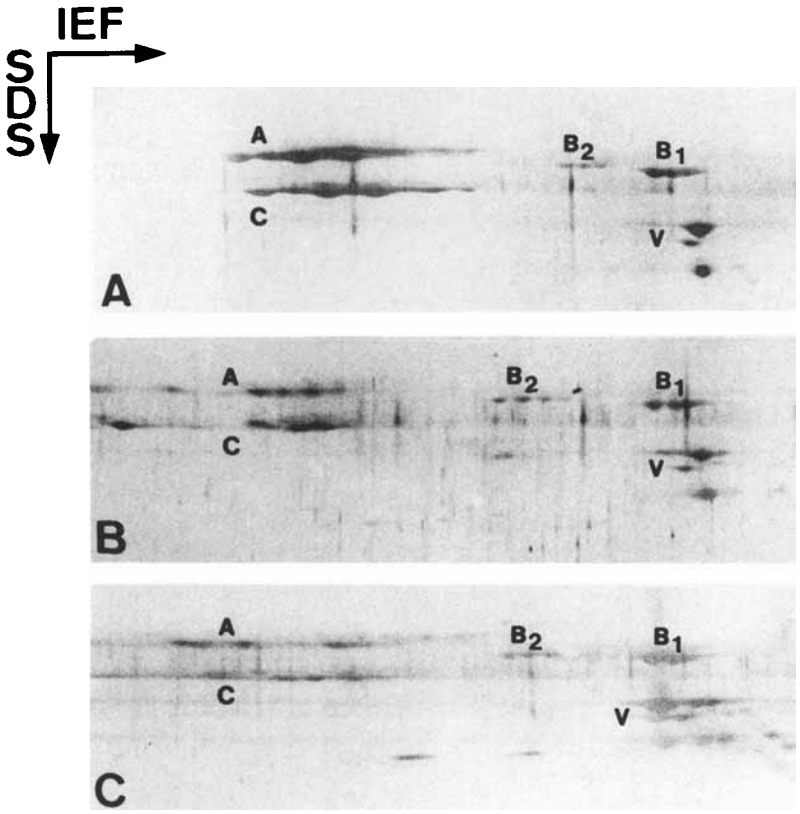


Fig. 6. Immunological identification of nuclear lamins and residual vimentin after two-dimensional isoelectrofocusing/SDS-polyacrylamide gel electrophoresis with the monoclonal anti-IF antibody [Pruss et al., 1981]. Proteins separated in parallel gels were transferred to nitrocellulose filters and incubated with the antibody. Alkaline phosphatase-conjugated antimouse antibody was used as a secondary antibody. **A:** Nuclear lamina proteins from EAT cells grown at 37°C. **B:** The same proteins as in (A) were dephosphorylated with alkaline phosphatase prior to electrophoresis. **C:** Proteins from the nuclear skeletal fraction from heat-shocked cells (2 h at 42°C). The positions of lamins (A, B_1, B_2, and C) and residual vimentin and its possible cleavage products (V) are indicated. The nuclear lamina proteins display their typical heterogeneity.

TABLE I. Specific Radioactivities of the Electrophoretical Fractions of Nuclear Lamins From "Normal" (37°C) and Heat-Shocked (2 h at 42°C) EAT Cells*

Fraction	Specific radioactivity cpm/A_{605} 37°C	42°C	Ratio of 37° to 42°C
lamin A	14,377	6,468	2.22
lamin B	9,528	9,755	0.98
lamin C	15,021	10,330	1.45

*EAT cells were grown in medium containing [^{32}P]orthophosphate for 2 h at 37°C or at 42°C (see Materials and Methods). Proteins from nuclear lamina fractions were electrophoresed and specific radioactivity was determined by Coomassie blue binding [Fenner et al., 1975] and Cerenkov counting.

that of Martelli et al. [1991]. These authors demonstrated, performing in vitro experiments, a stable nuclear lamina layer and a different extent of expression of the internal nuclear matrix and the nucleolar skeleton after exposure of isolated nuclei to 37°C or heat-shock treatment of intact cells. They also found that the method used for the isolation of nuclei had only a limited influence on matrix morphology and that RNase treatment is essential for the demonstration of the residual nucleoli. In our experimental conditions, we have a relatively extreme case in that we isolate as a final, insoluble fraction only the nuclear lamina layer, without distinct internal matrix and nucleolar skeleton. In our system, heat-shock treatment either induces the appearance of these structures or stabilizes them, thereby permitting their isolation. In addition, the stable nuclear lamina loses its higher order of organization (Figs. 2, 3). Nevertheless, the nuclear lamina proteins remain in the insoluble fraction, thus preserving its peripheral localization (Fig. 3C). From these results, it is quite clear that temperature provokes events deter-

mining the structural stability of the nuclear skeletal framework.

We have demonstrated considerable qualitative and quantitative changes in the protein composition of the insoluble nuclear skeleton after heat-shock exposure of living cells. Previous reports concerning this problem are somewhat conflicting. Some authors observed marked differences in protein quality and composition [Evan and Hancock, 1985; McConnell et al., 1987; Ornelles and Penman, 1990], whereas Martelli et al. [1991] found mostly quantitative differences and explained this with destruction of the matrix during the addition of 2 M NaCl. McConnell et al. [1987] also found that $>90\%$ of total nuclear skeletal protein in Drosophila was solubilized by 1 M NaCl, and heat-shock apparently stabilized this structure. We can not agree with such a simple explanation for the following reasons. First of all, the nuclear skeleton is defined as a nuclear structure resistant to treatments with high salt solutions or other chromatin-depleting reagents (for example dextrane sulphate, heparin, lithium diiodosalicylate, etc.) and is found throughout the eucaryotic world, ranging from yeast to mammals and plants. Second, as seen in Figures 2C and 4B, nuclear lamins are not affected by the 2 M NaCl treatment and were not released into the soluble fraction. In our opinion, these discordant observations result from the fact that the reported isolation procedures lead to complex nuclear matrices: lamina, internal matrix, and nucleolar skeleton. The last two components are expressed and stabilized to different extents in these preparations and it is well documented that these skeletal components are easily affected by small changes in the isolation protocols.

The electrophoretical profile of the proteins from the insoluble skeletal fraction shows a high degree of complexity after in vivo induced heat-shock (Fig. 4). These results are in concordance with the morphological observations and agree quite well with the polypeptide profiles of nuclear matrices obtained by many authors. Since the protein composition of the internal nuclear matrix is not yet definitely established, with only preliminary results published [for example Fey and Penman, 1988; Stuurman et al., 1990; Hakes and Berezney, 1991], we are not able to compare it with the additional, heat-induced, insoluble proteins observed in this study. Still, we demonstrate that a great number of these proteins are phosphorylated (Fig. 4C). Our results support the idea that in vivo or in vitro thermal stabilization of the internal nuclear matrix can be a useful tool that will hopefully permit the settling of the current controversy concerning the protein composition and significance of this skeleton [McConnell et al., 1987; Martelli et al., 1991].

In the present work, we have concentrated our efforts on the nuclear lamins as they represent the best-characterized nuclear skeletal proteins. After exposure of cells to heat shock temperatures, lamins A and C undergo dephosphorylation without changing their insolubility, that is, they remain assembled in the nuclear lamina. Phosphorylation of nuclear lamins and the role of this protein modification on assembly/disassembly of the lamina layer has been the subject of a number of papers published during the last 5 years [for reviews, see Dessev, 1990; McKeon, 1991; Nigg, 1992]. It was established that hyperphosphorylation of lamin proteins is a characteristic feature of lamina disassembly during mitosis and is mediated by cdc 2 kinase. In the present study, we observed the opposite situation: dephosphorylation of lamina proteins after in vivo heat-shock. Similar results were described by Smith et al. [1987] with respect to the lamin isoforms in *Drosophila*. To our knowledge, this is the only publication which reports the modification of nuclear lamina proteins after heat treatment. These authors show that after heat-shock in vivo lamin Dm_2 is converted nearly quantitatively into lamin Dm_1. It has previously been found that during interphase lamins are in a partially phosphorylated state [Ottaviano and Gerace, 1985], a condition which is proposed to be necessary for the maintainance of lamina plasticity and lamina growth. Smith et al. [1987] speculate that after heat-shock the membrane fluidity can increase and, as a second component, the nuclear lamina provides structural integrity to the nuclear envelope in a compensatory fashion by increasing its structural rigidity through dephosphorylation. This hypothesis, while attractive, awaits experimental confirmation. Our results clearly confirm that heat-shock-induced dephosphorylation of lamina proteins also occurs in mammalian cells. This point is potentially not trivial since, among other differences, mammals are homeothermic and *Drosophila* is poikilothermic and therefore likely to possess various homeostatic mechanisms to cope with the wide variation in body temperatures that the organism might experi-

ence. In addition, our data show differential modification of lamins A and C but not of lamin B, suggesting that the effect is a specific one and not due to wholescale activation of phosphatases.

After heat-shock, the lamina layer exhibits a change in its integral stability to treatment with high salt solutions, but nonetheless its components remain insoluble. It is possible that the concomitant structural stabilization of the internal nuclear framework is a compensatory mechanism that provides a reasonable, acceptable level of nuclear stability. In this regard, it should be noted that lamin B is proposed to be the intermediate filament attachment site in the nuclear envelope [Georgatos and Blobel, 1987] and to fulfil a role in anchoring the lamina to the inner nuclear membrane [Gerace and Blobel, 1982]. So, if lamin B remains unmodified, the connections of the lamina to the membrane could be preserved, whereas lamins A and C are probably involved in chromatin reorganization during heat-shock response. This is in agreement with the hypothesis that A-type lamins are involved in chromatin organization and function and B-type lamins interact with the inner nuclear membrane [Nigg 1989; 1992].

The phosphorylation of the nuclear lamina proteins has been widely studied. In our opinion, it is fundamentally necessary to know more about the opposite reaction (the dephosphorylation of lamins) in order to better appreciate the role of the nuclear lamina in nuclear functions. Evidence for such a kind of conclusion descends from the recently published data about dephosphorylation of lamins A and C in cytomegalovirus infected human fibroblasts [Radsak et al., 1991]. It was also reported that heat-shock treatment decreased protein-tyrosine kinase activities [Bagi and Hidvegi, 1990] and, as oxidative stress, induced a gene encoding a protein-tyrosine phosphatase [Keyse and Emslie, 1992]. All these data, taken together with those reported in the present paper, lead to the assumption that the nuclear lamina is involved in the stress response of the eucaryotic cell. Furthermore, phosphorylation/dephosphorylation of the nuclear lamina proteins plays a principal role in these events. In this respect, the in vivo induced heat-shock response may be a suitable model for many future investigations of the structure and function of the nuclear skeleton.

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