ARTICLE
Journal of Cellular Biochemistry 105:1485–1500 (2008)

Journal of Cellular Biochemistry

Effects of Heat Shock on the Distribution and Expression Levels of Nuclear Proteins in HeLa S3 Cells

Nisrine Haddad and Micheline Paulin-Levasseur*

Department of Biology, University of Ottawa, 30 Marie Curie, Ottawa, Ontario, Canada K1N 6N5

ABSTRACT

Cumulating evidence has led to the idea that nuclear functions such as DNA replication, RNA transcription, RNA splicing and nucleocytoplasmic transport are facilitated by a proteinaceous architectural framework within the nuclear compartment and at the nuclear envelope. In the present study, we have used immunofluorescence microscopy and quantitative Western blotting to compare the distribution and expression levels of several nuclear proteins during the response of HeLa S3 cells to both mild and severe hyperthermia. Cells were exposed to mild (42°C) or severe (45°C) hyperthermia treatment for 90 min and left to recover at 37°C for 1–25 h. The cell response was monitored immediately after the heat stress and at different time intervals during the recovery period. Our observations indicate that inner nuclear membrane proteins, LAP2β and emerin, as well as major components of the nuclear lamina, lamins A/C and lamin B_1, maintain an overall normal distribution at the nuclear periphery throughout the cell response to mild or severe hyperthermia. The response was nevertheless characterized by significant changes in the expression levels of emerin following recovery from a mild stress and of lamin B_1 after recovery from a severe stress. Our results also provide evidence that the organization of functional domains within the nuclear interior such as nucleoli and splicing speckles differs between cells responding to a mild or a severe stress. Mild hyperthermia was accompanied by a significant decrease in the expression level of the nucleolar protein 2H12 whereas severe hyperthermia was characterized by a reduction in the expression of the nucleocytoplasmic shuttling protein 2A7. Our data underline the complexity of nuclear function/structure relationships and the needs for a better understanding of protein–protein interactions within the nuclear compartment. J. Cell. Biochem. 105: 1485–1500, 2008.

KEY WORDS: HEAT SHOCK; HeLa S3 CELLS; NUCLEAR ENVELOPE; NUCLEAR INTERIOR; NUCLEAR FUNCTION/STRUCTURE RELATIONSHIPS; NUCLEAR MATRIX

In eukaryotic cells, the nuclear envelope delimits the boundary of the nucleus and segregates spatially the genetic material from the cytoplasm. This structure consists of the inner nuclear membrane (INM) and the outer nuclear membrane (ONM), which are separated by the perinuclear space. Both membranes are joined together at nuclear pore complexes [for a review, see Tran and Wente, 2006]. The ONM is continuous with the endoplasmic reticulum (ER): it is typically decorated with ribosomes and its composition is similar to that of the ER. In contrast, the INM has a distinct composition and is closely associated with chromatin and the nuclear lamina [reviewed by Gruenbaum et al., 2000; Worman and Courvalin, 2000].

Apposed to the nucleoplasmic face of the INM, the nuclear lamina is an insoluble protein meshwork that provides mechanical strength to the nuclear envelope. The major constituents of this meshwork are the nuclear lamins which can be subdivided into two types, A-type and B-type, based on differences in their biochemical properties, primary sequence and expression patterns. A-type lamins have a developmentally regulated and cell type-specific expression pattern while B-type lamins are ubiquitous [Fisher et al., 1986; McKeon et al., 1986; reviewed by Bosman, 1999]. Lamins bind directly to DNA and evidence suggests that the nuclear lamina may act as an anchoring site for interphase chromosomes [Benavente and Krohne, 1986; Gerace and Burke, 1988; Ulitzur et al., 1992; Stierle et al., 2003].

Lamin-associated proteins (LAPs)2/thymopoietins, emerin and MAN1 are INM constituents that share a conserved functional domain designated the LEM module [Manilal et al., 1996; Nagano et al., 1996; Lin et al., 2000; Wilson, 2000]. In mammals, three major LAP2 isoforms, α, β, and γ, have been identified [Furukawa et al., 1995]. Although the three polypeptides contain identical amino-terminal domains, LAP2α does not possess a transmembrane segment and is distributed diffusely throughout the nucleoplasm [Dechat et al., 1998]. In contrast LAP2β and γ are type II integral

Grant sponsor: Natural Sciences and Engineering Research Council of Canada; Grant number: RGPIN/41859-2008.
*Correspondence to: Micheline Paulin-Levasseur, Department of Biology, University of Ottawa, 30 Marie Curie, Ottawa, Ontario, Canada K1N 6N5. E-mail: mplev@uottawa.ca
Received 16 April 2008; Accepted 17 September 2008 • DOI 10.1002/jcb.21968 • 2008 Wiley-Liss, Inc.
Published online 3 November 2008 in Wiley InterScience (www.interscience.wiley.com).

membrane proteins that may associate with chromatin directly or indirectly through binding affinities to lamins B and a number of DNA-binding proteins [Foisner and Gerace, 1993; Furukawa et al., 1995, 1997, 1998; Furukawa, 1999; Cai et al., 2001; Lee et al., 2001; Nili et al., 2001]. As another member of the LEM family, emerin which has been implicated in X-linked Emery-Dreifuss muscular dystrophy interacts with A-type and B-type lamins and associates with DNA-binding proteins such as the barrier to auto-integration factor (BAF) and germ cell-less (GCL) [Fairley et al., 1999; Sullivan et al., 1999; Clements et al., 2000; Haraguchi et al., 2000; Holaska et al., 2003].

The nuclear lamina and its associated proteins co-fractionate with nucleolar remnants and a yet ill-defined internal fibrogranular network during isolation of the nuclear matrix [reviewed by Pederson, 2000]. This nuclear proteinaceous fraction resembles the intact nucleus in size and shape, and has been implicated in the organization of DNA into specific domains. Over the past years, it has been a persistent suggestion that the nuclear matrix may play regulatory functions in the replication and transcription of DNA, as well as the processing of RNA molecules and their transport into the cytoplasm [reviewed by Berezney, 1991; Marshall et al., 1997; Nickerson, 1998; Pederson, 1998].

In attempt to gain better insights into the structure/function relationships within the mammalian nucleus, we have examined here the distribution and expression levels of several nuclear proteins during the response of HeLa S3 cells to mild and severe hyperthermia. This cell line was chosen because it had been extensively used in previous studies related to cellular stress response [see for instances: Reiter and Penman, 1983; Bond, 1988; Dynlacht et al., 1999; Zhu et al., 1999; Biamonti, 2004; Valgardsdottir et al., 2005, 2008]. Components of the nuclear envelope such as LAP2β, emerin and lamins were selected as markers to investigate changes in organization and/or composition occurring at the nuclear periphery throughout the response to either mild or severe hyperthermia. Likewise, nuclear interior components were selected as markers to assess changes within specific nucleoplasmic domains. These included fibrillarin [Ochs and Smetana, 1991], as well as the 2H12, 2A7, and 1B4 antigens previously characterized in our laboratory [Paulin-Levasseur et al., 1995; Luus and Paulin-Levasseur, 1997; Paulin-Levasseur and Julien, 1999]. Our results underline the complexity of nuclear function/structure relationships and the needs for a better understanding of protein–protein interactions within the nuclear compartment.

MATERIALS AND METHODS

TISSUE CULTURE

HeLa S3 cells were obtained from the American Type Culture Collection (ATCC: CCL2.2; Manassas, VA). Cells were cultured in α-modified Eagle's minimal essential medium (α-MEM; GIBCO BRL, Burlington, ON, Canada) supplemented with 10% fetal calf serum (FCS; GIBCO BRL), and 1% antibiotics (50 U/ml penicillin and 50 μg/ml streptomycin; GIBCO BRL).

HYPERTHERMIA TREATMENTS AND VIABILITY TESTS

Forty-eight hours after seeding, HeLa S3 cells were subjected to either mild (42°C) or severe (45°C) heat-shock treatments for 90 min using a Haake Circulator (Fisher Scientific Limited, Hampton, NH). Prior to heat shock, the old medium was replaced by new medium pre-warmed at 37°C. Viability of control and heat-shocked cells was assayed using Trypan blue exclusion [Celis, 1994]. This series of tests was also performed on cells allowed to recover from heat-shock treatments at 37°C for different time periods. In all cases, cells suspended in the cultured medium and cells attached to the dishes were harvested together and used in the counts.

PI STAINING AND FLOW CYTOMETRY

Control and treated cells were collected and assayed using Trypan blue exclusion as described above. Cells were then washed twice with phosphate buffer saline (PBS; 1.3 mM NaCl, 50 mM Na_2HPO_4, 15 mM KH_2PO_4, pH 7.0) and re-suspended in 500 μl of this buffer. Cells were fixed in 5 ml of cold ethanol and stored in fixative. When needed, cells were collected by low speed centrifugation and re-suspended in 800 μl of PBS containing 1% bovine serum albumin (BSA; BDH, Mississauga, ON, Canada). To each sample, 100 μl of a

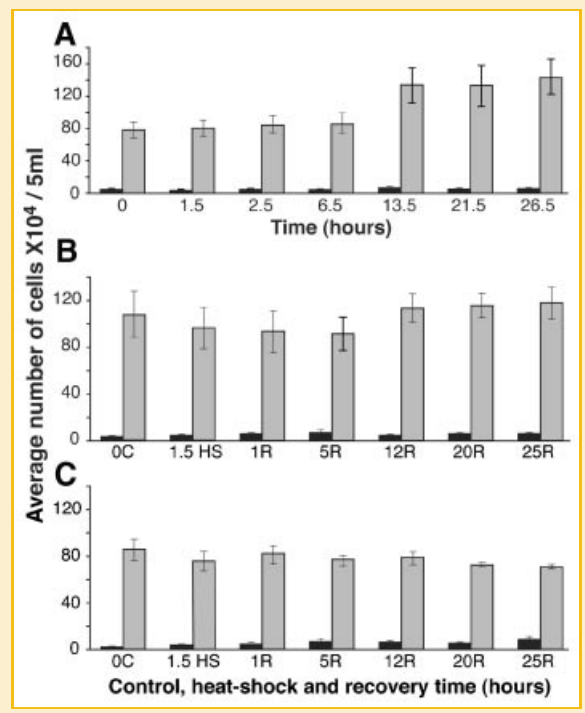

Fig. 1. Histograms depicting the viability and growth of control and heat-shocked HeLa S3 cell populations. Cells were cultured under standard conditions until they reached 60% confluence. Their viability and growth rate were then monitored over a period of 26.5 h. The behavior of control cell populations is shown in panel A. The behavior of cell populations (0C) which were subjected to either mild (42°C) or severe (45°C) hyperthermia for 90 min (1.5HS) and allowed to recover at 37°C for 1–25 h (1R–25R) is illustrated in panels B and C, respectively. Black bars represent dead cell counts whereas gray bars stand for live cell counts. The data for each time point are the mean of three separate experiments, comprising six counts each. Error bars show the standard error from the mean.

10X propidium iodide (PI) solution (500 μg/ml in 3.8×10^{-2} M sodium citrate, pH 7.0) were added. Boiled RNase A (10 mg/ml prepared in 10 mM Tris–HCl, pH 7.5) (RNase A; Sigma, St Louis, MO) was added at a quantity of 100 μl/sample. Cells were incubated at 37°C for 30 min prior to analysis. Cell cycle distribution was monitored using a LSR flow cytometer (Becton Dickinson, Franklin Lakes, NJ). For DNA analysis, a 488 nm Argon laser and a F12 detector equipped with 575/26 BP filter was used. For acquisition of analysis, CellQuest software was used.

LABELING WITH 5-BROMO-2′-DEOXY-URIDINE

5-Bromo-2′-deoxy-uridine (BrdU) was purchased from Roche Diagnostic, Inc. (Indianapolis, IN) and labeling of the cells was performed according the instructions provided by the manufacturer. BrdU was added directly to the medium of cell populations at a final concentration of 10 μM. For detection, cells were incubated with an anti-BrdU (Roche Diagnostic, Inc.) at a 1:50 dilution (for 30 min at room temperature) and then with a FITC-conjugated polyclonal goat anti-mouse IgG (Jackson ImmunoResearch, West Grove, PA) at a 1:150 dilution (for 30 min in the dark).

GEL ELECTROPHORESIS AND WESTERN BLOTTING

For sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), HeLa S3 cells were solubilized in SDS-sample buffer 2X (2XSB) [Laemmli, 1970]: 1×10^6 cells were solubilized in 250 μl of 2XSB. For protein analysis, HeLa S3 samples remained undiluted (2.4×10^4 cells per well) or were diluted further 1:5 (0.48×10^4 cells per well) in 1X SB. Separated polypeptides were electrophoretically transferred from gels onto nitrocellulose membranes. For Western blotting, nitrocellulose membranes were incubated with specific antibodies as recommended by Amersham Biosciences (Baie D'Urfé Québec, Canada). Immunoblots were developed using the chemi-luminescence reagents (Amersham Biosciences) and the reactivity was either visualized on hyperfilm ECL (Amersham Biosciences) or quantified by scanning laser densitometry.

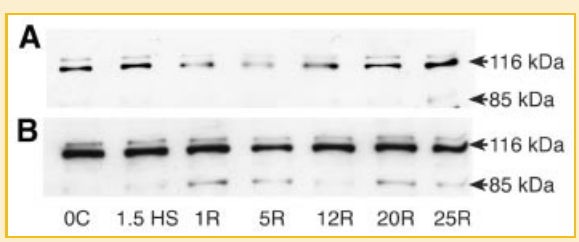

Fig. 2. Immunodection of PARP following mild (A) or severe (B) heat-shock treatment of HeLa S3 cells. Whole cell homogenates were prepared in 2X sample buffer, separated by SDS–PAGE, transferred onto nitrocellulose and then blotted with the PARP antibody. Chemiluminescence detection on radio-grams is shown here. The PARP antibody reacted with a protein species with a relative mobility of 116 kDa in all cell samples. The antibody also recognized a minor protein species with a relative mobility of 85 kDa in cell samples exposed to mild heat shock and allowed to recover for 25 h (A), as well as in samples subjected to and recovering from a severe heat shock (B).

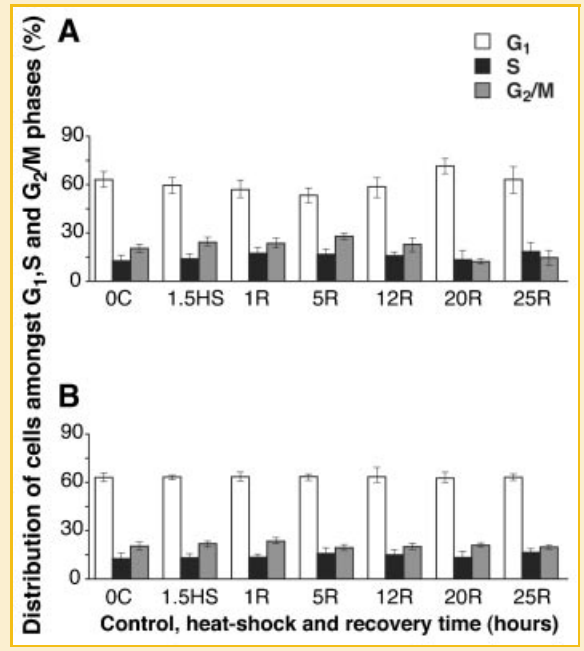

Fig. 3. Histograms depicting the cell cycle distribution of control and heat-shocked HeLa S3 cell populations. Control cell populations (0C) were subjected to either mild (A) or severe (B) hyperthermia for 90 min (1.5HS) and allowed to recover at 37°C for 1–25 h (1R–25R). For all experiments, cells were collected and counted using Trypan blue exclusion. They were then fixed with pure ethanol and processed for flow cytometry analysis of cell cycle distribution as described in "Materials and Methods" Section. White bars represent cells in G1 phase; black bars are for cell populations in S phase; and gray bars represent cells in G2 and M phases. The data for each time point are the mean of three separate experiments. Error bars show the standard error from the mean.

DENSITOMETRIC ANALYSIS

Scanning laser densitometry (CHEMI DOC™ Documentation System, BIO-RAD, Segrate, Milan, Italy) and image analysis (Quantity One quantitation software, Windows/McIntosh) were used to quantify protein expression on immunoblots reacted with specific probes. For each antigen, the reactivity of four independent series of samples was quantified by densitometry. Readings were always done on protein bands below the saturation level of the system. The values were then imported into Systat 10 for statistical analysis. Data for Residuals were analyzed with a general linear model and Tukey test for multiple comparisons. All tests were assessed at the 5% significance level.

TABLE I. Percentages (%) of Cells That Incorporated BrdU Amongst HeLa S3 Populations Subjected to Mild or Severe Heat-Shock Treatments

Time	Mild heat-shock (% of cells)	Severe heat-shock (% of cells)
0C	11.49	9.35
HS	1.09	0.16
1R	1.01	0.3
25R	1.29	1.0

The data for one experiment are summarized here. 0C, control cells; HS, cells subjected to hyperthermia for 90 min; 1R, cells allowed to recover at 37°C for 1 h; 25R, cells allowed to recover at 37°C for 25 h.

IMMUNOFLUORESCENCE AND NUCLEAR MATRIX PREPARATIONS

HeLa S3 cells were grown on sterile glass coverslips and heat-shocked as described above. Cells were then fixed, permeabilized and immunolabeled as previously described [Chaly et al., 1984]. Nuclear matrices were prepared and processed for immunofluorescence (IF) as described by Chaly et al. [1985].

FLUORESCENCE MICROSCOPY

Conventional epifluorescence was carried out on a Zeiss Axiophot microscope. Images were recorded on Ilford 400 ASA B&W film and scanned with a CanoScan 4000 US scanner (Canon, Mississauga, ON, Canada).

ANTIBODIES

The following primary antibodies were used against nuclear envelope antigens: anti-LAP2β, a mouse monoclonal IgG1 (clone 27) kindly provided by Dr. Roberto Campos (Transduction Laboratories, Lexington, KY) and used at 1:5,000 for Western blot (WB); anti-emerin, a mouse monoclonal IgG1 (clone 4G5) (Novocastra, Newcastle, UK) used at a dilution of 1:100 for WB.

The following primary antibodies were used against nuclear matrix proteins: anti-human lamin B, a mouse monoclonal IgG1 (clone 101-B7) (Matritech, Inc., Cambridge, UK) and used at 1:200 for IF or WB; anti-2A7, a mouse monoclonal IgG1 [Paulin-Levasseur and Julien, 1999] and used at 1:50 for IF or 1:20 for WB; anti-2H12, a mouse monoclonal IgG1 [Paulin-Levasseur et al., 1995] and used

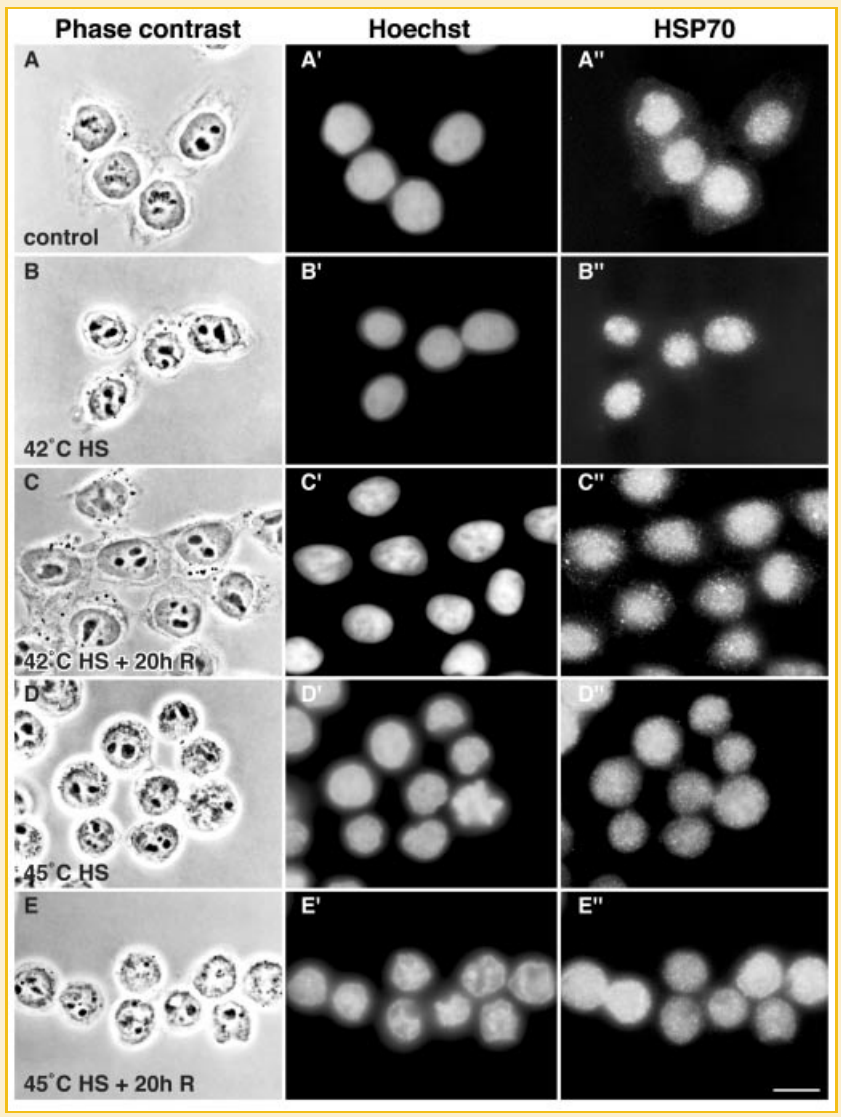

Fig. 4. Immunolocalization of HSP70 proteins following mild or severe heat-shock treatment of HeLa S3 cells. Cells were grown and subjected to experimental treatment on coverslips. They were fixed with 3% paraformaldehyde, permeabilized with Triton X-100 and labeled with the HSP70 antibody (A″–E″). They were also counterstained with Hoechst 33258 (A′–E′) and observed by phase-contrast microscopy (A–E). Monitored cell populations included: control HeLa S3 cells (A–A″); cells subjected to mild hyperthermia (42°C) for 90 min (B–B″); cells subjected to mild hyperthermia which had recovered at 37°C for 20 h (C–C″); cells subjected to severe hyperthermia (45°C) for 90 min (D–D″); and cells subjected to severe hyperthermia which had recovered at 37°C for 20 h (E–E″). Note the altered morphology of severely stressed cells (D and E). Bar, 10 μm.

at 1:20 for IF and pure for WB; anti-fibrillarin, a mouse monoclonal (provided by Dr. Krohne, University of Würzburg, Germany) and used at 1:25 for IF; anti-1B4, a mouse monoclonal IgE [Luus and Paulin-Levasseur, 1997] and used pure for IF.

The following primary antibody was used as a marker for hyperthermia: anti-Hsp70, a rabbit polyclonal (StressGen Biotechnologies Corp., Victoria, BC, Canada) and used at 1:2,000 for IF or 1:20,000 for WB. A mouse monoclonal IgG_{2a} against "proliferative cell nuclear antigen" (PCNA; Santa Cruz Biotechnologies, CA) was used 1:200 for WB.

The following CY3-conjugated secondary antibodies were used for IF: polyclonal donkey anti-mouse IgG (Jackson ImmunoResearch) used at 1:400; polyclonal donkey anti-rabbit IgG (Jackson ImmunoResearch) used at 1:400.

The following biotinylated secondary antibodies were used for WB: polyclonal sheep anti-mouse Ig (Amersham Biosciences) used at 1:5,000; polyclonal donkey anti-rabbit used at 1:5,000.

RESULTS

VIABILITY, GROWTH AND CELL CYCLE DISTRIBUTION OF HeLa S3 CELLS

To evaluate the impact of hyperthermia on the survival of HeLa S3 cells, two sets of experiments were performed: (1) the cell viability and growth were monitored; and (2) the distribution of HeLa S3 populations throughout the cell cycle was examined. Observed cell populations included: (a) untreated (control) HeLa S3 cells; (b) HeLa S3 cells heat-shocked at 42°C (mild) or 45°C (severe) for 90 min; and (c) heat-shocked cells that have been recovering at 37°C for 1, 5, 12, 20, and 25 h.

The viability and growth rate of control HeLa S3 populations were first monitored at different times after an initial 48-h period of culture (allowing cells to reach approximately 60% confluence, considered thereafter as control cultures at time 0), using Trypan blue exclusion. As depicted in Figure 1A, control cultures reached full confluence after 26.5 h when the number of cells had increased by a factor of 1.84. Over this period, the percentage of dead cells amongst the cultures did not increase significantly and remained low.

The effects of hyperthermia on cell viability and growth over an equivalent period, that is, 90 min of heat-shock stress followed by 25 h of recovery, are shown in Figure 1B,C. Whether mild (42°C) or severe (45°C), the heat-shock stress did not compromise HeLa S3 cell viability under our experimental conditions. However, in both cases, it appeared to inhibit the proliferation of treated populations for the whole experimental period including recovery. Statistical analysis of the behavior of both mild and severe stressed populations did not reveal any significant change in the numbers of either live or dead cells throughout the experimental period. The possibility that cells would massively undergo apoptosis following mild or severe hyperthermia appeared highly unlikely based on the following data. The proteolytic cleavage of the poly(ADP-ribose) polymerase (PARP), a 116 kDa protein, into two fragments of 85 and 26 kDa has previously been recognized as an indicator of apoptosis [Lamarre et al., 1988]. Using a specific antibody that reacts with both the full length and the larger (85 kDa) proteolytic fragment of the

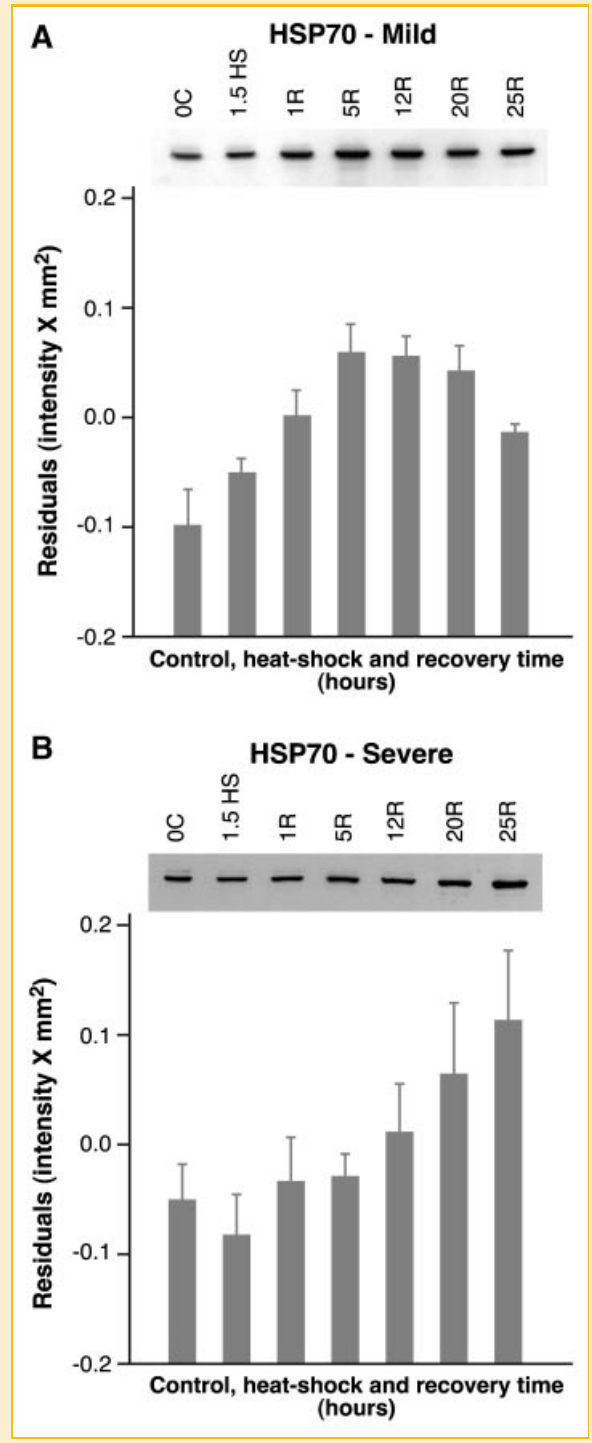

Fig. 5. Immunodetection of HSP70 proteins on radiograms and histograms depicting heat-induced quantitative changes in the expression levels of these proteins in control and heat-shocked HeLa S3 cell populations. Control cell populations (0C) were subjected to either mild (A) or severe hyperthermia (B) for 90 min (1.5HS) and allowed to recover at 37°C for 1–25 h (1R–25R). For all experiments, cells were processed for SDS–PAGE and Western blotting as described in "Materials and Methods" Section. Antibody reactivity was quantified by scanning laser densitometry on protein blots developed using chemiluminescence reagents. Four independent series of samples were quantified by densitometry. The data for each treatment is the mean of Residuals ($n = 4$). Error bars show the standard error from the mean. $^{*}P < 0.05$, Tukey.

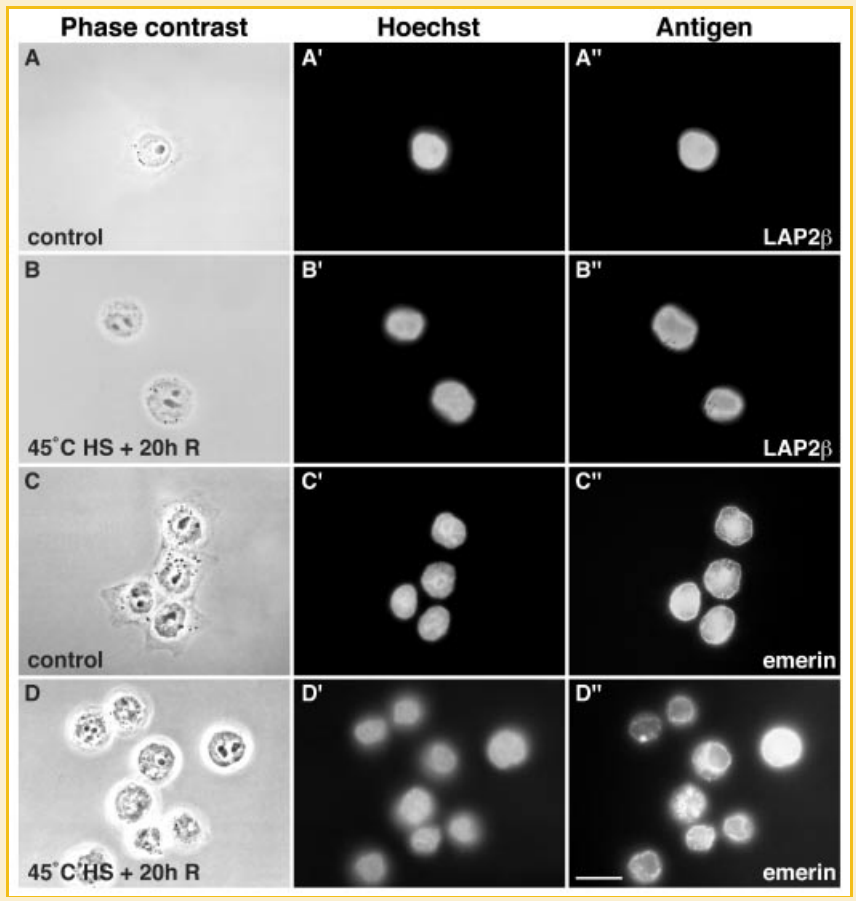

Fig. 6. Immunolocalization of nuclear envelope proteins following severe heat-shock treatment of HeLa S3 cells. Cells were grown and subjected to experimental treatment on coverslips. They were fixed with 3% paraformaldehyde, permeabilized with Triton X-100 and immunostained with the LAP2β antibody (A″ and B″) or the emerin antibody (C″ and D″). They were also counterstained with Hoechst 33258 (A′–D′) and observed by phase-contrast microscopy (A–D). Monitored cell populations included: control HeLa S3 cells (A–A″ and C–C″); and cells subjected to severe hyperthermia (45°C) for 90 min which had recovered at 37°C for 20 h (B–B″ and D–D″). Bar, 10 μm.

PARP, the occurrence of apoptosis within the control and treated cell populations was assayed by immunoblotting. As shown in Figure 2, a protein band with a relative mobility of 116 kDa, characteristic of the intact PARP, was detected in all samples. A second protein band of 85 kDa, which corresponds to the larger cleavage product of PARP, was also seen in samples from cells exposed to mild treatment after 25 h of recovery (Fig. 2A) as well as cells subjected to and recovering from a severe heat shock (Fig. 2B). Nevertheless, the intensity of the 85 kDa band was relatively low in all samples and did not increase noticeably throughout the time course of treatments.

Considering the above data, it appeared relevant to examine whether the cell cycle distribution of HeLa S3 cells was affected by the heat shock. Flow cytometry was therefore performed on control and treated cells labeled with the fluorescent DNA dye PI. The distribution profiles of PI fluorescence obtained are depicted in Figure 3. Surprisingly, the profiles generated from populations that had been exposed to either mild (Fig. 3A) or severe (Fig. 3B) hyperthermia for 90 min and allowed to recover over a period of 25 h remained closely similar to each other as well as to that of control populations (0C). Typically, 50–60% of the cells were transiting through G1 phase while 10–20% were undergoing S phase and 15–25% were progressing through G2/M phases. Statistical analysis of the profiles from treated populations relative to controls or amongst themselves did not reveal significant differences except in the following cases: (1) relative to the control, the percentage of cells found in G2/M phases after 20 and 25 h of recovery from mild hyperthermia was significantly lower ($P < 0.031$); and (2) compared to each other, the percentage of cells found in G2/M phases after either 5 or 20 h of recovery from mild hyperthermia was significantly different ($P < 0.036$). Interestingly, the incorporation of BrdU amongst treated populations was drastically reduced in comparison to controls (Table I), indicating that replication was impaired upon heat-shock stress. Nevertheless, cellular levels of PCNA in stressed versus control cells did not differ significantly (data not shown).

EFFECTS OF HYPERTHERMIA ON THE LOCALIZATION AND EXPRESSION LEVELS OF Hsp70 IN HeLa S3 CELLS

To further validate our experimental system, the effects of mild and severe hyperthermia on the localization and expression levels of the

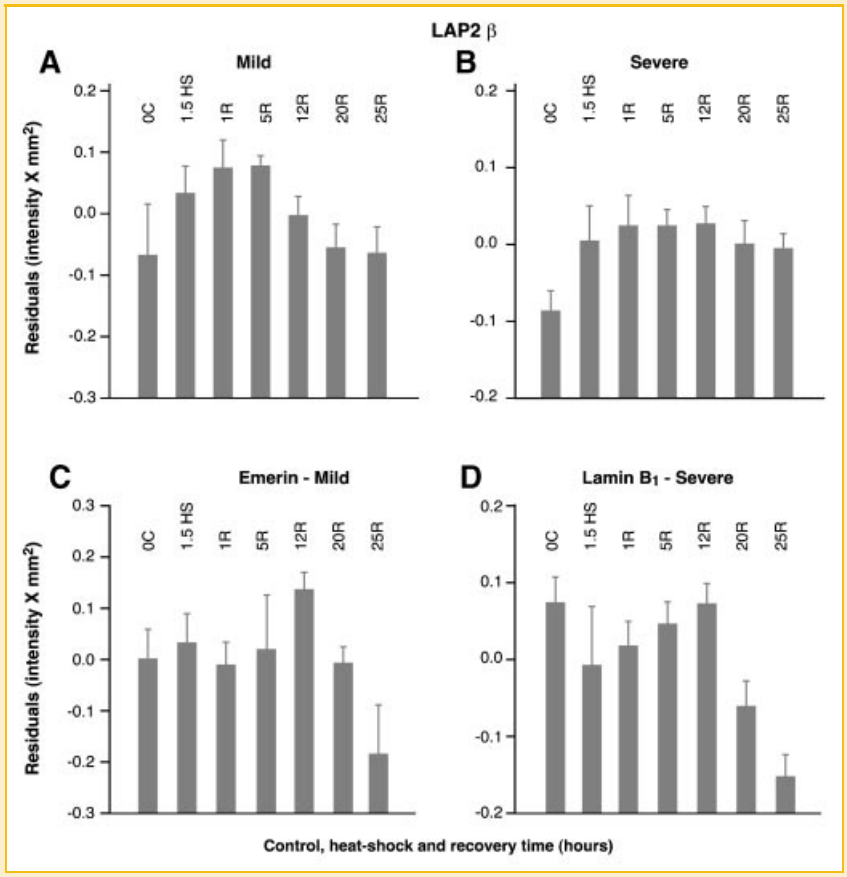

Fig. 7. Histograms depicting heat-induced quantitative changes in the expression levels of nuclear envelope proteins in control and heat-shocked HeLa S3 cell populations. Control cell populations (0C) were subjected to either mild (A and C) or severe hyperthermia (B and D) for 90 min (1.5HS) and allowed to recover at 37°C for 1 to 25 h (1R–25R). Histograms are shown for LAP2β (A and B), emerin (C) and lamin B1 (D). For all experiments, cells were processed for SDS–PAGE and Western blotting as described in "Materials and Methods" Section. Antibody reactivity was quantified by scanning laser densitometry on protein blots developed using chemiluminescence reagents. For each antigen, four independent series of samples were quantified by densitometry. The data for each treatment is the mean of Residuals (n = 4). Error bars show the standard error from the mean. $^{*}P < 0.05$, Tukey.

heat-shock protein (Hsp) 70 in HeLa S3 cells were examined by IF microscopy and quantitative immunoblotting analysis.

In control cells (Fig. 4A–A″), Hsp70 was distributed diffusely in the nucleus, including nucleoli, and could be found at lower levels throughout the cytoplasm. After exposure to a mild heat shock at 42°C for 90 min (Fig. 4B–B″), the localization of the protein became exclusively nuclear with more prominent staining of nucleoli. The general morphology of the cells was maintained upon these conditions. Early during recovery of these cells at 37°C, Hsp70 remained exclusively nuclear but its presence in nucleoli was less prominent (data not shown). As depicted in Figure 4C–C″, the protein had leaked back into the cytoplasm after 25 h of recovery.

In contrast, no nuclear mobilization of Hsp70 was observed in cells subjected to a severe heat shock at 45°C for 90 min (Fig. 4D–D″). The protein also maintained the same distribution throughout the whole recovery period (Fig. 4E–E″). Nevertheless, the morphology of these severely stressed cells was altered (compare Fig. 4A–C with Fig. 4D,E). The overall cell shape was rounder and the volume ratio of nucleus to cytoplasm was increased after severe heat shock.

Figure 5 summarizes the quantitative changes in the expression levels of Hsp70 during the response of HeLa S3 cells to mild or severe hyperthermia, as determined by densitometric analysis of immunoblots. There was an overall highly significant increase ($P < 0.0001$) in the expression of Hsp70 when cells were subjected to mild hyperthermia (Fig. 5A). The increase reached its maximum at 5 h post-heat shock. The expression then started to decrease slowly but remained significantly higher than in untreated cells. However, the trend was different for cells exposed to severe hyperthermia (Fig. 5B). Hsp70 expression appeared to decrease slightly after shock, although the difference could not be considered as statistically significant. The cellular content of these proteins then increased steadily during recovery to reach maximum expression at 25 h post-heat shock ($P < 0.033$, when compared to cells immediately after treatment).

EFFECTS OF HYPERTHERMIA ON THE LOCALIZATION AND EXPRESSION OF NUCLEAR ENVELOPE PROTEINS

To investigate the effects of hyperthermia on nuclear envelope structure, the behavior of two integral proteins of the INM, LAP2β

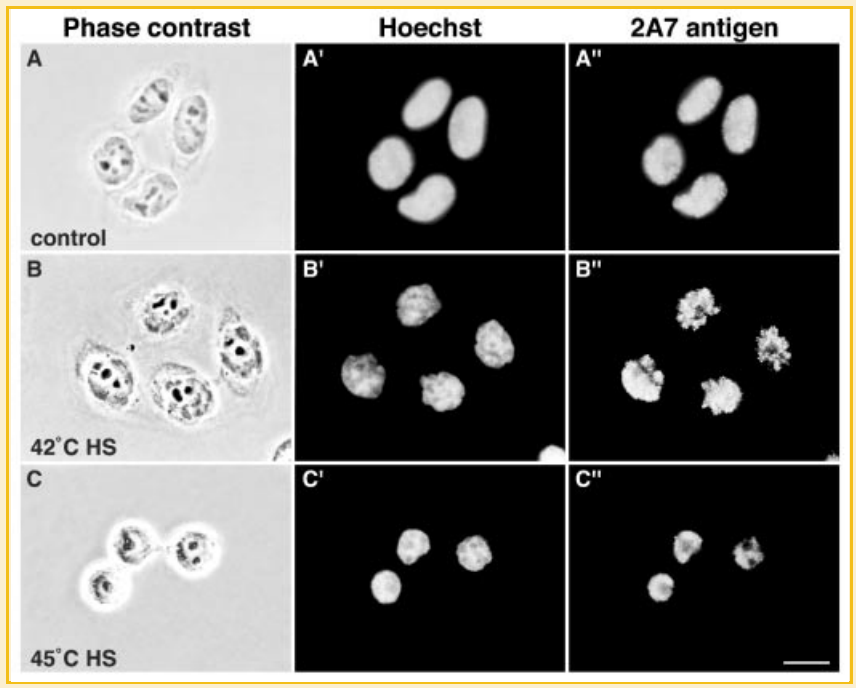

Fig. 8. Immunolocalization of nuclear interior protein 2A7 following mild or severe heat-shock treatment of HeLa S3 cells. Cells were grown and subjected to experimental treatment on coverslips. They were fixed with 3% paraformaldehyde, permeabilized with Triton X-100 and immunostained with the 2A7 antibody (A″–C″). They were also counterstained with Hoechst 33258 (A′–C′) and observed by phase-contrast microscopy (A–C). Monitored cell populations included: control HeLa S3 cells (A–A″); cells subjected to mild hyperthermia (42°C) for 90 min (B–B″); and cells subjected to severe hyperthermia (45°C) for 90 min (C–C″). Note the flower-shaped nuclei counterstained with Hoechst in figure B′. Bar, 10 μm.

and emerin, as well as the major constituents of the nuclear lamina in HeLa S3 cells, lamins A/C and lamin B_1, was monitored immediately after heat shock and over the time course of recovery. By IF, these proteins maintained their localization at the nuclear periphery upon mild or severe heat-shock treatment, as illustrated for LAP2β (Fig. 6A–B″). The only indication that hyperthermia might perturb the organization of nuclear envelope constituents was obtained on cells labeled for emerin following recovery from a severe treatment. In a large proportion of this cell population, the retention of emerin within the ER appeared to be increased relative to control samples (Fig. 6C–D″).

Further assessment of the impact of hyperthermia on nuclear envelope proteins was performed by quantitative densitometric analysis of immunoblots from total cellular homogenates of control and treated HeLa S3 populations. In all cases, although to different extents, changes in expression levels of these proteins upon stress and during subsequent recovery were detected. For instance, as illustrated in Figure 7, the expression level of LAP2β was increased after a 90-min exposure to either mild or severe hyperthermia. The cellular content in this protein resumed control levels late during recovery from mild treatment (Fig. 7A) but remained high following recovery from severe treatment (Fig. 7B). Statistical analysis of the data on LAP2β indicated that there were a significant difference amongst samples examined after mild treatment ($P < 0.049$) and a tendency to significance between severely treated samples ($P > 0.052$). However, the most striking effects of mild and severe treatments were monitored for emerin following recovery from mild hyperthermia (Fig. 7C) and for lamin B_1 following recovery from severe hyperthermia (Fig. 7D). The expression level of emerin appeared to increase early during the response to mild stress but then decreased abruptly and significantly ($P < 0.007$) after 25 h of recovery. In the case of lamin B_1, the expression level of proteins did not appear to be significantly affected upon the first 12 h of the response to severe stress but dropped drastically during late recovery, reaching highly significant differences when compared to control ($P < 0.002$) or 90 min-shocked samples ($P < 0.05$). Our analysis did not reveal any significant changes in the expression levels of emerin following a severe treatment nor in those of lamin B_1 after a mild treatment.

EFFECTS OF HYPERTHERMIA ON THE LOCALIZATION AND EXPRESSION OF NUCLEAR INTERIOR PROTEINS

The structural integrity of functional domains within the nuclear interior such as nucleoli and splicing speckles in cells responding to either mild or severe heat-shock stress was then examined by IF using specific antibodies.

As described previously (Paulin-Levasseur and Julien, 1999), the 2A7 antigen is a human nucleocytoplasmic shuttling protein that resides in the nucleoplasm and can be found also in nucleoli of interphase HeLa S3 cells (Fig. 8A–A″). Upon exposure of these cells to mild hyperthermia, the protein retained its nuclear location but had retracted towards the nuclear interior (Fig. 8B–B″). This rearrangement was particularly evident in treated cells

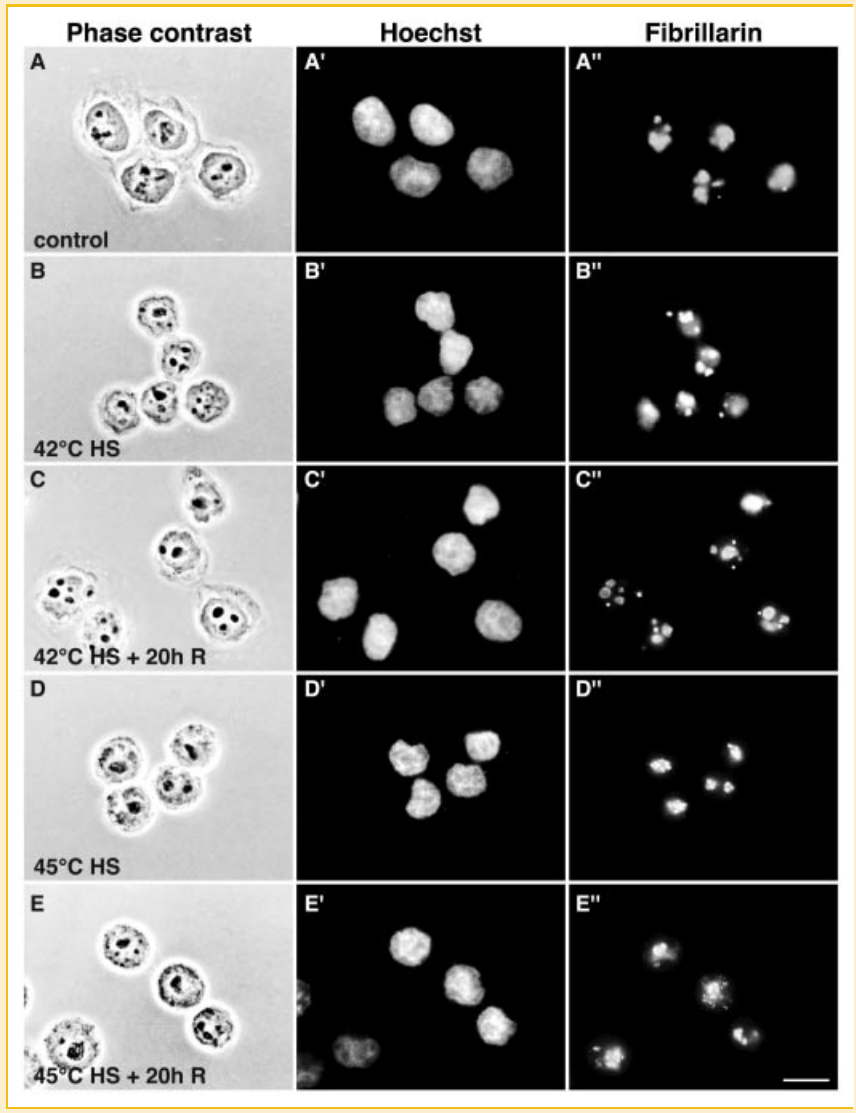

Fig. 9. Immunolocalization of nuclear interior protein fibrillarin following mild or severe heat-shock treatment of HeLa S3 cells. Cells were grown and subjected to experimental treatment on coverslips. They were fixed with 3% paraformaldehyde, permeabilized with Triton X-100 and immunostained with the fibrillarin antibody (A″–E″). They were also counterstained with Hoechst 33258 (A′–E′) and observed by phase-contrast microscopy (A–E). Monitored cell populations included: control HeLa S3 cells (A–A″); cells subjected to mild hyperthermia (42°C) for 90 min (B–B″); cells subjected to mild hyperthermia which had recovered at 37°C for 20 h (C–C″); cells subjected to severe hyperthermia (45°C) for 90 min (D–D″); and cells subjected to severe hyperthermia which had recovered at 37°C for 20 h (E–E″). Bar, 10 μm.

counterstained with Hoechst, in which the 2A7 nuclear staining appeared "flower-shaped" (Fig. 8B′ and B″), and it did not change over recovery (data not shown). The response was even more accentuated in cells that had been subjected to severe hyperthermia (Fig. 8C–C″). In these cells, the antigen appeared to have gained further inward compaction towards the nuclear interior and to be excluded from nucleoli. Again, the 2A7 reorganization persisted over the recovery period at 37°C (data not shown).

On the other hand, the 2H12 antigen and fibrillarin constitute two markers of the nucleolar compartment. The 2H12 antigen is a human specific phosphorylated variant of B23 [Paulin-Levasseur et al., 1995] which is located exclusively in nucleoli and may play a structural role in the organization of the nucleolar compartment. No change in the distribution of this antigen was observed, neither after a mild or a severe heat-shock stress (data not shown). Fibrillarin is well recognized for its role in ribosomal RNA processing [Ochs and Smetana, 1991] and is found in nucleoli and coiled bodies (also called Cajal bodies) of interphase HeLa S3 cells (Fig. 9A–A″). Throughout mild hyperthermia treatment and recovery, fibrillarin was retained within nucleoli but its inclusion within small nucleoplasmic corpuscles, presumably coiled bodies, became prominent (Fig. 9B–B″ and C–C″). A more drastic redistribution of this protein was observed following severe hyperthermia (Fig. 9D–D″). Immediately after shock, fibrillarin was arranged into small dot-like structures resembling "beads on a string" within the nucleoli (Fig. 9D″). These structures were still present after 20 h of recovery although fibrillarin was then also seen in the nucleoplasm (Fig. 9E–E″).

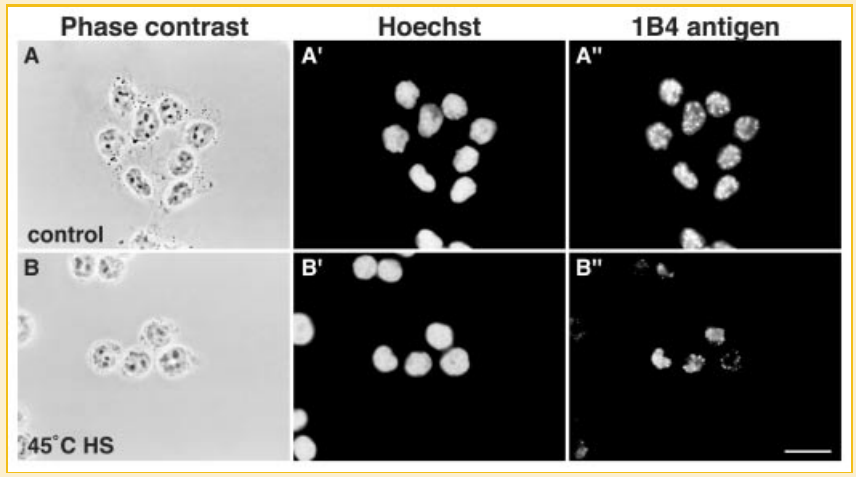

Fig. 10. Immunolocalization of nuclear interior protein 1B4 following mild or severe heat-shock treatment of HeLa S3 cells. Cells were grown and subjected to experimental treatment on coverslips. They were fixed with 3% paraformaldehyde, permeabilized with Triton X-100 and immunostained with the 1B4 antibody (A″–B″). They were also counterstained with Hoechst 33258 (A′–B′) and observed by phase-contrast microscopy (A–B). Monitored cell populations included: control HeLa S3 cells (A–A″) and cells subjected to severe hyperthermia (45°C) for 90 min (B–B″). Bar, 5 μm.

Finally, the 1B4 antigen which had been previously identified as a structural component of nuclear splicing speckles and shown to co-localize with SC35 proteins in HeLa S3 cells [Luus and Paulin-Levasseur, 1997] was used as a marker of nuclear speckles (Fig. 10A–A″). The distribution of these domains was not altered by a mild heat-shock treatment (data not shown) but appeared to undergo drastic changes in response to severe hyperthermia. As depicted in Figure 10B–B″, fewer numbers of nuclear speckles were detected following severe heat shock. Furthermore, the speckles were often displaced towards the periphery of the nucleus. This rearrangement was maintained over the recovery period (data not shown).

The expression levels of the 2A7 and 2H12 antigens in control versus mildly or severely stressed HeLa S3 populations were also analyzed by densitometry of immunoblots. No significant change in the cellular content of the 2A7 antigen was detected upon a mild heat-shock treatment but, as shown in Figure 11A, a severe heat-shock exposure resulted in a highly significant ($P < 0.0001$) reduction in the protein levels of this antigen. Our data indicate that the expression of the 2A7 antigen decreased steadily following a severe stress to reach its lowest level after 20 h of recovery. On the other hand, the mild heat-shock treatment did impact significantly on the cellular content of the 2H12 antigen after 25 h of recovery whereas the severe regimen did not. As illustrated in Figure 11B, the levels of 2H12 antigen decreased gradually but steadily following a mild stress ($P < 0.019$).

IMMUNOFLUORESCENCE MONITORING OF Hsp70, 2A7 AND FIBRILLARIN BEHAVIORS DURING IN SITU ISOLATION OF NUCLEAR MATRICES FROM HeLa S3 CELLS

The changes observed in the nuclear localization of some antigens in response to mild or severe stress prompted us to investigate their relationship to the nuclear matrix. This was achieved by monitoring the behavior of these proteins throughout the different steps of in situ isolation of nuclear matrices from control and heat-shocked HeLa S3 cells.

In the residual DNase 1/RNase A-digested nuclear matrices obtained from control HeLa S3 cells, Hsp70 maintained the same distribution as in intact cells (compare Fig. 12A–A″ with Fig. 4A–A″), indicating that this protein may be considered as a structural component of the nuclear interior. The protein was faintly detected in matrices prepared just after a mild stress (Fig. 12B–B′) but had regained association with this fraction following recovery (Fig. 12C–C′). In contrast, severe heat shock (Fig. 12D–D′) and subsequent recovery (Fig. 12E–E′) did not affect the association of Hsp70 proteins with nuclear matrices.

As reported previously [Paulin-Levasseur and Julien, 1999], the nucleoplasmic fraction of the 2A7 antigen in HeLa S3 was extracted during the first steps of in situ isolation of nuclear matrices, that is, permeabilization of intact cells with Triton X-100, severing of the chromatin with low concentrations of DNase I and treatment with a low-salt buffer. In contrast, the nucleolar fraction of the antigen resisted extraction throughout these steps and was further retained during treatment with high salts and digestion with high concentrations of DNase I, to be partially released only upon digestion with RNase A (Fig. 13A–A″), suggesting that this protein could be associated with the karyoskeletal framework of nucleoli through interactions with RNA moieties in control cells. Mild hyperthermia and subsequent recovery did not change the relationship of the 2A7 antigen to nuclear matrices (Fig. 13B–B′ and C–C′). Interestingly, the behavior of the 2A7 antigen was quite different upon severe hyperthermia and subsequent recovery: under these conditions, the nucleoplasmic fraction of the antigen was apparently not released by extraction any more (Fig. 13D–D′ and E–E′).

As shown in Figure 14A–A″, fibrillarin behaved as a structural component of nuclear matrices in control HeLa S3 cells, maintaining its localization within nucleolar remnants after extraction. The partitioning of this protein remained unchanged in cells subjected to mild stress (Fig. 14B–B′) and subsequent recovery (Fig. 14C–C′). However, since severe hyperthermia generated

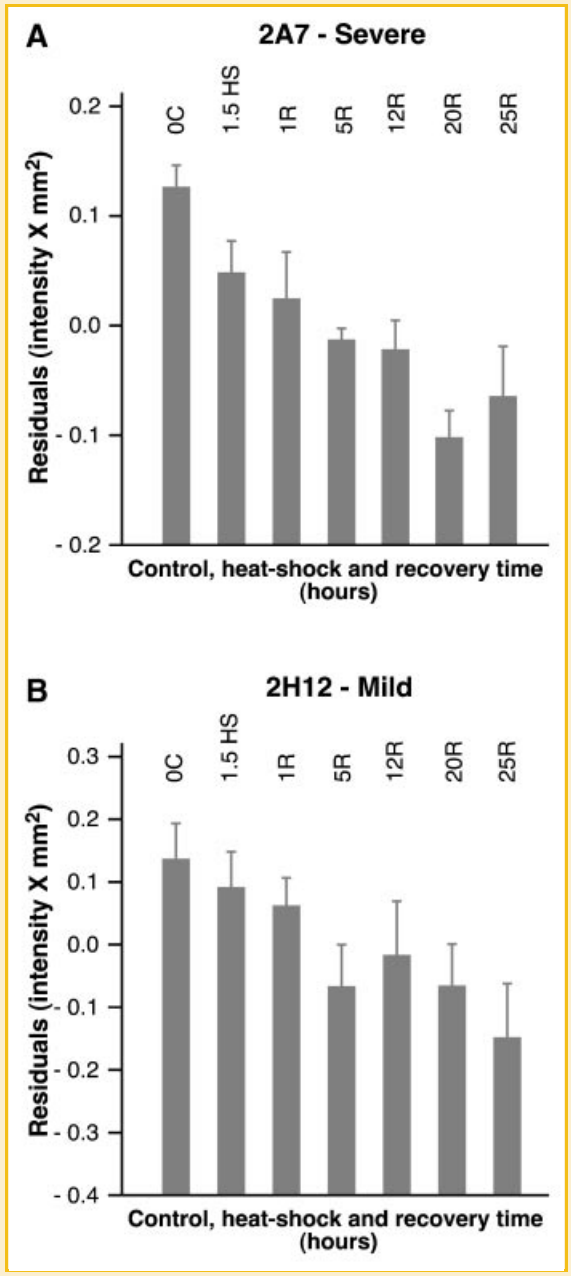

Fig. 11. Histograms depicting heat-induced quantitative changes in the expression levels of the nuclear interior proteins 2A7 and 2H12 in control and heat-shocked HeLa S3 cell populations. Control cell populations (0C) were subjected to either severe (A) or mild (B) hyperthermia for 90 min (1.5HS) followed by recovery at 37°C for 1–25 h (1R–25R). For all experiments, cells were processed for SDS–PAGE and Western blotting as described in "Materials and Methods" Section. Blots were probed with the 2A7 antibody (A) and the 2H12 antibody (B). Antibody reactivity was quantified by scanning laser densitometry on protein blots developed using chemiluminescence reagents. For each antigen, four independent series of samples were quantified by densitometry. The data for each treatment is the mean of Residuals ($n = 4$). Error bars show the standard error from the mean. $^{*}P < 0.05$, Tukey.

rearranged nucleoli with an appearance of "beads on a string," fibrillarin was found confined to such structures within nuclear matrices immediately after stress (Fig. 14D–D′) as well as following recovery (Fig. 14E–E′).

DISCUSSION

THE PROLIFERATION OF HeLa S3 CELL POPULATIONS IS INHIBITED IN RESPONSE TO BOTH MILD AND SEVERE HYPERTHERMIA

As shown in the present study, HeLa S3 cells survive an acute mild or severe heat-shock stress and remain fully viable throughout a 25-h recovery period. However, consistent with earlier reports [for reviews, see Kühl and Rensing, 2000; Park et al., 2005], the proliferation of treated cell populations was inhibited. The occurrence of apoptosis amongst these populations could not account for the proliferation arrest, as assessed by monitoring the proteolytic cleavage of PARP. Our data are in line with the suggestion that all cell cycle phases are delayed after hyperthermia while cells are mounting a protective response for survival [Read et al., 1984; Sugano et al., 1995; Fuse et al., 1996; Hut et al., 2005]. Our flow cytometric analysis demonstrates that stressed cells maintain a cell cycle distribution closely similar to control populations and failed to provide any evidence of a stage-specific block following hyperthermia.

In the present study, impairment of DNA replication amongst heat-shocked cells was indicated by a drastic decrease in their incorporation of BrdU even though their content in PCNA remained fairly comparable with controls over the experimentation period. These data suggest that the progression of replication forks might be affected by hyperthermia. An interesting proposal regarding the regulation of DNA replication after heat-shock stress has been put forward by Wang et al. [2001]. According to this proposal, the nucleolus might serve as a sensor of heat damage through the mobilization of nucleolar proteins into the nucleoplasm to compromise DNA replication activity by binding directly to the replication protein A (RPA). Such a scenario would be compatible with the transient assembly in HeLa cells of nuclear stress bodies that are located in the proximity of nucleoli following mild heat-shock exposure [Biamonti, 2004; Valgardsdottir et al., 2005, 2008]. It would also be consistent with our observations of rearrangements in the spatial distribution of nucleolar proteins following severe heat-shock stress in the present study.

EFFECTS OF HEAT SHOCK ON Hsp70

When cells encounter stress including hyperthermia, they mount active responses to resume homeostasis. Cell survival depends largely on the induced expression of a highly conserved set of polypeptides termed the classical HSPs [for a review, see De Maio, 1999]. The synthesis of HSPs is regulated transcriptionally [Li and Werb, 1982; Morimoto et al., 1990]. Our data on the distribution and expression of Hsp70 in HeLa S3 cells show that this protein behaves differently after a mild or severe heat-shock treatment. It is well recognized that multiple signaling pathways may contribute to build an efficient response to heat damage [Kühl and Rensing, 2000] and it appears possible that the stringency of the stress in HeLa S3 cells may be sensed via distinct mechanisms. Upon mild hyperthermia, the expression of Hsp70 was promptly up-regulated and the protein was rapidly recruited into the nuclear compartment, supporting the view that this chaperone may play specific functions within the nucleus to repair the heat-induced damage [Miyamoto et al., 2004]. In contrast, nuclear translocation of Hsp70 was not

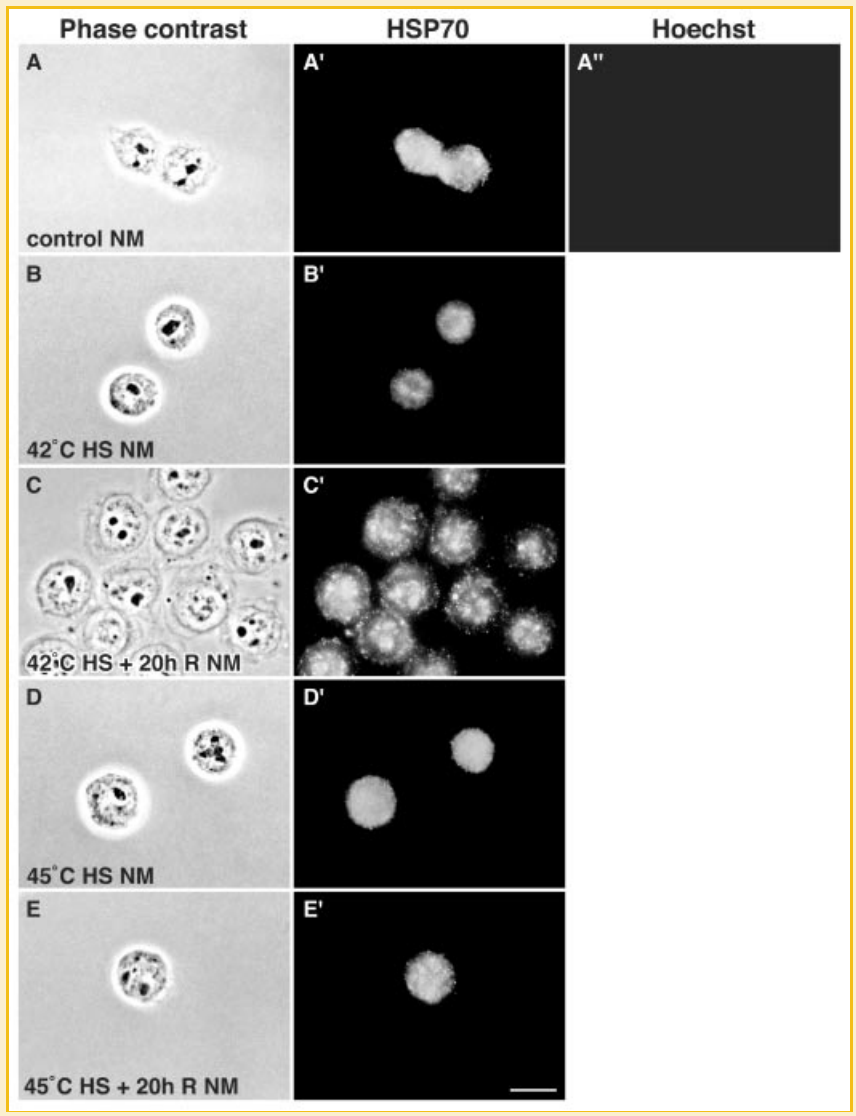

Fig. 12. Immunofluorescence monitoring of the behavior of the HSP70 proteins during in situ isolation of nuclear matrices (NM) from control and heat-shocked HeLa S3 cells. Cell samples were prepared as described in Chaly et al. (1985). Cells were labeled for IF with the HSP70 antibody (A′–E′), counterstained with Hoechst 33258 (only shown in A″), and observed by phase-contrast microscopy (A–E). Monitored cell populations included: control NM preparations digested with DNase I/RNase A (A–A″); NM preparations of cells subjected to a mild heat shock for 90 min at 42°C, and digested with DNase I/RNase A (B–B′); NM preparations of cells subjected to a mild heat shock followed by recovery at 37°C for 20 h, and digested with DNase I/RNase A (C–C′); NM preparations of cells subjected to a severe heat shock at 45°C for 90 min, and digested DNase I/RNase A (D–D′); and NM preparations of cells subjected to a severe heat shock followed by recovery at 37°C for 20 h, and digested with DNase I/RNase A (E–E′). Bar, 10 μm.

observed in cells subjected to severe hyperthermia and the expression of this polypeptide increased significantly only after 25 h of recovery at 37°C. In this case, the high stringency of the stress may call for a different response scenario.

EFFECTS OF HEAT SHOCK ON NUCLEAR PROTEINS

Our data first demonstrate that the INM proteins, LAP2β and emerin, as well as the major components of the nuclear lamina, lamins A/C and B_1, maintain an overall normal distribution at the nuclear periphery throughout the cell response to mild or severe hyperthermia. Nevertheless, increased retention of emerin in the ER was observed during late recovery from a severe heat shock. This change is most likely indicative of structural rearrangements at the NE. Indeed, our data provide evidence that several NE proteins undergo significant changes in their expression levels upon heat-shock stress. For instance, the cellular content of emerin was significantly decreased following 25 h of recovery from a mild heat shock and the expression of lamin B_1 was drastically reduced after 20–25 h of recovery from a severe stress. The results on lamin B_1 could not be predicted from previous studies as this protein had been reported to be significantly down-regulated following a 1 h mild stress at 43°C [Caizergues-Ferrer et al., 1984] but also to be promptly up-regulated upon a brief (40 min) severe heat-shock stress at 45.5°C [Dynlacht et al., 1999; Zhu et al., 1999]. Such discrepancies cannot be explained at present but may be due to differences in experimental conditions (temperatures, treatment times) and/or

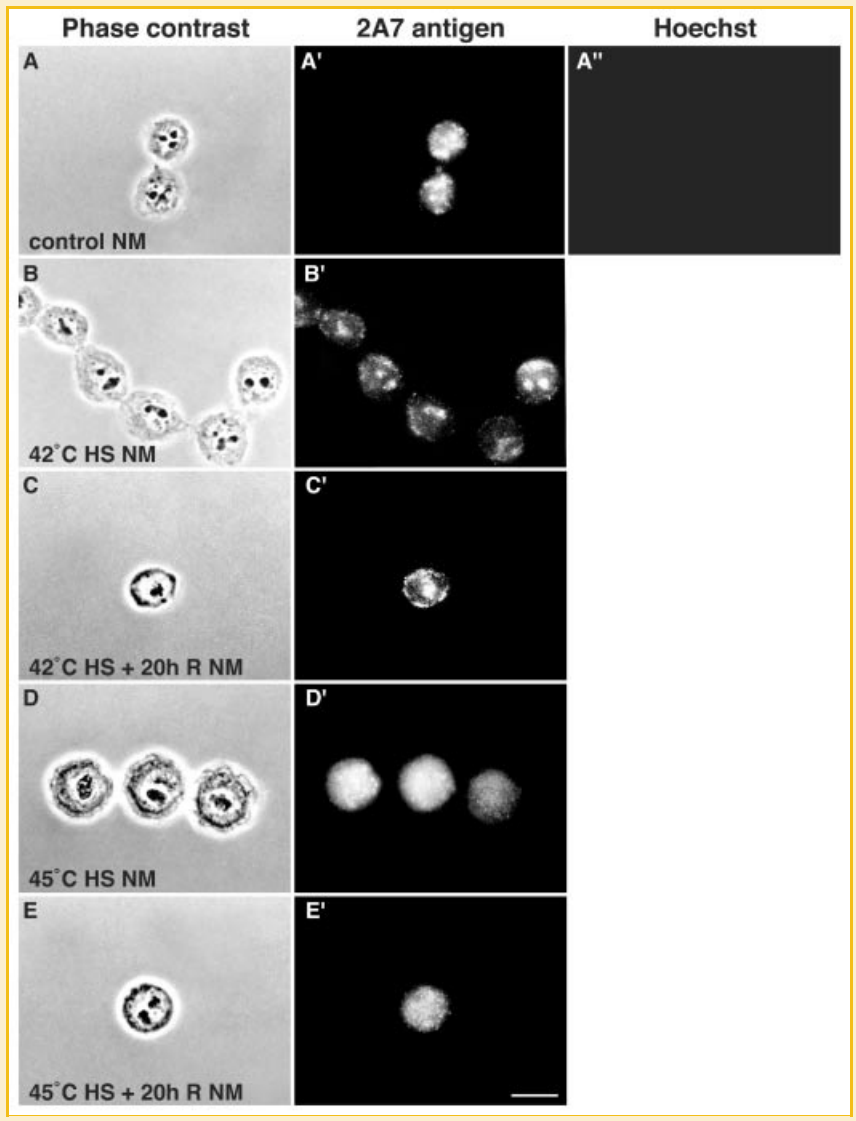

Fig. 13. Immunofluorescence monitoring of the behavior of the 2A7 antigen during in situ isolation of nuclear matrices (NM) from control and heat-shocked HeLa S3 cells. Cell samples were prepared as described in Chaly et al. (1985). Cells were labeled for IF with the 2A7 antibody (A′–E′), counterstained with Hoechst 33258 (only shown in A″), and observed by phase-contrast microscopy (A–E). Monitored cell populations included: control NM preparations digested with DNase I/RNase A (A–A″); NM preparations of cells subjected to a mild heat shock for 90 min at 42°C, and digested with DNase I/RNase A (B–B′); NM preparations of cells subjected to a mild heat shock followed by recovery at 37°C for 20 h, and digested with DNase I/RNase A (C–C′); NM preparations of cells subjected to a severe heat shock at 45°C for 90 min, and digested DNase I/RNase A (D–D′); and NM preparations of cells subjected to a severe heat shock followed by recovery at 37°C for 20 h, and digested with DNase I/RNase A (E–E′). Bar, 10 μm.

cellular systems. However, they do emphasize the need for standardization of stress conditions in enabling comparison of data between research laboratories. Nevertheless, our data put in question the classification of lamin B_1 as a "prompt heat shock" protein [Dynlacht et al., 1999; Zhu et al., 1999]. "Prompt heat shock" proteins would be synthesized rapidly upon exposure to hyperthermia: their heat-induced synthesis is not affected by the transcription inhibitor actinomycin D and, therefore, these proteins are translationally regulated [Reiter and Penman, 1983; Ornelles and Penman, 1990; Dynlacht et al., 1999; Zhu et al., 1999]. It is interesting to note that, in the present study, LAP2β was the only NE protein to show an overall increased expression during the stress response. The cellular content in LAP2β increased transiently following a mild treatment and regained levels similar to control samples at 20 h post-heat shock. A severe hyperthermia treatment resulted also in higher expression levels of this antigen but the increase was maintained afterward throughout the whole recovery period. Further investigation would be required to determine whether LAP2β could be considered as a "prompt heat shock" protein.

Our data provide also evidence that the organization of functional domains within the nuclear interior such as nucleoli and splicing speckles differs between cells responding to a mild or a severe stress. For instance, while the distribution of the nucleolar protein 2H12 was apparently maintained in both mildly- and severely stressed cells, the expression level of this antigen was reduced significantly

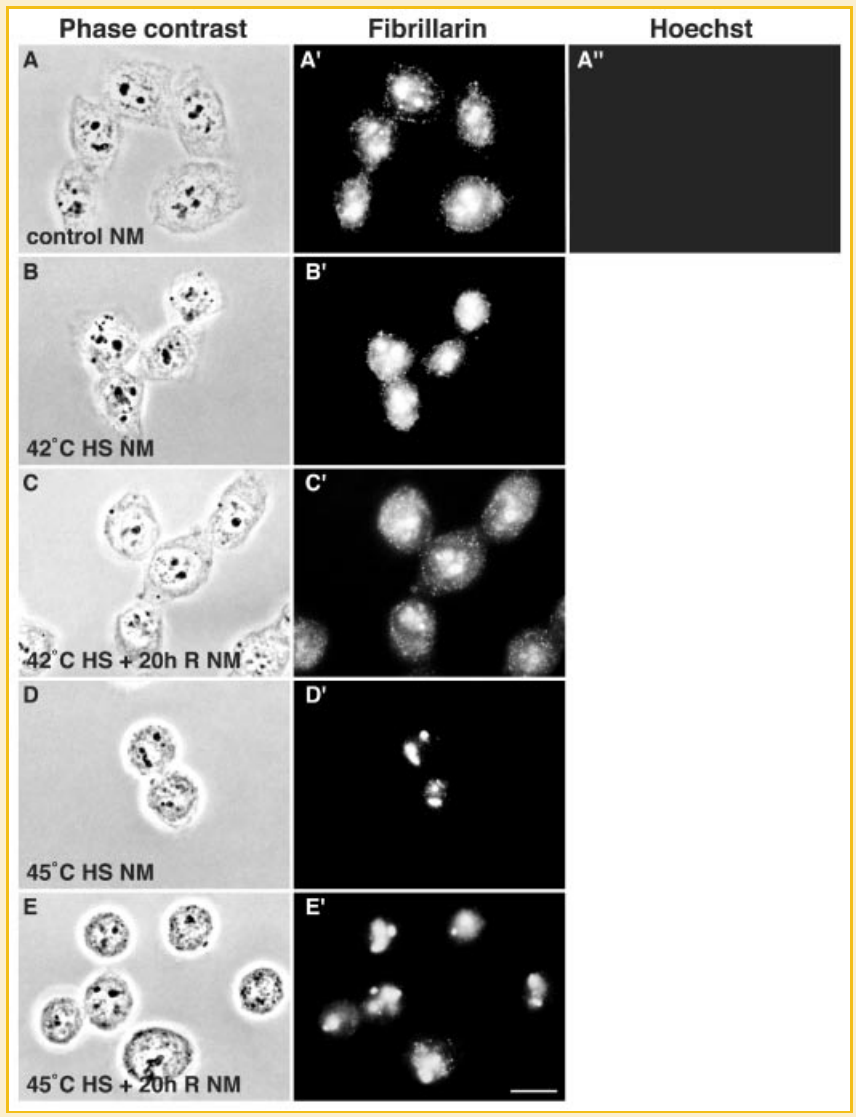

Fig. 14. Immunofluorescence monitoring of the behavior of fibrillarin during in situ isolation of nuclear matrices (NM) from control and heat-shocked HeLa S3 cells. Cell samples were prepared as described in Chaly et al. (1985). Cells were labeled for IF with the anti-fibrillarin antibody (A′–E′), counterstained with Hoechst 33258 (only shown in A″), and observed by phase-contrast microscopy (A–E). Monitored cell populations included: control NM preparations digested with DNase I/RNase A (A–A″); NM preparations of cells subjected to a mild heat shock for 90 min at 42°C, and digested with DNase I/RNase A (B–B′); NM preparations of cells subjected to a mild heat shock followed by recovery at 37°C for 20 h, and digested with DNase I/RNase A (C–C′); NM preparations of cells subjected to a severe heat shock at 45°C for 90 min, and digested DNase I/RNase A (D–D′); and NM preparations of cells subjected to a severe heat shock followed by recovery at 37°C for 20 h, and digested with DNase I/RNase A (E–E′). Bar, 10 μm.

following the mild heat shock but not upon the severe regimen. As well, the response of severely- versus mildly stressed cells was typically characterized by a more extensive reorganization of both the nucleocytoplasmic shuttling protein 2A7 and the nucleolar protein fibrillarin. The inward nuclear compaction of the 2A7 antigen following exposure to severe hyperthermia was accompanied by a highly significant reduction in its cellular content whereas no significant change in the expression level of this protein could be detected in mildly stressed cells. Analysis of fibrillarin content in control and heat-treated HeLa S3 cells remains to be assessed as the antibody used in this study did not react with the denatured protein.

All together, our results underline the complexity of the ''nuclear response'' to stress. It appeared therefore of interest to investigate the relationship of some antigens, amongst those monitored above, to the nuclear matrix under control conditions as well as upon hyperthermia treatment. Several studies have suggested that hyperthermia induces an increase in nuclear matrix-associated protein mass [see references therein Dynlacht et al., 1999]. However, controversy still persists on the artifactual versus functional nature of the phenomenon. The observations reported here on the behavior of Hsp70 proteins, 2A7 antigen and fibrillarin do not support the possibility of a non-specific, artifactual, aggregation of proteins within the nuclear matrix to increase protein mass upon heat induction. Considering these data, it becomes obvious that future research into protein–protein interactions prior to and following heat shock will be crucial to better understand the structure/function relationships prevailing in the nucleus.

REFERENCES

Benavente R, Krohne G. 1986. Involvement of nuclear lamins in postmitotic reorganization of chromatin as demonstrated by microinjection of lamin antibodies. J Cell Biol 103:1847–1854.

Berezney R. 1991. The nuclear matrix: A heuristic model for investigating genomic organization and function in the cell nucleus. J Cell Biochem 47:109–123.

Biamonti G. 2004. Nuclear stress bodies: A heterochromatin affair? Nat Rev Mol Cell Biol 5:493–498.

Bosman FT. 1999. The nuclear matrix in pathology. Virchows Arch 435:391–399.

Bond U. 1988. Heat shock but no other stress inducers leads to the disruption of a sub-set of snRNPs and inhibition of in vitro splicing in HeLa cells. EMBO J 7:3509–3518.

Cai M, Huang Y, Ghirlando R, Wilson KL, Craigie R, Clore GM. 2001. Solution structure of the constant region of nuclear envelope protein LAP2 reveals two LEM-domain structures: One binds BAF and the other binds DNA. EMBO J 20:4399–4407.

Caizergues-Ferrer M, Dousseau F, Gas N, Bouche G, Stevens B, Almaric F. 1984. Induction of new proteins in the nuclear matrix of CHO cells by a heat shock: Detection of a specific set in the nucleolar matrix. Biochem Biophys Res Commun 118:444–450.

Celis JE. 1994. Cell biology. A Laboratory Handbook. London, UK: Academic Press Limited. pp 387–393.

Chaly N, Bladon T, Setterfield G, Little JE, Kaplan JG, Brown DL. 1984. Changes in distribution of nuclear matrix antigens during the mitotic cycle. J Cell Biol 99:661–671.

Chaly N, Little J, Brown DL. 1985. Localization of nuclear antigens during preparation of nuclear matrices in situ. Can J Biochem Cell Biol 63:644–653.

Clements L, Manilal S, Love DR, Morris GE. 2000. Direct interaction between emerin and lamin A. Biochem Biophys Res Commun 267(3): 709–714.

Dechat T, Gotzmann J, Stockinger A, Harris CA, Talle MA, Siekierka JJ, Foisner R. 1998. Detergent-salt resistance of LAP2 alpha in interphase nuclei and phosphorylation-dependent association with chromosomes early in nuclear assembly implies functions in nuclear structure dynamics. EMBO J 17:4887–4902.

De Maio A. 1999. Heat shock proteins: Facts, thoughts and dreams. Shock 11:1–12.

Dynlacht JR, Story MD, Zhu W-G, Danner J. 1999. Lamin B is a prompt heat shock protein. J Cell Physiol 178:28–34.

Fairley EA, Kendrick-Jones J, Ellis JA. 1999. The Emery-Dreifuss muscular dystrophy phenotype arises from aberrant targeting and binding of emerin at the inner nuclear membrane. J Cell Sci 112:2571–2582.

Fisher DZ, Chaudary N, Blobel G. 1986. cDNA sequencing of nuclear lamins A and C reveals primary and secondary structural homology to intermediate filament proteins. Proc Natl Acad Sci USA 83:6450–6454.

Foisner R, Gerace L. 1993. Integral membrane proteins of the nuclear envelope interact with lamins and chromosomes, and binding is modulated by mitotic phosphorylation. Cell 73:1267–1279.

Furukawa K. 1999. LAP2 binding protein I (L2BP1/BAF) is a candidate mediator of LAP2-chromatin interaction. J Cell Sci 112:2485–2492.

Furukawa K, Fritze CE, Gerace L. 1998. The major nuclear envelope targeting domain of LAP2 coincides with its lamin binding region but is distinct from its chromatin interaction domain. J Biol Chem 273:4213–4219.

Furukawa K, Glass C, Kondo T. 1997. Characterization of the chromatin binding activity of lamina-associated polypeptide (LAP) 2. Biochem Biophys Res Commun 238(1): 240–246.

Furukawa K, Pante N, Aebi U, Gerace L. 1995. Cloning of a cDNA for lamina-associated polypeptide 2 (LAP2) and identification of regions that specify targeting to the nuclear envelope. EMBO J 14:1626–1636.

Fuse T, Yamada K, Asai K, Kato T, Nakanishi M. 1996. Heat shock-mediated cell cycle arrest is accompanied by induction of p21 CKI. Biochem Biophys Res Comm 225:759–763.

Gerace L, Burke B. 1988. Functional organization of the nuclear envelope. Annu Rev Cell Biol 4:335–374.

Gruenbaum Y, Wilson KL, Harel A, Goldberg M, Cohen M. 2000. Review: Nuclear lamins—Structural proteins with fundamental functions. J Struct Biol 129(2–3): 313–323.

Haraguchi T, Koujin T, Hayakawa T, Kaneda T, Tsutsumi C, Imamoto N, Akazawa C, Sukegawa J, Yoneda Y, Hiraoka Y. 2000. Live fluorescence imaging reveals early recruitment of emerin, LBR, RanBP2, and Nup153 to reforming functional nuclear envelopes. J Cell Sci 113:779–794.

Holaska JM, Lee KK, Kowalski AK, Wilson KL. 2003. Transcriptional repressor germ cell-less (GCL) and barrier to integration factor (BAF) compete for binding to emerin in vitro. J Biol Chem 278(9): 6969–6975.

Hut HMJ, Kampinga HH, Sibon OCM. 2005. Hsp70 protects mitotic cells against heat-induced centrosome damage and division abnormalities. Mol Biol Cell 16:3776–3785.

Kühl NM, Rensing L. 2000. Heat shock effects on cell cycle progression. Cell Mol Life Sci 57:450–461.

Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685.

Lamarre D, Talbot B, de Murcia G, Laplante C, Leduc Y, Mazen A, Poirier GG. 1988. Structural and functional analysis of poly(ADP ribose) polymerase: An immunological study. Biochim Biophys Acta 950(2): 147–160.

Lee KK, Haraguchi T, Lee RS, Koujin T, Hiroaka Y, Wilson KL. 2001. Distinct functional domains in emerin bind lamin A and DNA-bridging protein BAF. J Cell Sci 114:4567–4573.

Li GC, Werb Z. 1982. Correlation between synthesis of heat shock proteins and development of thermotolerance in Chinese hamster fibroblasts. Proc Natl Acad Sci USA 79:3218–3222.

Lin F, Blake DL, Callebaut I, Skerjanc IS, Holmer L, McBurney MW, Paulin-Levasseur M, Worman HJ. 2000. MAN1, an inner nuclear membrane protein that shares the LEM domain with lamina-associated polypeptide 2 and emerin. J Biol Chem 275:4840–4847.

Luus L, Paulin-Levasseur M. 1997. Characterization of the 1B4 antigen. Mol Biol Cell 8:217a.

Manilal S, Nguyen TM, Sewry CA, Morris GE. 1996. The Emery-Dreifuss muscular dystrophy protein, emerin, is a nuclear membrane protein. Hum Mol Genet 5:801–808.

Marshall WF, Fung JC, Sedat JW. 1997. Deconstructing the nucleus: Global architecture from local interactions. Curr Opin Genet Dev 7:259–263.

McKeon FD, Kirshner MW, Caput D. 1986. Homologies in both primary and secondary structure between nuclear envelope and intermediate filaments proteins. Nature 319:463–468.

Miyamoto Y, Saiwaki T, Yamashita J, Yasuda Y, Kotera I, Shibata S, Shigeta M, Hiraoka Y, Haraguchi T, Yoneda Y. 2004. Cellular stresses induce the nuclear accumulation of importin α and cause a conventional nuclear import block. J Cell Biol 165:617–623.

Morimoto RI, Tissieres A, Georgopoulos C, editors. 1990. The stress response, function of the proteins, and perspectives. In: Stress proteins in biology and medicine. New York: Cold Spring Harbor Laboratory Press. pp 1–36.

Nagano A, Koga R, Ogawa M, Kurano Y, Kawada J, Okada R, Hayashi YK, Tsukahara T, Arahata K. 1996. Emerin deficiency at the nuclear membrane in patients with Emery-Dreifuss muscular dystrophy. Nat Genet 12:254–259.

Nickerson JA. 1998. Nuclear dreams: The malignant alteration of nuclear architecture. J Cell Biochem 70:172–180.

Nili E, Cojocaru GS, Kalma Y, Ginsberg D, Copeland NG, Gilbert DJ, Jenkins NA, Berger R, Shaklai S, Amariglio N, Brok-Simoni F, Simon AJ, Rechavi G. 2001. Nuclear membrane protein LAP2beta mediates transcriptional repres-

sion alone and together with its binding partner GCL (germ-cell-less). J Cell Sci 114:3297–3307.

Ochs RL, Smetana K. 1991. Detection of fibrillarin in nucleolar remnants and the nucleolar matrix. Exp Cell Res 197:183–190.

Ornelles DA, Penman S. 1990. Prompt heat-shock and heat-shifted proteins associated with the nuclear matrix-intermediate filament scaffold in *Drosophila melanogaster* cells. J Cell Sci 95:393–404.

Park HG, Han SI, Oh SY, Kang HS. 2005. Cellular responses to mild heat stress. Cell Mol Life Sci 62:10–23.

Paulin-Levasseur M, Julien M. 1999. Characterization of the 2A7 antigen as a 85-kDa human nucleocytoplasmic shuttling protein. Exp Cell Res 250:439–451.

Paulin-Levasseur M, Julien M, Horner M, Chen G. 1995. Characterization of the 2H12 antigen as a nonshuttling human isoelectric variant of the nucleolar protein B23. Exp Cell Res 219:514–526.

Pederson T. 1998. Thinking about a nuclear matrix. J Mol Biol 277:147–159.

Pederson T. 2000. Half a century of ''the nuclear matrix''. Mol Biol Cell 11(3): 799–805.

Read RA, Fox MH, Bedford JS. 1984. The cell cycle dependence of thermotolerance. 2. CHO cells heated at 45°C. Radiat Res 98:491–505.

Reiter T, Penman S. 1983. ''Prompt'' heat shock proteins: Translationally regulated synthesis of new proteins associated with the nuclear matrix-intermediate filaments as an early response to heat shock. Proc Natl Acad Sci USA 80:4737–4741.

Stierle V, Couprie J, Ostlund C, Krimm I, Zinn-Justin S, Hossenlop P, Worman HJ, Courvalin J. 2003. The carboxyl-terminal region common to lamins A and C contains a DNA binding domain. Biochem 42(17): 4819–4828.

Sugano T, Nitta M, Ohmori H, Yamaizumi M. 1995. Nuclear accumulation of p53 in normal human fibroblasts is induced by various cellular stresses which evoke the heat shock response, independently of the cell cycle. Jpn Cancer Res 86:415–418.

Sullivan T, Escalante-Alcade D, Bhatt H, Anver M, Bhat N, Nagashima K, Stewart CL, Burke B. 1999. Loss of A-type lamin expression compromises nuclear envelope integrity leading to muscular dystrophy. J Cell Biol 147: 913–920.

Tran EJ, Wente SR. 2006. Dynamic nuclear pore complexes: Life on the edge. Cell 125(6): 1041–1053.

Ulitzur N, Harel A, Feinstein N, Gruenbaum Y. 1992. Lamin activity is essential for nuclear envelope assembly in a Drosophila embryo cell-free extract. J Cell Biol 119:17–25.

Valgardsdottir R, Chiodi I, Giordano M, Cobianchi F, Riva S, Biamonti G. 2005. Structural and functional characterization of noncoding repetitive RNAs transcribed in stressed human cells. Mol Biol Cell 16:2597–2604.

Valgardsdottir R, Chiodi I, Giordano M, Rossi A, Bazzani S, Ghigna C, Riva S, Biamonti G. 2008. Transcription of Satellite III non-coding RNAs is a general stress response in human cells. Nucleic Acids Res 36(2): 423–434.

Wang Y, Guan J, Wang H, Wang Y, Leeper D, Iliakis G. 2001. Regulation of DNA replication after heat shock by replication protein A-nucleolin interactions. J Biol Chem 276:20579–20588.

Wilson KL. 2000. The nuclear envelope, muscular dystrophy and gene expression. Trends Cell Biol 10:125–129.

Worman HJ, Courvalin J-C. 2000. The inner nuclear membrane. J Membr Biol 177:1–11.

Zhu W-G, Roberts ZV, Dynlacht JR. 1999. Heat-induced modulation of lamin B content in two different cell lines. J Cell Biochem 75:620–628.