Journal of Cellular Biochemistry 75:620–628 (1999)

Heat-Induced Modulation of Lamin B Content in Two Different Cell Lines

Wei-Guo Zhu, Zachary V. Roberts, and Joseph R. Dynlacht*

Indiana University School of Medicine, Department of Radiation Oncology, Indiana Cancer Pavilion, Indianapolis, Indiana 46202

Abstract Previous work in our laboratory indicates that the nuclear matrix protein lamin B is a "prompt" heat shock protein, which increases significantly when human U-1 melanoma and HeLa cells are exposed to 45.5°C for 5–40 min. Using Western blotting, we found that the lamin B content in U-1 and HeLa cells increased to a greater extent during post-heat incubation at 37°C than during the heat dose itself. When HeLa cells were heated at 45.5°C for 30 min, and then incubated at 37°C for up to 7 h, lamin B content was increased significantly (1.69-fold maximum increase at 3 h) compared to unincubated heated cells. Also, thermotolerant HeLa cells showed a greater increase (up to 1.72-fold) in lamin B content during subsequent heating compared to nontolerant cells. The increase in lamin B content in thermotolerant cells, or when heated cells were incubated at 37°C, was also observed in U-1 cells. HeLa cells heated in the presence of glycerol (a heat protector) showed a 1.21–1.72-fold increase in lamin B content compared to cells heated for 10–30 min without glycerol. In contrast, lamin B content decreased 1.23–1.85-fold when cells were heated for 10–30 min in the presence of procaine (a heat sensitizer) compared to cells heated without procaine. These data suggest that lamin B may play an important role in the heat shock response, and that modulation of lamin B content by heat sensitizers or protectors may play a role in regulation of heat sensitivity. J. Cell. Biochem. 75:620–628, 1999.

Key words: lamin B; heat shock; nuclear matrix proteins; thermotolerance

Data from several laboratories indicate that the nuclear matrix is a target for hyperthermic cell killing [Warters, 1988; Roti Roti et al., 1997; Burgman and Konings, 1992]. Several changes in the organization of the nuclear matrix occur when mammalian cells are heated [Warters, 1988; Roti Roti et al., 1997; Warters et al., 1993]. Heat induces aggregation and/or unfolding of nuclear proteins, some of which are normally soluble but may co-isolate with the nuclear matrix after heat-shock [see Roti Roti et al., 1997, 1998, and references therein]. The increased binding of nuclear proteins to the nuclear matrix has been suggested to be a result of protein denaturation, though it is still unclear as to whether it is the soluble nuclear proteins or the nuclear matrix proteins which become denatured. Aggregation of protein to the nuclear matrix is believed to be associated with the disruption of cellular metabolism and ultimately may lead to cell death [Roti Roti et al., 1997; Burgman and Konings, 1992]. On the other hand, the nuclear matrix itself also appears to be a determinant for resistance to heat [Roti Roti et al., 1997]. The nuclear matrix has been identified as a site for transcription [Berezney et al., 1995]. In particular, the heat shock protein 70 (HSP 70) gene family is located at the nuclear matrix [Roti Roti et al., 1997], typical of actively transcribed genes, giving strength to the claim that the nuclear matrix is involved in protection against subsequent heat shock.

The nuclear matrix consists of the nuclear pore-lamina complex, residual nucleoli, and the internal nuclear matrix [Berezney, 1991]. The major proteins of the nuclear pore-lamina complex are the nuclear lamins, lamin A, B, and C [Kaufmann et al., 1983]. The lamina provides

Abbreviations used: HEPES, N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid; HSP, heat shock proteins; MARs, matrix-attached DNA regions; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; TT, thermotolerant.

Grant sponsor: National Cancer Institute; Grant number: CA64674.

*Correspondence to: Joseph R. Dynlacht, Indiana University School of Medicine, Department of Radiation Oncology, Indiana Cancer Pavilion, RT 041, 535 Barnhill Drive, Indianapolis, IN 46202.

Received 1 February 1999; Accepted 18 May 1999

structural support for the nucleus [Berezney, 1991]. Nuclear lamins may also be involved in the organization of chromatin, DNA replication, and nuclear envelope reassembly [Newport and Forbes, 1987]. Our previous work indicates that lamin B might be involved in the heat-shock response [Dynlacht et al., 1996, 1999]. Since the synthesis of lamin B in U-1 human melanoma and HeLa cells occurred during heating, lamin B appears to be induced by a different mechanism than the conventional heat shock proteins, which are synthesized when heated cells are returned to 37°C for several hours [Li and Werb, 1982]. Therefore, lamin B has been defined as a "prompt" heat shock protein. "Prompt" heat shock proteins were first documented by Reiter and Penman [1983]. They reported that at least 50 polypeptides are synthesized in HeLa cells during heating. These polypeptides are synthesized from pre-existing mRNA, translated during heat shock and associated with the nuclear matrix-intermediate filament fraction. However, the biological functions of these "prompt" heat shock proteins remain to be determined. The modulation of lamin B content that occurs during heating does not appear to be directly involved in the cell killing process [Dynlacht et al., 1999]. In U-1 cells, Western blotting analysis shows a significant increase in lamin B content within the first 5 min of heating at 45.5°C, reaching a plateau after 10 min of heating. No significant cell killing is observed when cells are heated for 5 min; however, after 5 min of heating, cell survival becomes an exponential function of the heat dose [Dynlacht et al., 1999]. These data have led us to propose that lamin B might therefore be involved in the recovery of cells after heat shock, or the induction of thermotolerance.

Several chemicals that modulate hyperthermic cell killing also modulate heat-induced nuclear protein content [Roti Roti and Wilson, 1984; Henle and Warters, 1982]. The local anesthetic procaine has been shown to sensitize cells to heat shock [Yatvin, 1977]. Although the cell membrane is thought to be the target for procaine [Nicolson et al., 1976], heating in the presence of procaine causes many effects, including an increase in chromatin protein content [Roti Roti and Wilson, 1984], an increase in chromosomal aberrations [Dewey et al., 1990] and inhibition of thermotolerance induction [Konings, 1985]. Glycerol protects cells when present during heating [Henle and Warters, 1982], presumably by raising the transition temperature for protein denaturation below 60°C [Lepock et al., 1988]. Glycerol has been reported to decrease the amount of heat-induced nuclear protein [Henle and Warters, 1982]. However, the exact mechanism by which glycerol and procaine modulate hyperthermic cell killing is not known.

It was the aim of this study to investigate the changes in lamin B content when cells were returned to 37°C after heating at 45.5°C and in thermotolerant cells. We also investigated the effects of glycerol or procaine on lamin B content during heating. Our results support the notion that lamin B may play a crucial role in recovery of cells from heat shock, and the increased synthesis of lamin B may confer resistance to subsequent heat-induced cell killing. Further study of the role of specific proteins of the nuclear matrix in the heat response may provide insight into the mechanism by which heat induces cell killing.

MATERIALS AND METHODS

Cell Culture and Hyperthermic Treatment

U-1 human melanoma and HeLa CCL2 cells were maintained as monolayers and grown in 25-cm^2 tissue culture flasks containing 5 ml McCoy's 5A medium supplemented with 10% iron-supplemented calf serum (Hyclone, Inc., Logan, UT). Cells were maintained in a humidified incubator (95% air, 5% CO_2) at 37°C. Unless otherwise indicated, 3 days prior to the experiments, 2.5×10^5 cells were plated into 25-cm^2 tissue culture flasks (T-25) so as to yield $\sim 10^6$ log phase cells per flask at the start of each experiment. Medium was changed one hour prior to experiments. Cells were heated in monolayers in T-25 flasks containing 5 ml of medium. For heat treatments, flasks were submerged in a temperature controlled (45.5 ± 0.1°C) water bath for various times, and either incubated at 37°C or cells were trypsinized and counted for subsequent measurement of cell survival, or analysis of changes in lamin B content.

Induction of Thermotolerance and Modulation of Heat Sensitivity

For samples in which thermotolerance effects were to be studied, 4.5×10^5 cells were plated into T-25 flasks 2 days prior to induction of

thermotolerance. Cell medium was changed 1 h prior to experiments, then cells were heated at 45.5°C for 5 min. The heated cells were then incubated at 37°C for 15 h prior to reheating. Since the 5 min priming dose, though virtually non-toxic [Dynlacht et al., 1999], causes a slight lag in cell cycle progression, a greater number of cells were plated initially so that both nontolerant and thermotolerant flasks would yield $\sim 10^6$ log phase cells per flask after the heat challenge and prior to protein extraction (see below). Glycerol or procaine was dissolved in McCoy's 5A medium modified with 25 mM HEPES buffer in order to maintain a constant pH (Gibco BRL, Life Technologies, Inc., Grand Island, NY). Cells were then incubated in the presence of 7% glycerol or 4 mM procaine at 37°C for 5–35 min, or heated at 45.5°C for 10–30 min. Chemically treated medium was added to the cells 5 min prior to heating and was allowed to equilibrate at 37°C.

Clonogenic Cell Survival Assay

After heating at 45.5°C under various conditions, U-1 or HeLa cells were counted with an electronic cell counter (Particle Data, Inc., Elmhurst, IL) and plated in triplicate into T-25 flasks. Flasks were kept in a humidified incubator (95% air, 5% CO_2) at 37°C for 12–14 days to allow for colony formation. Colonies were then fixed with methanol/acetic acid (3:1) and stained with crystal violet. Calculations of cell survival were corrected for plating efficiency of unheated cells, which was 30–50%.

Western Blotting

Detection of lamin B protein content was carried out as described previously [Dynlacht et al., 1999]. Briefly, after various treatments, cells were trypsinized and washed once in cold phosphate buffered saline (PBS) at 4°C. Then cells were lysed in Laemmli's Sample Buffer (Bio-Rad Laboratories, Hercules, CA) containing 5% beta-mercaptoethanol at a final cell concentration of 4×10^6/ml. Lysed samples were heated at 100°C for 10 min, and then passed through a 26.5-gauge needle three times. Protein extract (8×10^4 cells per lane) was then loaded in a 10% SDS-polyacrylamide gel and electrophoresed at 125 V for 15 min and then at 200 V for 45–50 min. The proteins were transferred to a Hibond-ECL nitrocellulose membrane (Amersham, Arlington Heights, IL) with a 250-mA current at 4°C for 2.5 h. Membranes were then blocked in blocking solution (50 mM Tris, pH 7.5; 0.9% NaCl; 0.05% Tween-20; 3% nonfat dry milk) at room temperature for 2 h. Lamin B primary antibody (Oncogene Research Products, Cambridge, MA) was added to blocking buffer (1:100 dilution) and incubated with the membrane at 4°C overnight. After incubation with the primary antibody, the membrane was washed three times with blocking solution for 15 min each and incubated with secondary antibody (1:3,500 dilution) (horseradish peroxidase-conjugated sheep anti-mouse, Amersham) at 4°C for 1 h. The membrane was washed in blocking solution three times for 15 min each and then rinsed twice with cold PBS for 5 min each. The lamin B band was detected by using a chemiluminescent detection system (Amersham) as described by the manufacturer. The relative protein content of individual bands in immunoblots was determined by scanning densitometry. Optical densities obtained for each band were normalized to the respective control bands from unheated cells.

RESULTS

Representative Western blots show that lamin B content increases significantly and reaches a maximum at 30 min for HeLa cells (Fig. 1A) or 20 min for U-1 cells (Fig. 1B) during heating at 45.5°C. To investigate whether lamin B content remains elevated after heat shock, cells were incubated at 37°C for various intervals after heating at 45.5°C for 30 min (HeLa cells) or 20 min (U-1 cells). Representative Western blots illustrating the changes in

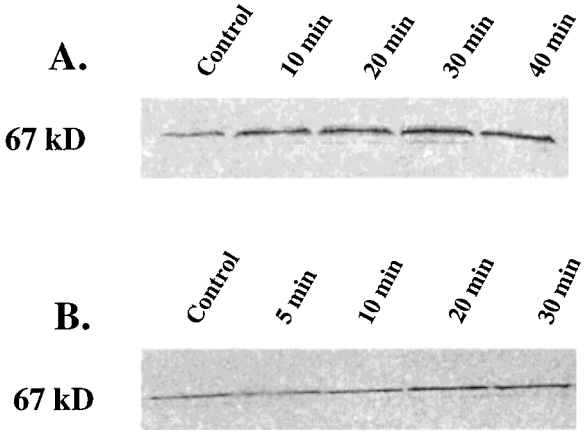

Fig. 1. Representative Western blots illustrating the heat-induced increase in lamin B content in HeLa (**A**) and U-1 melanoma (**B**) cells immediately after heating at 45.5°C for 5–40 min.

lamin B content when cells were returned to 37°C for 30 min to 7 h after heating at 45.5°C are shown in Figure 2A (HeLa cells) and Figure 2B (U-1 cells). Lamin B content continued to increase in both cell lines when cells were returned to 37°C after heating at 45.5°C. Lamin B content was quantified with a densitometer and then plotted as the ratio of the heated samples to the unheated control (Fig. 3A,B). Lamin B content increased gradually at 37°C and reached a maximum at 3 h when HeLa cells were incubated at 37°C after heating at 45.5°C for 30 min (Fig. 3A). When cells were incubated at 37°C for 3 h after heating at 45.5°C for 30 min, for example, the maximum increase in lamin B content is 1.69-fold compared to cells that were processed immediately after heating at 45.5°C for 30 min (Fig. 3A, $P < 0.05$). In U-1 cells, an increase in lamin B content was also observed when heated cells were returned to 37°C for 0.5 h to 7 h (Fig. 3B). Lamin B content decreased between 3–5 h incubation at 37°C in both cell lines (Fig. 3A,B), but failed to return to the level in the unheated control cells even after incubation at 37°C for 24 h (data not shown).

We also compared the lamin B content of thermotolerant cells to nontolerant cells in both cell lines. As shown in Figure 4, thermotolerant HeLa cells were more resistant to heat killing than nontolerant cells. Western blots show a greater increase in lamin B content in thermotolerant cells when thermotolerant cells were exposed to subsequent heating (Fig. 5A, HeLa cells; Fig. 5B, U-1 cells). Densitometer analysis indicated lamin B content in thermotolerant HeLa cells was 1.39 times higher than in non-

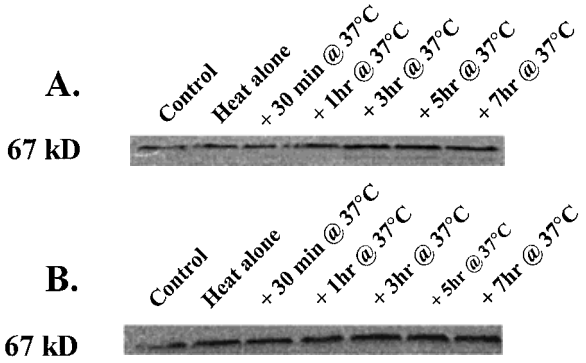

Fig. 2. Representative Western blots illustrating the increase in lamin B content when cells were returned to 37°C for 30 min to 7 h after heating at 45.5°C for 30 min (**A,** HeLa cells) or 20 min (**B,** U-1 cells).

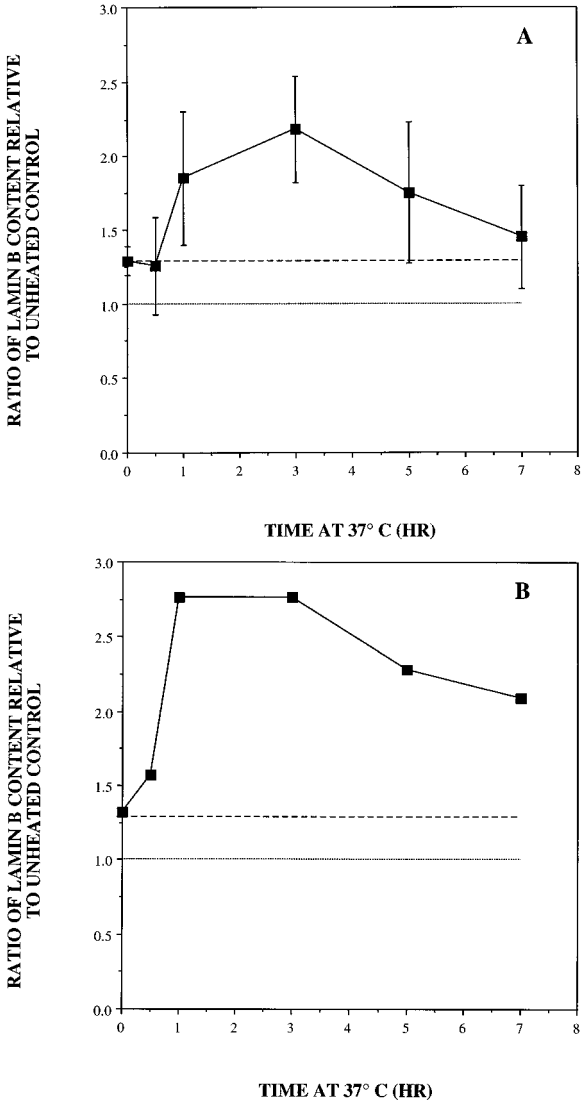

Fig. 3. Quantitative analysis of heat-induced changes in lamin B content in HeLa and U-1 cells. Lamin B bands were quantified with a densitometer, and the data were then plotted as the ratio of heated samples to the unheated control sample. **A:** The closed squares indicate the changes in lamin B content when HeLa cells were returned to 37°C after heating at 45.5°C for 30 min. The dashed line indicates the lamin B content of HeLa cells that were heated at 45.5°C for 30 min alone (no incubation; 1.29 ± 0.1). The dotted line indicates lamin B content in unheated control cells. The error bars represent the standard error of the mean (SEM) for four to six separate experiments. **B:** The closed squares indicate the increase in lamin B content when U-1 cells were returned to 37°C after heating at 45.5°C for 20 min. The dashed line indicates the lamin B content of U-1 cells that were heated at 45.5°C for 20 min alone (no incubation; 1.32-fold increase compared to unheated cells). The dotted line indicates the lamin B content in the unheated control sample. Data shown are from one representative experiment.

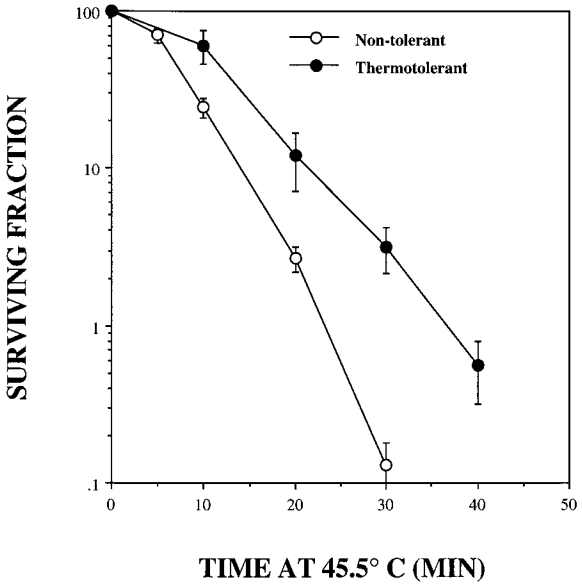

Fig. 4. Survival of thermotolerant or nontolerant HeLa cells heated at 45.5°C. Thermotolerance was induced by heating cells for 5 min at 45.5°C followed by incubation at 37°C for 15 h. The error bars represent the SEM for four separate experiments.

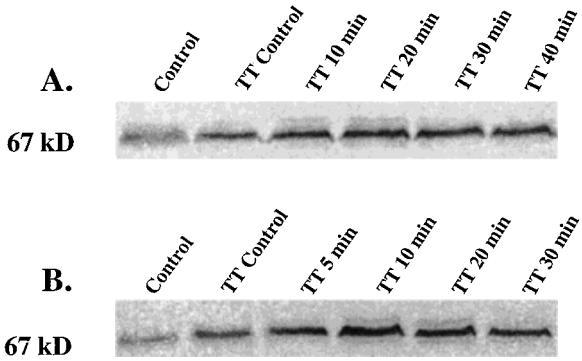

Fig. 5. Representative Western blots showing an increase in lamin B content in thermotolerant cells. Thermotolerant (TT) cells were heated at 45.5°C for 5–40 min and then lysed for Western blotting. **A:** HeLa cells. **B:** U-1 cells.

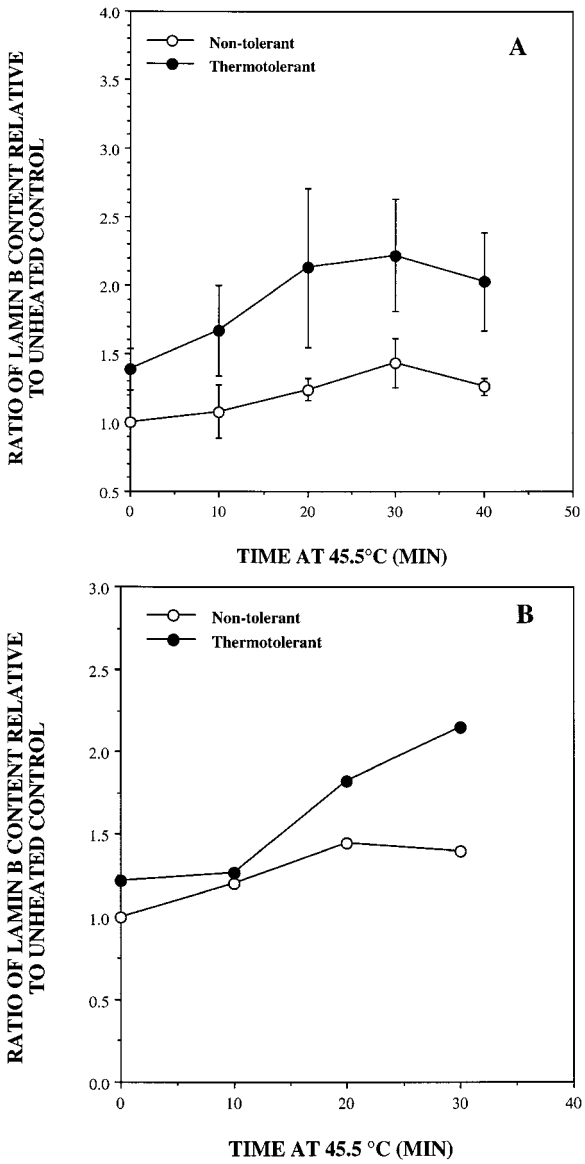

Fig. 6. Quantitative analysis of heat-induced changes in lamin B content in thermotolerant cells. Lamin B bands were quantified with a densitometer, and the data were then plotted as the ratio of the heated samples to an unheated control sample. **A:** Changes in lamin B content in thermotolerant or nontolerant HeLa cells. The error bars represent the SEM for four to six separate experiments. **B:** Changes in lamin B content in thermotolerant or nontolerant U-1 cells heated at 45.5°C for 5–30 min (data shown are from one representative experiment).

tolerant cells (Fig. 6A, $P < 0.05$). When thermotolerant HeLa cells were heated at 45.5°C, lamin B content increased to a greater extent than in nontolerant cells. For example, the maximum increase in lamin B content in thermotolerant HeLa cells was 1.82-fold that of nontolerant cells when heated at 45.5°C for 40 min (Fig. 6A, $P < 0.05$). In thermotolerant U-1 cells, initial lamin B content was also found to be higher than in nontolerant cells, and increased to a greater extent when cells were subsequently heated at 45.5°C for an additional 20 or 30 min (Fig. 6B).

Previous studies have shown that in cells treated with glycerol or procaine, cell survival increases or decreases significantly, respectively [Henle and Warters, 1982; Lepock et al., 1988]. Thus, we hypothesized that glycerol and procaine could modulate lamin B levels as well as heat sensitivity. Treatment with 7% glycerol or 4 mM procaine (in HEPES-buffered medium)

at 37°C for 40 min did not alter cell survival (plating efficiency of cells incubated for 40 min with 4 mM procaine or 7% glycerol was not significantly different from control cells). However, procaine (4 mM) enhanced hyperthermic cell killing significantly (Fig. 7). Cell survival decreased, for example, from 28.6 to 8.63% and from 4.1 to 0.14% when cells were heated at 45.5°C for 10 and 20 min with procaine compared to cells heated without procaine (Fig. 7, $P < 0.01$). Glycerol had a significant protective effect on heat-induced cell killing when HeLa cells were heated at 45.5°C (Fig. 7). Cell survival increased, for example, from 28.6 to 71.1% and from 4.1 to 17.2% when cells were heated at 45.5°C for 10 and 20 min with glycerol compared to cells heated without glycerol (Fig. 7, $P < 0.01$). Using Western blotting, we found that lamin B content increased during the first 15 min (increasing 1.22- to 1.44-fold) when cells were incubated with glycerol at 37°C; then lamin B content returned to control levels for the following 15–35 min (Fig. 8A). When cells were heated with glycerol at 45.5°C, lamin B content increased greatly compared to cells treated with heat alone or with glycerol at 37°C (Fig. 8A). The increases in lamin B content in cells heated with 7% glycerol are 1.72-, 1.47-, and 1.34-fold greater, respectively, than in cells heated without glycerol (Fig. 8A, $P < 0.01$). When cells were incubated in the presence of procaine (4 mM) at 37°C, no change in lamin B content is observed during the first 15 min (Fig. 8B), but there was a 1.3–1.67-fold decrease in lamin B content when cells were incubated for longer periods compared to control cells (Fig. 8B). Also, no obvious change in lamin B content was observed when cells are heated at 45.5°C in the presence of procaine compared to unheated control cells (Fig. 8B, $P > 0.05$). However, lamin B content was found to be decreased significantly when cells were heated at 45.5°C for 30 min

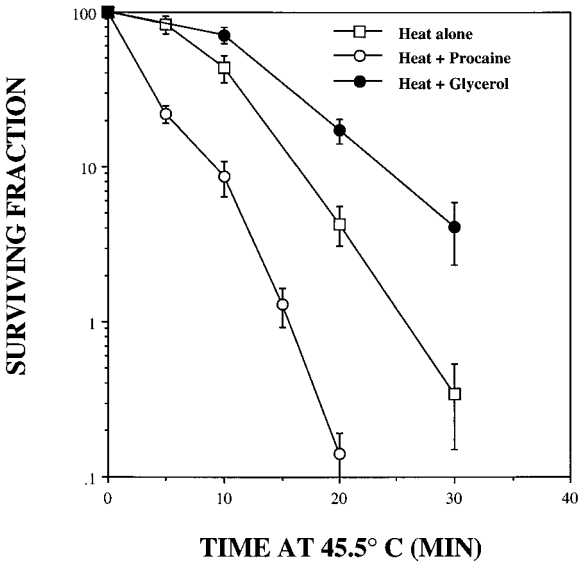

Fig. 7. A comparison of cell survival between HeLa cells heated at 45.5°C alone, or heated at 45.5°C in the presence of glycerol or procaine. The error bars represent the SEM for four to six separate experiments. All cells were heated in HEPES-buffered McCoy's 5A medium.

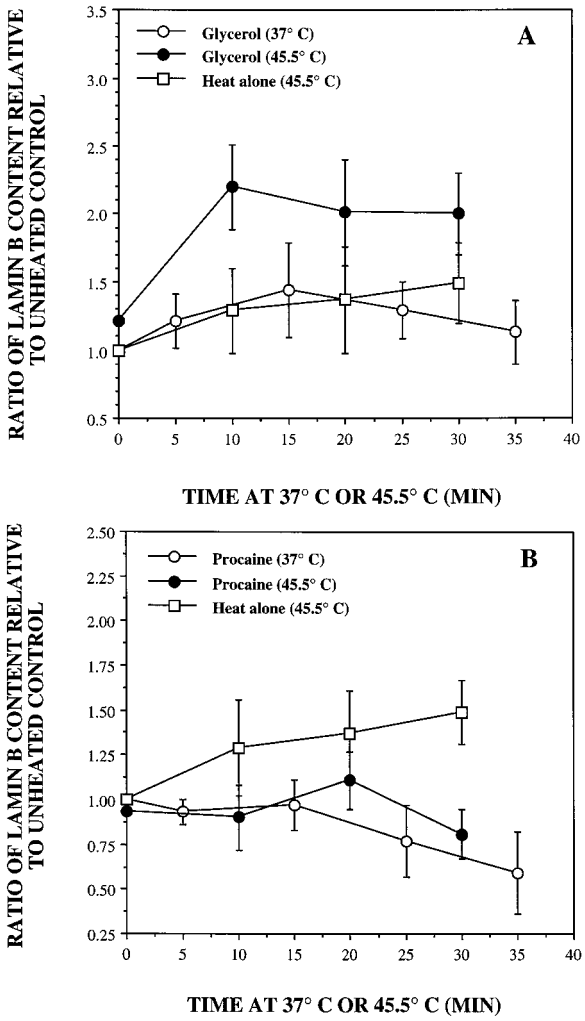

Fig. 8. Quantitative analysis of lamin B content in HeLa cells treated with glycerol or procaine at 37°C or 45.5°C. **A:** HeLa cells were incubated with glycerol (7%) at 37°C for 5–35 min or heated in the presence of glycerol (7%) at 45.5°C for 10–30 min. The error bars represent the SEM for four separate experiments. **B:** HeLa cells were incubated with procaine (4 mM) at 37°C for 5–35 min or heated with procaine (4 mM) at 45.5°C for 10–30 min. The error bars represent the SEM for four separate experiments. As a control, cells without any chemicals were heated at 45.5°C for 10–30 min in HEPES-buffered McCoy's 5A medium.

with 4 mM procaine compared to heated cells alone (Fig. 8B, decrease 1.85 times, $P < 0.01$). Nontolerant HeLa cells heated in the absence of drugs for as long as 40 min at 45.5°C showed up to a 1.4–1.5-fold increase in lamin B content compared to unheated cells. Though lamin B content was always significantly higher in heated cells compared to unheated cells in the experiments done for this study, for unknown reasons, the maximum increase was less than the 2.2-fold increase reported in a previous study [Dynlacht et al., 1999].

DISCUSSION

Returning cells to a normal temperature is essential for recovery of cellular metabolism and induction of thermotolerance. In response to heat shock, the processes of cellular metabolism, such as RNA and protein synthesis, and DNA replication, are severely inhibited [Laszlo, 1992; Higashikubo and Roti Roti, 1993; Henle and Leeper, 1979]. In CHO cells, the rate of protein synthesis is only 3% of the normal rate after 10 min of heating at 45°C [Henle and Leeper, 1979]. In HeLa cells, for example, RNA synthesis is reduced to 40% of control levels immediately following a heat exposure of 43°C for 15 min [Higashikubo and Roti Roti, 1993]. The RNA synthesis rate then gradually recovers with post-heat incubation at 37°C, reaching control levels by 5–6 h post-heat shock. The time scale for heat-induced changes in lamin B content and recovery of cellular metabolism are quite different from one another after heated cells are returned to 37°C. Lamin B is promptly synthesized during heating at 45.5°C in both cell lines (Fig. 1A,B). However, lamin B content increases further when cells are returned to 37°C after heating at 45.5°C (Fig. 2A,B), and sustains a higher level than in unheated cells even after 24 h post-heating (data not shown). These data imply that lamin B may not only play a role in response to acute heat shock, but also may play a special role in cell recovery from heat shock.

Depending on the length of exposure and the cell line being heated, hyperthermia may perturb the cell cycle, leading to an accumulation of cells in S and G_2 phase after heat exposure [Coss, 1986; Wong et al., 1989]. Interestingly, lamin B appears to be synthesized only in S phase [Foisy and Bibor-Hardy, 1988; Jost and Johnson, 1981]. Thus, synchronization of cells could explain the observed increase in lamin B content seen in bulk populations of cells at various times after heating. To prove this was not the case, we measured the changes in the cell cycle by flow cytometry, either when cells were incubated at 37°C for 15 h after heating at 45.5°C for 5 min in both cell lines (the same conditions used for the induction of thermotolerance), or when cells were incubated at 37°C for 7 h after heating at 45.5°C for 20 min (U-1 cells) or 30 min (HeLa cells). No obvious cell cycle changes were found (data not shown). Hence, cell cycle redistribution could not account for the observed changes in the lamin B content of cells during incubation at 37°C after heating or in thermotolerant cells.

Our data show that lamin B may be involved in the recovery of cells from heat shock (Figs. 2A,B, 3A,B). Furthermore, increased synthesis of lamin B may provide cells with the ability to resist subsequent heat-induced cell killing (Figs. 7, 8A,B). However, additional studies will be required to confirm this.

The induction of thermotolerance is tightly correlated with the synthesis of heat shock proteins (HSPs) [Li and Werb, 1982; Li et al., 1995]. At the transcriptional level, heat shock turns on a set of heat shock genes [Lindquist, 1986]. However, the synthesis of HSPs occurs when heated cells are returned to 37°C for several hours [Higashikubo and Roti Roti, 1993; Chirico et al., 1988; Laszlo and Li, 1985]. Our data show that lamin B synthesis is an early event in the cellular response to heat-shock (Fig. 1A,B). The continual increase in lamin B content when cells are returned to 37°C after heating implies that lamin B may be involved with the recovery of cellular metabolic processes. Since lamin B content in thermotolerant cells is much higher than in nontolerant cells (Fig. 6A,B), and lamin B content continues to increase when heated cells are incubated at 37°C, we speculate that lamin B may be involved in the induction of thermotolerance.

Lamin B may form nucleoplasmic foci that are associated with DNA [Moir et al., 1994]. Lamin B binds to matrix attachment regions (MARs) which have been proposed to serve as origins of replication [Luderus et al., 1992, 1994]. Lamin B has been shown to form a scaffold upon which replication factors bind, either directly or through lamin-associated proteins, to form a replication center [Berezney et al., 1995; Goldman et al., 1992; Newport et al., 1990]. Again, further experiments are neces-

sary to determine whether synthesis of lamin B or restructuring of the lamina is necessary for the resumption of DNA synthesis after heat shock.

The mechanisms by which glycerol and procaine influence the synthesis of lamin B are not known. There are many reports indicating that the modulation of heat sensitivity by procaine or glycerol may be correlated with changes in cell structure or the induction of thermotolerance. Cryobiology and biochemistry studies have shown glycerol can stabilize cell membranes, microtubules, and protein structure [Lin et al., 1981]. Glycerol may protect some of the proteinaceous cellular components, which, if undamaged, could result in increased cell survival after heat treatment [Lin et al., 1981]. Heat sensitization by procaine is considered to be caused by heat-induced membrane lesions [Nicolson et al., 1976]. However, heat-sensitization by procaine has been reported to proceed by other mechanisms as well, such as by alteration of the association of nuclear protein with chromatin after heat shock [Roti Roti and Wilson, 1984]. Several researchers reported that procaine could inhibit the triggering as well as the induction of thermotolerance [Konings, 1985; Rastogi et al., 1987]. Similarly, through inhibition of heat-induced lamin B synthesis, procaine may increase susceptibility to heat-induced cell killing.

In conclusion, we believe that the modulation of lamin B content may be implicated in the modulation of heat sensitivity by chemicals or induction of thermotolerance. However, whether the newly synthesized pool of lamin B becomes incorporated into the nuclear lamina remains to be determined, as does the extent and kinetics of its incorporation.

ACKNOWLEDGMENTS

We thank Daphne Farrington and Josh Seno for their technical assistance.

REFERENCES

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.

Berezney R, Mortillaro MJ, Ma H, Wei X, Samarabandu J. 1995. The nuclear matrix: a structural milieu for genomic function. In: Berezney R, Jeon KW, editors. Nuclear matrix: structural and functional organization. San Diego: Academic Press. p 2–54.

Burgman PW, Konings AWT. 1992. Heat-induced protein denaturation in the particulate fraction of HeLa S3 cells: effect of thermotolerance. J Cell Physiol 153:88–94.

Chirico WJ, Waters MG, Blobel G. 1988. 70K heat shock related proteins stimulate protein translocation into microsomes. Nature 332:805–810.

Coss RA. 1986. Decay of thermal resistance following acute heating is independent of the G_1- to S-phase transition. Radiat Res 107:143–146.

Dewey WC, Li XL, Wong RS. 1990. Cell killing, chromosomal aberrations, and division delay as thermal sensitivity is modified during the cell cycle. Radiat Res 122:268–274.

Dynlacht JR, Henthorn J, O'Nan C, Dunn ST, Story MD. 1996. Flow cytometric analysis of nuclear matrix proteins: method and potential applications. Cytometry 24: 348–359.

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

Foisy S, Bibor-Hardy V. 1988. Synthesis of nuclear lamins in BHK-21 cells synchronized with aphidicolin. Biochem Biophys Res Commun 156:205–210.

Goldman AE, Moir RD, Montag-Lowy M, Stewart M, Goldman RD. 1992. Pathway of incorporation of microinjected lamin A into the nuclear envelope. J Cell Biol 119:725–735.

Henle KJ, Leeper DB. 1979. Effects of hyperthermia (45 degrees) on macromolecular synthesis in Chinese hamster ovary cells. Cancer Res 39:2665–2674.

Henle KJ, Warters RL. 1982. Heat protection by glycerol in vitro. Cancer Res 42:2171–2176.

Higashikubo R, Roti Roti JL. 1993. Alterations in nuclear protein mass and macromolecular synthesis following heat shock. Radiat Res 134:193–201.

Jost E, Johnson RT. 1981. Nuclear lamina assembly, synthesis and disaggregation during the cell cycle in synchronized HeLa cells. J Cell Sci 47:25–53.

Kaufmann SH, Gibson W, Shaper JH. 1983. Characterization of the major polypeptides of the rat liver nuclear envelope. J Biol Chem 258:2710–2719.

Konings AWT. 1985. Development of thermotolerance in mouse fibroblast LM cells with modified membranes and after procaine treatment. Cancer Res 45:2016–2019.

Laszlo A, Li GC. 1985. Heat-resistance variants of Chinese hamster fibroblasts altered in expression of heat shock protein. Proc Natl Acad Sci USA 82:8029–8033.

Laszlo A. 1992. The effects of hyperthermia on mammalian cell structure and function. J Cell Prolif 25:59–87.

Lepock JR, Frey HE, Rodahl AM, Kruuv J. 1988. Thermal analysis of CHL V79 cells using differential scanning calorimetry: implications for hyperthermic cell killing and the heat shock response. J Cell Physiol 137:14–24.

Li GC, Mivechi NF, Weitzel G. 1995. Heat shock proteins, thermotolerance, and their relevance to clinical hyperthermia. Int J Hyperthermia 11:459–488.

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 PS, Kwock L, Hefter K. 1981. Protection of heat induced cytotoxicity by glycerol. J Cell Physiol 108:439–443.

Lindquist S. 1986. The heat-shock response. Annu Rev Biochem 55:1151–1191.

Luderus ME, de Graff A, Mattia E, den Blaauwen JL, Grande MA, de Jong L, van Driel R. 1992. Binding of matrix attachment regions to lamin B_1. Cell 70:949–959.

Luderus ME, den Blaauwen JL, de Smith OJ, Compton DA, van Driel R. 1994. Binding of matrix attachment regions to lamin polymers involves single-stranded regions and the minor groove. Mol Cell Biol 14:6297–6305.

Moir RD, Montag-Lowy M, Goldman RD. 1994. Dynamic properties of nuclear lamins: lamin B is associated with sites of DNA replication. J Cell Biol 135:1201–1212.

Newport JW, Forbes DJ. 1987. The nucleus: structure, function and dynamics. Annu Rev Biochem 56:535–565.

Newport JW, Wilson KL, Dunphy WG. 1990. A lamin independent pathway for nuclear envelope assembly. J Cell Biol 111:2247–2259.

Nicolson GL, Smith JR, Poste G. 1976. Effects of local anesthetics on cell morphology and membrane-associated cytoskeletal organization in BALB/3T3 cells. J Cell Biol 68:395–402.

Rastogi D, Henle KJ, Nagle WA, Moss AJ, Neilan BA, Bastogi SP. 1987. Development of thermotolerance in CHO cells: modification by procaine. Int J Hyperthermia 3:63–70.

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.

Roti Roti JL, Wilson CF. 1984. The effects of alcohols, procaine and hyperthermia on the protein content of nuclei and chromatin. Int J Radiat Biol 46:25–33.

Roti Roti JL, Wright WD, VanderWaal R. 1997. The nuclear matrix: a target for heat shock effects and a determinant for stress response. Crit Rev Eukary Gen Express 7:343–360.

Roti Roti JL, Kampinga HH, Malyapa RS, Wright WD, vanderWaal RP, Xu M. 1998. Nuclear matrix as a target for hyperthermic killing of cancer cells. Cell Stress Chaperones 3:245–55.

Warters RL. 1988. Hyperthermia blocks DNA processing at the nuclear matrix. Radiat Res 115:258–272.

Warters RL, Chu GL, Wong RS, Lyons BW, Dewey WC. 1993. Nuclear protein redistribution in heat-shocked cells. J Cell Physiol 154:402–409.

Wong RS, Borrelli MJ, Thompson LL, Dewey WC. 1989. Mechanism of killing Chinese hamster ovary cells heated in G_1: effects on DNA synthesis and blocking in G_2. Radiat Res 118:295–310.

Yatvin MB. 1977. The influence of membrane lipid composition and procaine on hyperthermic death of cells. Int J Radiat Biol 32:513–521.