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Mistargeting of B-type Lamins at the End of Mitosis: Implications on Cell Survival and Regulation of Lamins A/C Expression

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Abstract. We previously showed that targeting of protein phosphatase 1 (PP1) to the nuclear envelope (NE) by the A-kinase anchoring protein, AKAP149, correlates with nuclear assembly of B-type lamins in vitro. We demonstrate here that failure of AKAP149-mediated assembly of B-type lamins into the nuclear lamina at the end of mitosis is followed by apoptosis, and induces expression of the gene encoding A-type lamins in cells that normally do not express lamins A/C. In HeLa cells, inhibition of PP1 association with the NE mediated by a peptide containing the PP1-binding domain of AKAP149 results in failure of B-type lamins to assemble, and in their rapid caspase-dependent proteolysis. However, assembly of lamins A/C is not affected. Nonetheless, apoptosis follows within hours of nuclear reformation after mitosis. In lymphoid KE37 cells, which do not express lamins A/C, inhibition of B-type lamin assembly triggers rapid synthesis and nuclear assembly of both lamins A and C before apoptosis takes place. The results indicate that nuclear assembly of B-type lamins is essential for cell survival. They also suggest that mistargeting of B-type lamins at the end of mitosis elicits a tentative rescue process to assemble a nuclear lamina in lymphoid cells that normally do not express lamins A/C.

Key words: AKAP149 • apoptosis • lamin • mitosis • nuclear envelope

Introduction

The nuclear lamina consists of a peri- and intranuclear meshwork of intermediate filaments called lamins (Gruenbaum et al., 2000). Lamins have been grouped into A and B types based on their isoelectric points. Lamins A and C are splice variants of a single lamin A (LMNA) gene, whereas lamins B1 and B2 are encoded by two separate LMNB1 and LMNB2 genes. A- and B-type lamins differ in several ways, including their behavior at mitosis, post-translational processing and expression patterns. B-type lamins are expressed in all somatic cells, whereas A-type lamins are absent in some nonterminally differentiated cells (Guilly et al., 1987, 1990; Lin and Worman, 1997). Nuclear lamins are implicated in various nuclear functions (Ellis et al., 1997; Spann et al., 1997) and in severe forms of muscular and lipodystrophies (Wilson, 2000).

The nuclear envelope (NE)[1] is a dynamic structure that breaks down at mitosis and reforms in an ordered manner as a result of reversible phosphorylations of membrane, lamina, and chromatin proteins (Collas and Courvalin, 2000). In an investigation of the mechanisms regulating NE dynamics, we showed that the nuclear membrane A-kinase anchoring protein, AKAP149, targets chromatin-bound protein phosphatase 1 (PP1) to the NE upon nuclear reformation in vitro, and that this correlates with nuclear assembly of B-type lamins (Steen et al., 2000). In vivo, A- and B-type lamins seem to assemble via distinct pathways (Moir et al., 2000); however, the molecular mechanism of lamin targeting to the NE is not elucidated.

Apoptosis is a regulated cell death process required for tissue homeostasis (Kaufmann and Earnshaw, 2000). Apoptosis can be triggered by various external stimuli, but intracellular execution signals are conserved (Ashkenazi and

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[1]*Abbreviations used in this paper:* AKAP, A-kinase anchoring protein; LBR, lamin B receptor; NE, nuclear envelope; PARP, poly(ADP)ribosyl polymerase; PP1, protein phosphatase 1; TUNEL, terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling.

Dixit, 1998). Apoptosis is manifested by cell shrinkage and chromatin condensation. Chromatin condensation is secondary to a series of nuclear events (Earnshaw, 1995) such as DNA fragmentation and early caspase-dependent proteolytic degradation of specific nuclear proteins that include lamins (Lazebnik et al., 1995; Takahashi et al., 1996).

In this study we investigated the consequences of PP1 mistargeting to the NE at the end of mitosis, using peptide transfections into dividing fibroblasts and lymphoblastic cells. We show that nuclear assembly of B-type lamins is essential for cell survival, and that inhibition of lamin B assembly triggers synthesis lamins A/C in lymphoblastic cells that normally do not express the LMNA gene.

Materials and Methods

Antibodies

Anti-AKAP149 mAbs against human AKAP149 were from Transduction Laboratories. mAb E-19 against PP1 was from Santa Cruz Biotechnology, Inc. Rabbit antibodies against human LBR and a peptide of human lamin B, and mAb XB10 (BAbCO) against human lamins A/C, were gifts from B. Buendia and J.-C. Courvalin (Institut J. Monod, Paris, France) (Chaudhary and Courvalin, 1993; Buendia and Courvalin, 1997). Anti-lamin B antibodies did not distinguish between the lamin B1 and B2 isoforms, which were globally referred to as "lamin B" in the text. Anti-emerin mAb MANEM 5 was donated by G. Morris (N.E. Wales Institute, Wrexham, UK) (Manilal et al., 1999). Antibodies against poly(ADP)ribosyl polymerase (PARP) were from Boehringer.

Cells and Nuclei

HeLa cells were grown and synchronized in M phase with 1 μM nocodazole for 18 h. The human lymphoblastic KE37 cell line (gift from M. Bornens, Institut Curie, Paris, France) was grown in suspension in RPMI 1640 (GIBCO BRL) (Guilly et al., 1987) and synchronized in M phase as for HeLa cells. Nuclei and NEs were isolated from unsynchronized HeLa and KE37 cells as described elsewhere (Collas et al., 1999).

Peptide Transfection

Synthetic PP1-BD (SSPKGVLFSS) and PP1-BD(V155A) (SSPKGALFSS) peptides were transfected into synchronized M phase HeLa or KE37 cells using the transfection reagent DOTAP® (Roche). Peptides (2 mM stocks) were diluted to 100 μM by mixing 1 μl peptide, 9 μl H_2O, and 2 μl 20 mM Hepes (pH 7.5) with a premixed solution of 4 μl Hepes (pH 7.5) and 4 μl DOTAP. The mixture was incubated for 15 min in the dark, 10^5 cells (at 10^6 cells/ml) were added and incubated for 1.5 h at 37°C in a 5% CO_2 atmosphere. 1 ml of PBS was added and cells were washed twice by suspension and sedimentation. Cells were either cultured further (2–6 h, as indicated) to allow exit from mitotic arrest, processed for immunofluorescence or terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) analysis, or dissolved in SDS sample buffer. Routinely, 90–100% of the cells incorporated the peptides, as judged by uptake of fluorescein-conjugated PP1-BD(V155A) (data not shown).

Immunological Procedures

Western blotting analysis was performed as described earlier (Collas et al., 1999) using anti-lamin B (1:1,000 dilution), anti-lamins A/C (1:500), anti-PP1 (1:500), anti-AKAP149 (1: 250), and anti-PARP antibodies (1:250). Immunoprecipitations of AKAP149 and PP1 from NEs were performed as described (Steen et al., 2000), or after solubilization of the NEs in RIPA buffer (10 mM Tris-HCl, pH 7.5, 100 mM NaCl, 1% Triton X-100, 1 mM EDTA, 0.1% SDS, 0.1% sodium deoxycholate, 1 mM dithiothreitol) (see Fig. 1 A). Immunofluorescence analysis of methanol-fixed cells was performed as described previously (Steen et al., 2000). Antibodies were used at a 1:100 dilution, and DNA was stained with 0.1 μg/ml Hoechst 33342. In some experiments, proportions of fluorescently labeled cells were determined in >300 cells per treatment in 3–4 replicates. Image analysis was performed using the AnalySIS software (Soft Imaging Systems).

TUNEL Analysis

HeLa and KE37 cells were fixed with 3% paraformaldehyde. Fragmented DNA was labeled with fluorescein-conjugated dUTP using the Boehringer In Situ Cell Death Detection Kit according to instructions. Cells were examined by epifluorescence microscopy using an FITC filter, and images were analyzed with the AnalySIS software.

Results and Discussion

Targeting of PP1 to the Nuclear Envelope by AKAP149 at the End of Mitosis

We first tested the NE association of AKAP149 and PP1 in HeLa cells and human T lymphoblastic cells (line KE37), which are deficient in lamins A/C. Both AKAP149 and PP1 were detected on Western blots of HeLa and KE37 cell NEs (Fig. 1 A). In addition, PP1, lamin B, and lamins A/C coprecipitated with anti-AKAP149 antibodies from solubilized HeLa NEs (Fig. 1 A, left panel). PP1 and lamin B also coprecipitated with AKAP149 from KE37 NEs (Fig. 1 A, right panel). Thus, AKAP149 interacts with the nuclear lamina, and this interaction does not depend on lamins A/C.

Whether AKAP149 was implicated in targeting PP1 to

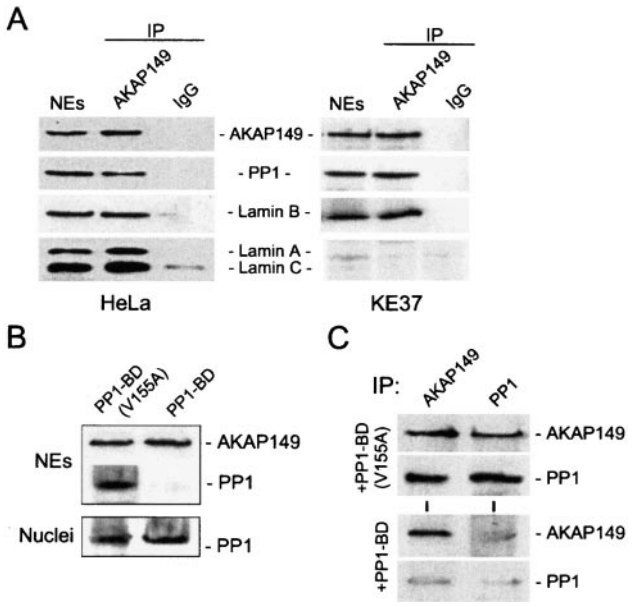

Figure 1. AKAP149 targets PP1 to the nuclear envelope. (A) PP1, lamin B, and lamins A/C coimmunoprecipitate with AKAP149. NEs were purified from interphase HeLa or KE37 cells, solubilized, and AKAP149 was immunoprecipitated (IP). Control immunoprecipitations were done using preimmune mouse IgGs. Precipitates were analyzed by immunoblotting using anti-AKAP149, anti-PP1, anti-lamin B and anti-lamins A/C antibodies. (B) HeLa cells synchronized in mitosis were incubated with PP1-BD(V155A) or PP1-BD. Excess peptide was removed and cells were cultured for 2 h. Nuclei and NEs were purified, and association of AKAP149 and PP1 with the NE was analyzed by immunoblotting. (C) AKAP149 or PP1 were immunoprecipitated from NEs solubilized from HeLa cells exposed to PP1-BD(V155A) or PP1-BD. Immune precipitates were immunoblotted using anti-AKAP149 or anti-PP1 antibodies.

the NE at the end of mitosis was investigated by introducing, into synchronized mitotic HeLa cells, a competitor peptide containing the PP1-binding domain of AKAP149 (PP1-BD: SSPKGVLFSS), or a mutated control peptide (PP1-BD[V155A]) that does not bind PP1 (Steen et al., 2000). Peptides were mixed with the transfection reagent DOTAP and introduced directly into cells synchronized in mitosis. Cells were washed to remove unincorporated peptide, and cultured for 2 h to allow exit from mitosis. Association of PP1 with purified NEs was assessed by immunoblotting. Fig. 1 B shows that whereas the control PP1-BD(V155A) peptide did not impair association of PP1 with the NE, PP1-BD completely abolished NE targeting of PP1. This was verified by the failure of AKAP149 and PP1 to coimmunoprecipitate from solubilized NEs, whereas both proteins coprecipitated in control cells exposed to PP1-BD(V155A) (Fig. 1 C). We concluded that AKAP149 targets PP1 to the NE at the end of mitosis.

Failure to Recruit PP1 to the NE at the End of Mitosis Abolishes Assembly of B-type Lamins

To address the significance of PP1 targeting to the NE by AKAP149, we introduced PP1-BD or the noncompetitor PP1-BD(V155A) peptide into mitotic HeLa cells, and assessed NE reassembly 2 h after exit from mitosis, by immunofluorescence using antibodies against lamin B, lamins A/C, and the inner nuclear membrane protein, LBR. Strikingly, whereas control cells exposed to no peptide, PP1-BD(V155A), or DOTAP alone displayed expected perinuclear staining of the three markers, the majority of cells exposed to PP1-BD (>80% out of 300 examined) reformed nuclei devoid of detectable lamin B (Fig. 2 A). Little or no lamin B was detected in the cytoplasm of these cells (Fig. 2 A; see below). Nevertheless, the cells displayed perinuclear staining of lamins A/C and LBR (Fig. 2 A), indicating that these nuclei were enclosed by an envelope. Further evidence for the assembly of nu-

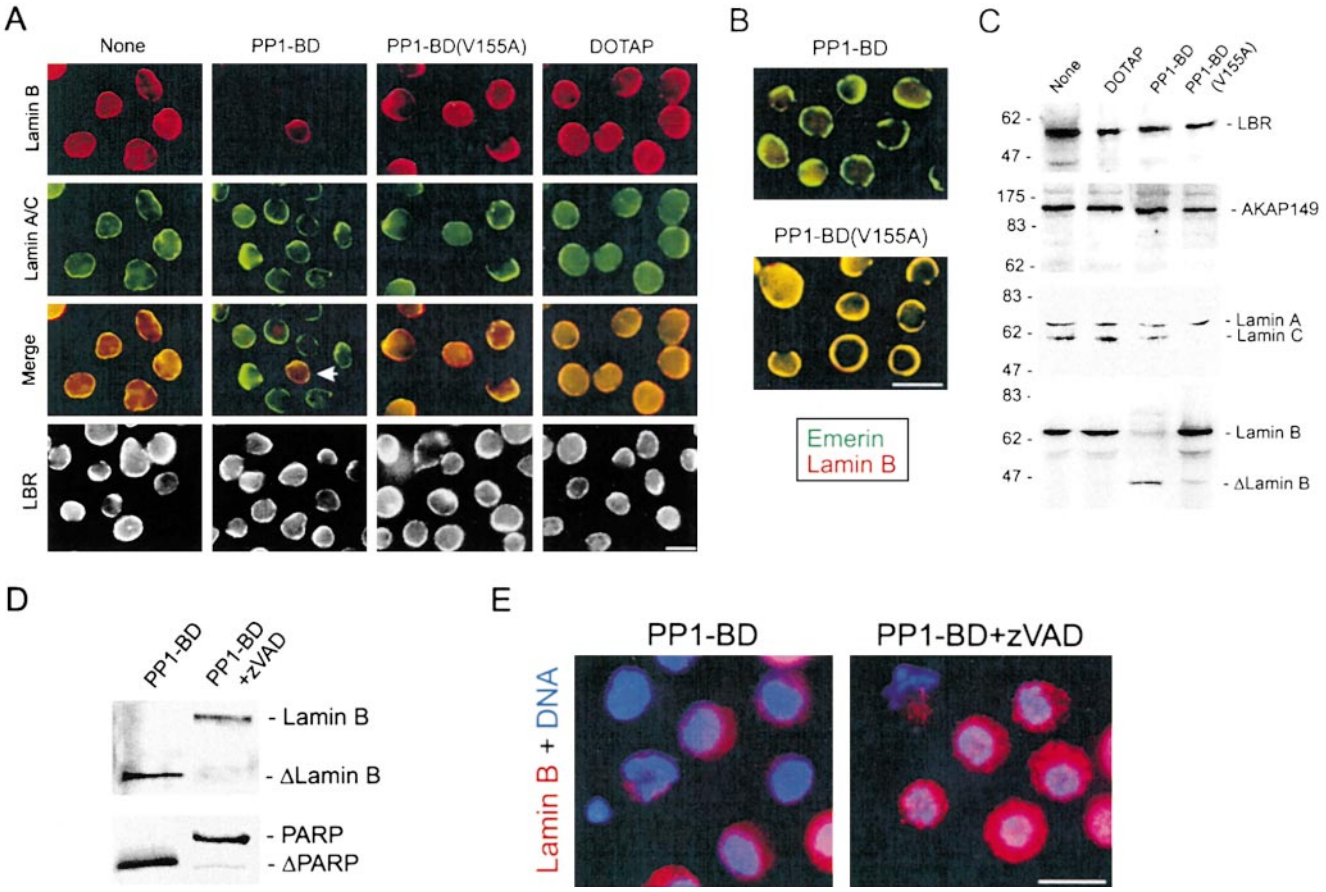

Figure 2. Inhibition of PP1 targeting to the NE abolishes assembly of lamin B, but not lamins A/C or nuclear membranes. Mitotic HeLa cells were exposed to no peptide, PP1-BD, PP1-BD(V155A), or the transfection reagent DOTAP alone, washed, and released from mitotic arrest for 2 h as in the legend to Fig. 1. (A) Distribution of lamin B, lamins A/C, and LBR was analyzed by immunofluorescence. Arrow points to a PP1-BD–treated cell that probably escaped peptide inhibition and displayed lamin B staining. (B) Dual immunofluorescence labeling of emerin (green) and lamin B (red) in HeLa cells exposed to PP1-BD or PP1-BD(V155A) as in A. Merged images are shown. (C) Proteolysis of lamin B not assembled into the NE. Mitotic HeLa cells were exposed to no peptide, DOTAP alone, PP1-BD, or PP1-BD(V155A) as in A, and extracts were immunoblotted using anti-LBR, anti-AKAP149, anti-lamins A/C, and anti-lamin B antibodies. Δlamin B indicates a 45-kD lamin B fragment. (D) The caspase inhibitor zVAD-FMK prevents proteolytic degradation of unassembled lamin B, but does not rescue nuclear reassembly of lamin B. Mitotic HeLa cells were treated with PP1-BD alone or together with 100 μM zVAD-FMK. Excess peptide was removed and cells were cultured for 2 h with zVAD-FMK. Proteolysis of lamin B and PARP was analyzed by immunoblotting. (E) Distribution of lamin B in cells exposed to PP1-BD or PP1-BD plus zVAD-FMK was examined by immunofluorescence (red). DNA was stained with Hoechst 33342 (blue). Bars, 10 μm.

clear membranes in the absence of lamin B targeting to the NE was lent by dual immunofluorescence analysis of another integral protein of the inner nuclear membrane, emerin, and lamin B (Fig. 2 B). Additionally, Western blotting analysis of PP1-BD–treated cells revealed that LBR, AKAP149, and lamins A/C remained intact, as judged by their expected migration in SDS-PAGE (Fig. 2 C). However, lamin B was proteolyzed into a 45-kD product (Fig. 2 C, Δlamin B), reminiscent of the lamin B fragment generated by caspase activity in apoptotic cultured cells (Lazebnik et al., 1995; Takahashi et al., 1996). Therefore, inhibition of PP1 targeting to the NE at the end of mitosis abolishes nuclear assembly of lamin B, and elicits its proteolysis. However, assembly of nuclear membranes and lamins A/C is not affected. Moreover, the data argue that LBR apparently does not depend on B-type lamins for assembly into the NE.

The correlation between failure of lamin B assembly and its degradation raised the issue of whether failure to assemble was a due to degradation of the lamin or, conversely, whether it caused proteolytic processing of the lamin. Fig. 2 D shows that lamin B degradation was prevented in HeLa cells treated with PP1-BD together with 100 μM of the broad caspase inhibitor, zVAD-FMK, and cultured for another 2 h with zVAD-FMK after release from mitotic arrest. zVAD-FMK also inhibited proteolysis of the control caspase 3 substrate, PARP, induced by PP1-BD (Fig. 2 D). When lamin B distribution was examined in cells exposed to PP1-BD or PP1-BD and zVAD-FMK, we found that in neither case did the cells display perinuclear lamin B staining (Fig. 2 E). The labeling was distributed in the cytoplasm, in a punctate manner and with variable intensity. These results indicate that lamin B that fails to assemble into a lamina as a result of PP1-BD treatment is proteolyzed in a caspase-sensitive manner. Caspase-dependent degradation of unassembled lamin B is rapid, and suggests that these cells entered apoptosis (see below). Moreover, although lamin B degradation is prevented with zVAD-FMK, its assembly into the NE remains abolished with the PP1-BD peptide. We concluded that lamin B failed to assemble into a nuclear lamina as a consequence of inhibiting PP1 targeting to the NE, rather than as a result of degradation of the lamin.

Mistargeting of PP1 and Lamin B by PP1-BD Is Followed by Apoptosis

HeLa cells exposed to PP1-BD as described above were monitored at 6 h after release from mitotic arrest. DNA staining revealed nuclear fragmentation in all cells (Fig. 3 A). Moreover, LBR, lamin B, and lamins A/C, normally restricted to the NE in control cells (Fig. 3 A; bottom), were redistributed into the cytoplasm after PP1-BD treatment (top). No obvious alteration in AKAP149 distribution was observed, most likely owing to the NE-ER localization of the protein in healthy cells.

LBR, lamin B, lamins A/C, and AKAP149 were extensively proteolyzed in PP1-BD–treated cells (Fig. 3 B). LBR was cleaved into a 20-kD fragment (ΔLBR), as in apoptotic avian cells (Duband-Goulet et al., 1998). Lamin B remained as a 45-kD product, and lamins A/C were extensively proteolyzed into labile products (Fig. 3 B) (see Lazebnik et al., 1995 for proteolytic lamins A/C fragments). Degradation of AKAP149 into a major 67-kD polypeptide was also observed (Fig. 3 B), as well as complete proteolysis of lamina-associated polypeptide 2β, another integral protein of the inner nuclear membrane (data not shown).

TUNEL analysis revealed extensive DNA fragmentation in nearly 100% of PP1-BD–, but not PP1-BD(V155A)–treated cells (Fig. 3, C and D). This fragmentation was inhibited with 100 μM zVAD-FMK (Fig. 3 D). The similarity of nuclear events observed with the PP1-BD peptide and in apoptotic cultured cells (Lazebnik et al., 1995; Takahashi et al., 1996; Duband-Goulet et al., 1998) establishes the apoptotic nature of cell death resulting from PP1 and lamin B mistargeting to the NE. This observation supports the view that B-type lamins are essential for cell survival. This is consistent with data from *Caenorhabditis elegans*, which encodes a single lamin of B-type and exhibits a null lamin phenotype that is embryonic lethal (Liu et al., 2000). Note, however, that we do not at present exclude the possibility that the PP1-BD inhibitory peptide, by binding to PP1, sequesters the phosphatase and prevents it from dephosphorylating additional targets that are directly responsible for inducing apoptosis.

Mistargeting of Lamin B in Lamins A/C–deficient Lymphoblastic Cells Elicits De Novo Synthesis of Lamins A/C

The effect of preventing lamin B assembly at the end of mitosis in the lamins A/C–deficient KE37 cell line was investigated using the DOTAP-mediated peptide transfection approach developed for HeLa cells. As in HeLa cells, exposure of mitotic KE37 cells to PP1-BD, but not PP1-BD(V155A), abolished lamin B targeting to the NE (Fig. 4 A). As expected, no lamins A/C were detected in control cells; however, PP1-BD–treated cells surprisingly displayed distinct perinuclear labeling of lamins A/C (Fig. 4 A). Synthesis of intact lamins A and C (apparent M_r 70 and 60 kD, respectively) in PP1-BD–treated KE37 cells was confirmed on immunoblots (Fig. 4 B). The blots also showed proteolytic processing of lamin B, whereas LBR and AKAP149 remained intact 2 h after mitotic release (Fig. 4 B). Nevertheless, despite NE targeting of lamins A/C, all KE37 cells exposed to PP1-BD underwent apoptosis within 6 h of mitotic release (Fig. 4 C). This indicates that, as in HeLa cells, inhibition of PP1 recruitment to the NE by AKAP149 in KE37 cells abolishes lamin B assembly. However, inhibition of lamin B assembly in KE37 cells correlates with synthesis and nuclear assembly of lamins A/C. Note that no alteration in the level of lamin A/C proteins was detected upon inhibition of lamin B assembly in HeLa cells (Fig. 2, A and C).

Differential Regulation of A/C- and B-type Lamins

Our results provide evidence for distinct pathways of assembly of lamins A/C and B into the NE. Inhibition of AKAP149-mediated PP1 targeting to the reforming NE prevents assembly of B-type lamins, whereas that of lamins A/C is not affected. Thus, only B-type lamins require AKAP149-mediated concentration of PP1 at the nuclear membrane for assembly. At mitosis, lamins A/C are more soluble than B-type lamins (which remain mostly

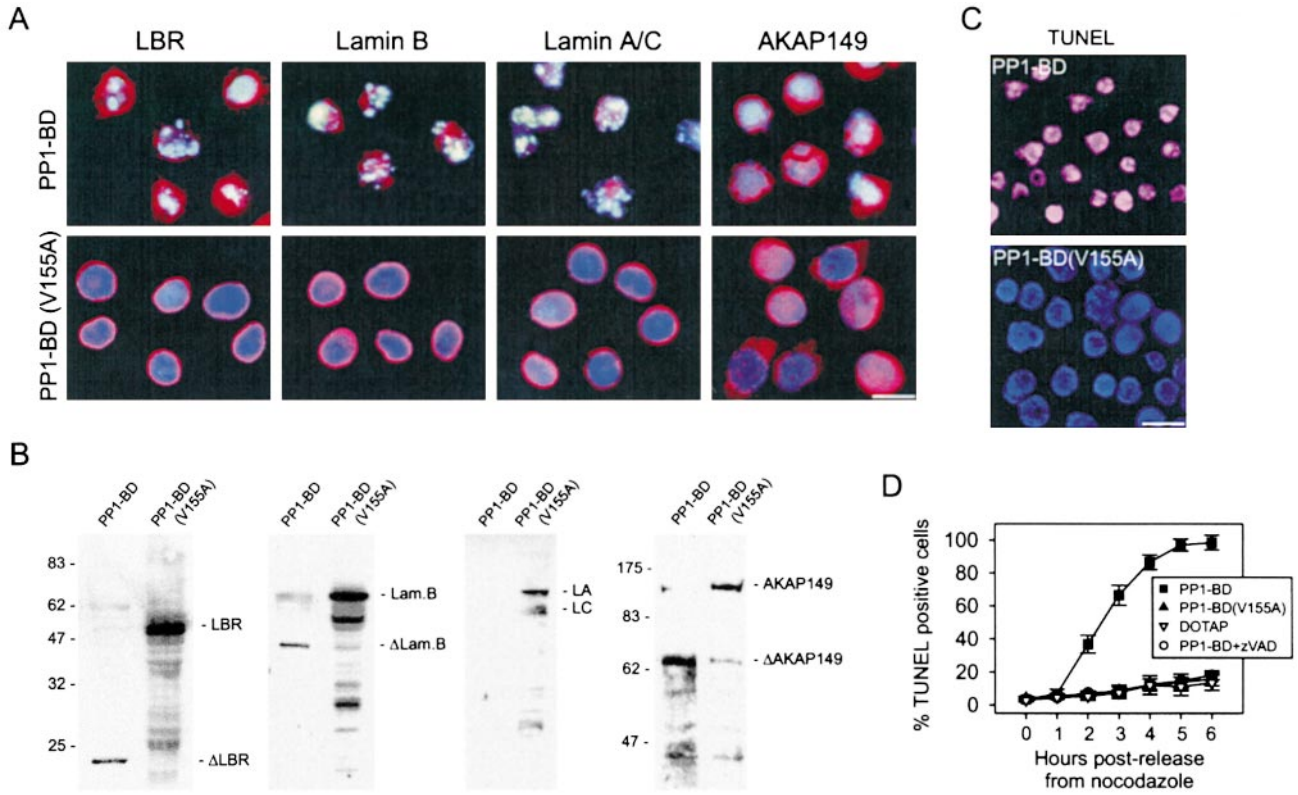

Figure 3. Cells exposed to PP1-BD undergo apoptosis. (A) Mitotic HeLa cells exposed to PP1-BD or PP1-BD(V155A) were washed and cultured for 6 h. Distribution of LBR, lamin B, lamins A/C, and AKAP149 was examined by immunofluorescence (red label). DNA was stained with Hoechst 33342 (blue) to assess nuclear morphology. (B) Cells treated with PP1-BD or PP1-BD(V155A) were analyzed by immunoblotting using anti-LBR, anti-lamin B, anti-lamins A/C, and anti-AKAP149 antibodies. (C) TUNEL analysis of HeLa cells treated with PP1-BD or PP1-BD(V155A). Fluorescein dUTP label was colored in red and was merged with Hoechst DNA staining (blue) in both panels. (D) Proportions of TUNEL-positive cells over time after exposure to either PP1-BD, PP1-BD(V155A), DOTAP alone or PP1-BD plus 100 μM zVAD-FMK (mean ± SD). Bars, 10 μm.

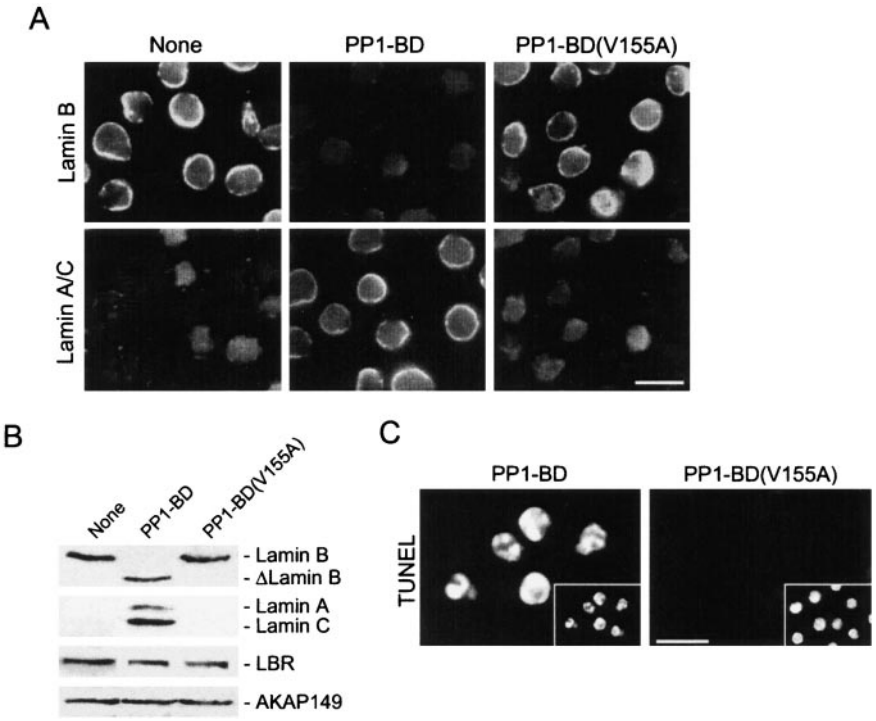

Figure 4. Synthesis of lamins A/C after inhibition of lamin B assembly by PP1-BD in KE37 cells. (A) Mitotic KE37 cells were exposed to either no peptide, PP1-BD, or PP1BD(V155A), washed, and cultured for 2 h. Distribution of lamins B and A/C was analyzed by dual immunofluorescence. (B) Cells were also analyzed by immunoblotting using anti-lamin B, anti-lamins A/C, anti-LBR, and anti-AKAP149 antibodies. (C) TUNEL analysis of KE37 cells exposed to PP1-BD or PP1-BD(V155A) as in A. Insets, DNA staining with Hoechst 33342. Bars, 10 μm.

membrane bound), and thus may not require spatially restricted, elevated concentration of phosphatase activity to be dephosphorylated and polymerize. Additional support for a distinct assembly pathway for A- and B-type lamins emerges from detailed imaging observations of transfected lamins A and B1 assembly in living mammalian cells (Moir et al., 2000). This study showed that whereas lamin B1 concentrates on the chromosome surface in telophase, lamin A is first found in an unpolymerized form in the nucleoplasm before polymerizing into the lamina (Goldman et al., 1992; Moir et al., 2000). Moreover, A- and B-type lamins bind to distinct ligands in the inner nuclear membrane or the chromatin (Wilson, 2000), suggesting distinct targeting during NE reformation. Recently identified lamin-binding proteins such as Narf (Barton and Worman, 1999), AKAP149 (Steen et al., 2000), and lamina-associated polypeptide 2 isoforms (Dechat et al., 2000) suggest that lamina dynamics may be regulated by components in specific nuclear domains.

Differential assembly of A- and B-type lamins at the end of mitosis raises the issue of whether the lamins depend on each other for their assembly. Several nonterminally differentiated cells only harbor B-type lamins (Guilly et al., 1990; Lin and Worman, 1997); therefore, lamins A/C are not required for lamin B assembly. Moreover, we show that lamins A/C assemble even when NE targeting of B-type lamins is abolished, and thus NE targeting of lamins A/C does not require preassembly of a lamina structure. Thus, it becomes clear that A- and B-type lamins do not depend on each other for their assembly. Yet, lamin B–deficient cells undergo apoptosis regardless of nuclear assembly of lamins A/C, indicating that NE targeting of B-type lamins is sine qua non for cell survival.

In contrast to the LMNB1 gene, the LMNA gene is differentially expressed. Although both human LMNB1 and LMNA genes lack typical TATA boxes in their 5′ proximal promoter regions, the LMNA gene contains two atypical TATA-like domains further upstream (Lin and Worman, 1997). These regions may be target sites for negative regulatory elements, as suggested by the role of 5′ proximal promoter regions in the cell type–specific transcription of intermediate filament genes (Oshima, 1992). Under normal conditions, the LMNA gene is not transcribed in KE37 cells (Guilly et al., 1987). Our results suggest a signaling mechanism that derepresses the LMNA gene in response to a mislocalization of B-type lamins at the end of mitosis. The interaction of lamins and lamin-binding proteins with transcriptional activators (Cohen et al., 2001) supports this attractive hypothesis.

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References

Ashkenazi, A., and V.M. Dixit. 1998. Death receptors: signaling and modulation. *Science.* 281:1305–1308.

Barton, R.M., and H.J. Worman. 1999. Prenylated prelamin A interacts with Narf, a novel nuclear protein. *J. Biol. Chem.* 274:30008–30018.

Buendia, B., and J.-C. Courvalin. 1997. Domain-specific disassembly and reassembly of nuclear membranes during mitosis. *Exp. Cell Res.* 230:133–144.

Chaudhary, N., and J.-C. Courvalin. 1993. Stepwise reassembly of the nuclear envelope at the end of mitosis. *J. Cell Biol.* 122:295–306.

Cohen, M., Y. Gruenbaum, K.K. Lee, and K.L. Wilson. 2001. Transcriptional repression, apoptosis, human disease and the functional evolution of the nuclear lamina. *Trends Biochem. Sci.* 26:41–47.

Collas, P., and J.-C. Courvalin. 2000. Sorting of nuclear membrane proteins at mitosis. *Trends Cell Biol.* 10:5–8.

Collas, P., K. Le Guellec, and K. Tasken. 1999. The A-kinase anchoring protein, AKAP95, is a multivalent protein with a key role in chromatin condensation at mitosis. *J. Cell Biol.* 147:1167–1180.

Dechat, T., S. Vlcek, and R. Foisner. 2000. Review: lamina-associated polypeptide 2 isoforms and related proteins in cell cycle-dependent nuclear structure dynamics. *J. Struct. Biol.* 129:335–345.

Duband-Goulet, I., J.C. Courvalin, and B. Buendia. 1998. LBR, a chromatin and lamin binding protein from the inner nuclear membrane, is proteolyzed at late stages of apoptosis. *J. Cell Sci.* 111:1441–1451.

Earnshaw, W.C. 1995. Nuclear changes in apoptosis. *Curr. Opin. Cell Biol.* 7:337–343.

Ellis, D.J., H. Jenkins, W.G. Whitfield, and C.J. Hutchison. 1997. GST-lamin fusion proteins act as dominant negative mutants in *Xenopus* egg extract and reveal the function of the lamina in DNA replication. *J. Cell Sci.* 110:2507–2518.

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

Gruenbaum, Y., K.L. Wilson, A. Harel, M. Goldberg, and M. Cohen. 2000. Review: nuclear lamins--structural proteins with fundamental functions. *J. Struct. Biol.* 129:313–323.

Guilly, M.-N., A. Bensusan, J.F. Bourge, M. Bornens, and J.-C. Courvalin. 1987. A human T lymphoblastic cell line lacks lamins A and C. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:3795–3799.

Guilly, M.-N., J.-P. Kobl, F. Gosti, F. Godeau, and J.-C. Courvalin. 1990. Lamins A and C are not expressed at early stages of human lymphocyte differentiation. *Exp. Cell Res.* 189:145–147.

Kaufmann, S.H., and W.C. Earnshaw. 2000. Induction of apoptosis by cancer chemotherapy. *Exp. Cell Res.* 256:42–49.

Lazebnik, Y.A., A. Takahashi, R.D. Moir, R.D. Goldman, G.G. Poirier, S.H. Kaufmann, and W.C. Earnshaw. 1995. Studies of the lamin proteinase reveal multiple parallel biochemical pathways during apoptotic execution. *Proc. Natl. Acad. Sci. USA.* 92:9042–9046.

Lin, F., and H.J. Worman. 1997. Expression of nuclear lamins in human tissues and cancer cell lines and transcription from the promoters of the lamins A/C and B1 genes. *Exp. Cell Res.* 236:378–384.

Liu, J., T.R. Ben-Shahar, D. Riemer, M. Treinin, P. Spann, K. Weber, A. Fire, and Y. Gruenbaum. 2000. Essential roles for *Caenorhabditis elegans* lamin gene in nuclear organization, cell cycle progression, and spatial organization of nuclear pore complexes. *Mol. Biol. Cell.* 11:3937–3947.

Manilal, S., C.A. Sewry, A. Pereboev, N. Man, P. Gobbi, S. Hawkes, D.R. Love, and G.E. Morris. 1999. Distribution of emerin and lamins in the heart and implications for Emery-Dreifuss muscular dystrophy. *Hum. Mol. Genet.* 8:353–359.

Moir, R.D., M. Yoon, S. Khuon, and R.D. Goldman. 2000. Nuclear lamins A and B1. Different pathways of assembly during nuclear envelope formation in living cells. *J. Cell Biol.* 151:1155–1168.

Oshima, R.G. 1992. Intermediate filament molecular biology. *Curr. Opin. Cell Biol.* 4:110–116.

Spann, T.P., R.D. Moir, A.E. Goldman, R. Stick, and R.D. Goldman. 1997. Disruption of nuclear lamin organization alters the distribution of replication factors and inhibits DNA synthesis. *J. Cell Biol.* 136:1201–1212.

Steen, R.L., S.B. Martins, K. Tasken, and P. Collas. 2000. Recruitment of protein phosphatase 1 to the nuclear envelope by A-kinase anchoring protein AKAP149 is a prerequisite for nuclear lamina assembly. *J. Cell Biol.* 150: 1251–1262.

Takahashi, A., E.S. Alnemri, Y.A. Lazebnik, T. Fernandes-Alnemri, G. Litwack, R.D. Moir, R.D. Goldman, G.G. Poirier, S.H. Kaufmann, and W.C. Earnshaw. 1996. Cleavage of lamin A by Mch2 alpha but not CPP32: multiple interleukin 1 beta-converting enzyme-related proteases with distinct substrate recognition properties are active in apoptosis. *Proc. Natl. Acad. Sci. USA.* 93:8395–8400.

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