Comment

Lamins and Apoptosis: A Two-Way Street?

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The nuclear lamina is a thin (20 nm) yet insoluble protein meshwork that, in higher cells, lines the nucleoplasmic face of the nuclear envelope (NE).[1] The lamina is intimately associated with both the inner nuclear membrane and underlying chromatin, while at the same time providing anchoring sites for nuclear pore complexes (Gerace and Burke, 1988). Because of these extensive interactions, the lamina has long been considered to play an important role in the maintenance of nuclear architecture. This notion has been lent considerable weight in recent years by the findings that lamina and NE defects are linked to a number of human diseases (Wilson, 2000). Steen and Collas (2001) (this issue) now provide some tantalizing data that links nuclear lamina organization to cell survival.

The major components of the nuclear lamina are the A- and B-type lamins. These are intermediate filament protein family members (Stuurman et al., 1998) that feature a central coiled-coil flanked by nonhelical head and tail domains. In mammalian somatic cells, there are four major lamins, A, B1, B2, and C. The B-type lamins are encoded by separate genes (LMNB1 and LMNB2) and, as a class, are found in the nuclei of all mammalian somatic cells. Lamins A and C, in contrast, arise through alternative splicing of the same primary transcript encoded by the LMNA gene, expression of which is developmentally regulated. In the mouse, lamins A and C are absent from the early embryo and only appear later during development (Stewart and Burke, 1987; Roeber et al., 1989). Indeed, some cell types never express A-type lamins. Clearly then, A-type lamins are not strictly required for the formation of a nuclear lamina and NE. However, neither are they entirely dispensable. While ablation of the LMNA gene in mice causes no overt developmental abnormalities, it does lead to seriously retarded postnatal growth linked to cardiomyopathy and muscular dystrophy (Sullivan et al., 1999). This phenotype is associated with large-scale changes in nuclear architecture. Similar effects have been observed in both *Drosophila* and *Caenorhabditis elegans*, where changes in lamin expression lead to gross nuclear structural abnormalities (Lenz-Böhme et al., 1997; Liu et al., 2000). In humans, defects in the LMNA gene have now been linked to forms of muscular dystrophy and cardiomyopathy as well as to partial lipodystrophy, a disorder affecting adipocyte function (Cohen et al., 2001).

During mitosis in higher cells, the NE must be disassembled for the condensed chromosomes to gain access to the mitotic spindle (Moir et al., 2000a). Disassembly of the lamina is initiated by phosphorylation of S and T residues at either end of the lamin coiled-coil domain (Heald and McKeon, 1990; Peter et al., 1990; Ward and Kirschner, 1990). This eventually leads to dispersal of A- and B-type lamin homooligomers throughout the mitotic cell (Gerace and Blobel, 1980). In telophase, the dispersed lamins are recycled to form NEs in each daughter cell. Steen et al. (2000) have previously shown that reassembly of B-type lamins is under the control of both protein phosphatase 1 (PP1) and an A-kinase anchoring protein, AKAP149. The latter is a membrane protein localized to both the ER and nuclear membranes, and contains a specific binding site for PP1. During mitosis, PP1 appears largely chromatin bound, but in telophase it is recruited to the nuclear periphery to dephosphorylate B-type lamins and thereby enable polymerization and lamina assembly. Steen et al. (2000) have demonstrated in vitro that PP1 targeting to the NE involves binding to AKAP149. If this binding is inhibited using a short peptide corresponding to the AKAP149 PP1-binding domain (PP1-BD), then PP1 recruitment to the nuclear periphery does not occur and B-type lamin assembly is effectively abolished. A control peptide containing a V to A substitution [PP1-BD(V155A)] has no effect on this process. This model (Fig. 1) predicts that B-type lamins can only polymerize after the reforming nuclear membranes reassociate with chromatin, since membranes are needed to provide AKAP149 activity. In fact, lagging assembly of B-type lamins has been reported in studies using green fluorescent protein–tagged lamin B (Moir et al., 2000b).

Steen and Collas (2001) have now extended these studies to include intact cells. The approach taken was to employ lipid micelles to introduce either inhibitory or control peptides into HeLa cells arrested in mitosis. Their remarkable results have implications for apoptosis (programmed
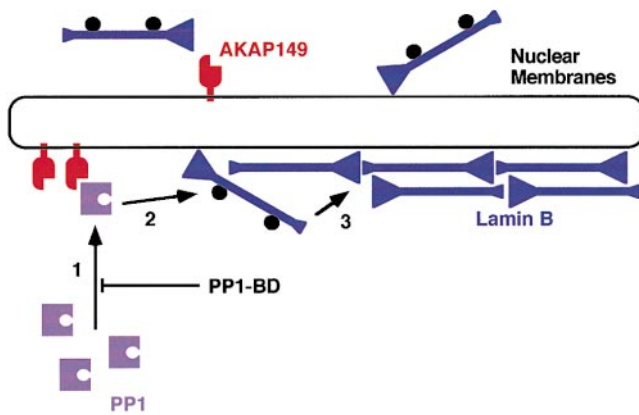
Figure 1. The proposed roles of PP1 in nuclear lamina reformation. In mitotic telophase, PP1 is recruited to the nuclear periphery (1) via its interaction with AKAP149, a step that is blocked by PP1-BD. PP1 then dephosphorylates B-type lamins (2), allowing them to assemble (3). Disassembled B-type lamins are depicted as predominantly membrane associated during mitosis. (●) Phosphorylated sites within lamin B.

cell death), as well as nuclear assembly. After introduction of PP1-BD and subsequent release from mitosis, they observed that association of PP1 with the nuclear envelope was blocked and there was a profound inhibition of B-type lamin reassembly. At the same time, A-type lamin assembly was unaffected and a NE still formed. These results exactly match their earlier in vitro data. However, in PP1-BD–treated cells that entered early G1, B-type lamins were rapidly degraded in a caspase-dependent manner. Inhibition of caspase activity revealed that the bulk of the lamin B (both B1 and B2) was mislocalized to the cytoplasm, excluding the trivial possibility that failure to incorporate lamin B into the nuclear lamina was due to lamin degradation. Other NE proteins, including A-type lamins and both emerin and lamin B receptor (two integral inner nuclear membrane proteins), remained intact and were correctly localized in the presence of PP1-BD. However, ~6 h after release from mitosis, proteolysis of these proteins commenced. This was accompanied by DNA and nuclear fragmentation and the appearance of highly condensed chromatin, all hallmarks of apoptosis. Thus, PP1-BD treatment of cells before exit from mitosis elicited a delayed apoptotic response. Steen and Collas (2001) speculate that the failure to assemble B-type lamins directly triggers apoptosis, although an additional PP1-dependent process unrelated to lamin assembly cannot yet be ruled out. This is clearly an issue that needs to be addressed.

Since a NE does assemble in PP1-BD–treated HeLa cells, there can be at best only a minimal requirement for B-type lamins in this process. To determine whether a lamina was required at all for NE reformation, Steen and Collas (2001) examined the effects of PP1-BD on KE37 lymphoblasts, a cell type that does not express A-type lamins. As in HeLa cells, PP1-BD blocked lamin B reassembly during telophase, and the lymphoblasts underwent apoptosis 6 h later. Then came a surprise: when they labeled the KE37 cells with antibodies against lamins A and C they found both of these proteins to be present at the nuclear periphery of those cells that had received PP1-BD,

but not in cells treated with the control peptide! It would appear that failure to recruit PP1 to the nuclear periphery at the end of mitosis resulted in the induction of A-type lamin synthesis! Although yet to be demonstrated conclusively, this effect is likely attributable to the inhibition of B-type lamin assembly. It is as if the cell attempts to compensate for failure to assemble B-type lamins by upregulating A-type lamin expression. However, this is ultimately a futile exercise since the cells are committed to apoptosis. This is the first example of LMNA gene activation outside the context of differentiation. The implication is that lamin gene expression may be regulated by the assembly state of the nuclear lamina and/or by unassembled lamin proteins.

The last few years have witnessed a surge of interest in the biology of the NE driven in part by the findings that several human diseases are linked to defects in both the LMNA and emerin genes. It has become increasingly clear that the nuclear lamina plays a key role in maintenance not only of nuclear envelope integrity but of nuclear architecture as a whole (Cohen et al., 2001). Loss of lamin gene expression has been linked to gross changes in nuclear shape and redistribution of heterochromatin (Sullivan et al., 1999). These findings, plus lamin interactions with transcriptional repressors such as Rb, further suggest that lamins could potentially modify global patterns of transcription (Cohen et al., 2001). Now Steen and Collas (2001) have provided some compelling evidence for a link between lamin B status and the induction of LMNA expression. In cells programmed to die, caspase-dependent degradation of lamins has been recognized as a prelude to nuclear destruction (Lazebnik et al., 1995). This new work further suggests that not only is lamin degradation a feature of apoptosis, but that failure to correctly assemble a nuclear lamina is actually a trigger of apoptosis. Clearly, there is still a lot we have to learn about nuclear lamin function.

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