

# What makes a cell tick? The A, B and C of the matter

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**Nuclear lamina in an eukaryotic cell is primarily composed of the lamins A, B and C. The A type lamins are found only in differentiated cell types while the B type lamins are present both in differentiated and undifferentiated cells. Lamin B interacts with the inner nuclear membrane. In recent years there have been extensive studies on the relationship between the dynamic state of lamin B and the nuclear envelope integrity with respect to the fate of a particular cell. In this article, we have analysed the recent developments and have considered the sequence of events that might be contributing to the fate of a cell either to undergo normal cell division or uncontrolled cellular proliferation or apoptosis.**

A variety of morphological and biochemical studies carried out over the years have established that the nuclear lamins play an important role in nuclear structure and dynamics. The nuclear lamina forms a fibrous structure at the periphery of the nucleus and is physically interposed between the inner surface of the nuclear envelope and chromatin. Its critical position in the nucleus attributes various important functions to this structure<sup>1</sup>. Lamina is generally believed to provide structural support in maintaining and modulating the nuclear shape and volume. During interphase, the lamina is a plausible site for the attachment of chromatin to the nuclear envelope which helps in maintaining chromosomes in separate domains within the nucleus. The lamins belong to the intermediate family of proteins being similar to the latter, both in their primary amino acid sequence and secondary structure<sup>2</sup>. They are organized into three well-defined domains, namely, the central  $\alpha$ -helical rod (made up of three  $\alpha$ -helical coils 1A, 1B and 2), an amino terminal head and a carboxy terminal tail (Figure 1). Mammalian cells contain three major proteins, lamins A, B and C along with a fourth minor protein B<sub>2</sub> (ref. 3). Lamins A and C arise by alternate splicing of the same gene, and are classified under A type lamins<sup>4</sup>. Lamin B is coded by a separate gene. The A type lamins are found only in differentiated cell types, while the B type lamins are found both in differentiated and undifferentiated cells<sup>5</sup>.

## Differential expression of lamin subtypes

Cell type expression of different lamins was first noted in *Xenopus*<sup>6</sup> which was subsequently documented in a study using mouse embryogenesis<sup>7</sup>. The acquisition of nuclear lamin polypeptides A/C varied according to the developmental stage and tissue type. Using monoclonal antibodies specific to each of the lamins, they were localized by immunofluorescence technique in different tissues. At days 9 to 11, all tissues of the developing embryo lacked lamins A/C. Their expression was detected only at the time when tissue differentiation had commenced. Moreover, lamins A/C were not simultaneously expressed in all organs at the same time. However, at days 9 to 11 lamin B was expressed. So clearly, while a single lamin was sufficient to form a nuclear lamina in some cell types, the acquisition of lamins A/C during

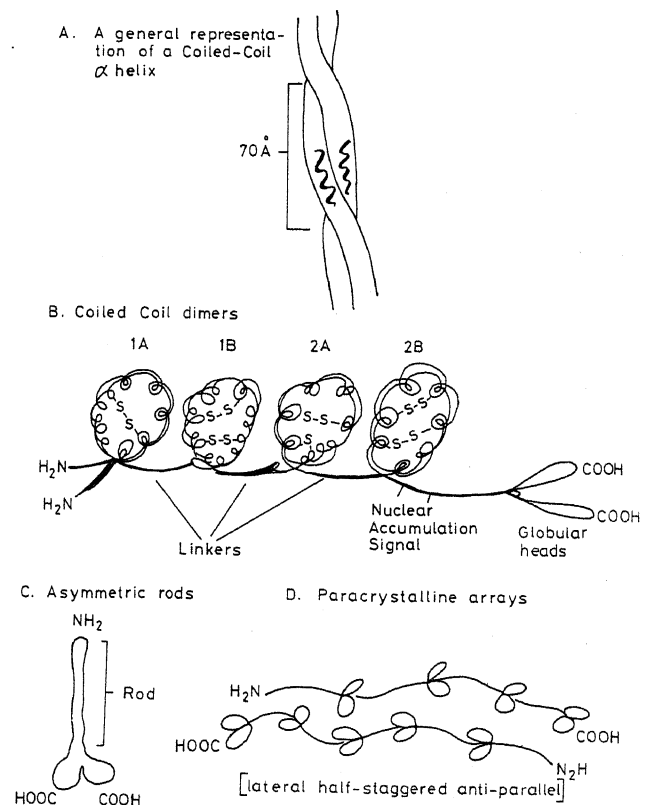


Figure 1. Structural details of lamin polypeptides.

development may be actually responsible for significant changes in the nuclear architecture and chromatin organization. Recently, additional B type lamins have been discovered which are germ cell specific. Earlier studies by Stick and Schwarz<sup>8</sup> demonstrated that there are no remnants of the lamina structure in the meiotic prophase pachytene spermatocytes. Nor could they detect any lamina structure in the post-meiotic haploid round spermatids. All these studies used antibodies raised against somatic lamins. Our laboratory, however, had identified a 110 kDa protein specific to nuclear matrix of pachytene spermatocytes of rat<sup>9</sup>. Subsequently, this protein was shown to be a dimer of a 60 kDa protein which was similar but not identical to lamin B, based on peptide finger-printing analysis<sup>10</sup>. Interestingly, this protein was absent in the somatic tissues but could be detected in female rat germ cells<sup>11</sup>. At about the same time Furukawa and Hotta<sup>12</sup> cloned a mouse spermatocyte lamin B, which they have termed as lamin B<sub>3</sub>. Interestingly, the carboxy terminal domain and coil 2 subdomain are identical to the corresponding regions of lamin B<sub>1</sub> and B<sub>2</sub> but the short head domain and coils 1a and 1b are completely replaced by an unrelated sequence. Ectopic expression of mouse lamin B<sub>3</sub> resulted in a hook-shaped nucleus. We showed by using monoclonal antibodies to the 60 kDa rat germ cell specific lamin, that it was localized to ends of the paired homologous chromosomes<sup>13</sup> suggesting that it may be providing attachment sites for the paired chromosomes. The structural aspects of various lamins have been discussed in detail<sup>14</sup>. In this article we have discussed briefly the recent developments on the dynamics of lamina structure during 'cell cycle' and 'apoptosis'.

### Phosphorylation of lamins during cell cycle

Mitosis in higher eukaryotes is characterized by a dramatic reorganization of cellular architecture including the formation of a mitotic spindle, chromatin condensation and nuclear envelope breakdown. At the onset of mitosis lamins are phosphorylated. This leads to solubilization of lamins A and C resulting in the breakdown of the nuclear envelope. On the other hand lamin B remains bound to the membrane vesicles<sup>15</sup>. Among the various kinases identified so far, p34cdc2/cyclin B kinase (MPF) is a likely candidate for phosphorylating the lamins at a conserved Ser-23 residue in several systems<sup>16</sup>. However, recent *in vitro* studies have demonstrated that direct phosphorylation of human lamin B1 at Ser<sub>395</sub> and Ser<sub>405</sub> by human  $\beta$ Ipkc could also cause lamin solubilization and subsequent nuclear envelope breakdown<sup>17</sup>. Further, these investigations show that although both  $\beta$ Ipkc as well as p34cdc2 kinase could phosphorylate soluble lamin B at similar rates *in vitro*,  $\beta$ Ipkc phosphorylated interphase nuclear envelope lamin B at a rate 200 times greater than p34cdc2/cyclin

B kinase. The possible involvement of  $\beta$ Ipkc in the phosphorylation of lamin B is also supported by the observation that this kinase translocates into the nucleus during G2/M phase transition<sup>18</sup>.

While the role of lamin phosphorylation during mitosis is reasonably well understood, the functional significance of lamin phosphorylation in interphase is not clear. *In vitro* studies indicate that several protein kinases are capable of phosphorylating lamins like protein kinase C, protein kinase A and MAP kinase<sup>19</sup>. *In vivo* picture of phosphorylation of lamins became clear in 1993 when it was found that the major phosphorylation site was located in the C-terminus of chicken lamin B<sub>2</sub> (ref. 20). These sites are phosphorylated both *in vitro* and *in vivo* by protein kinase C. The phosphorylation of PKC itself (subject to hormonal regulation) caused the inhibition of nuclear transport rate of lamin B<sub>2</sub>.

### Isoprenylation of lamins

Lamins also undergo another important post-translational modification at the C-terminus. Mature lamins A and B differ in the extent to which they interact with the inner nuclear membrane. Lamins A and B have a conserved C-terminus sequence CAAX (A, aliphatic and X, any amino acid). This sequence is the major determinant site for isoprenylation and carboxymethylation<sup>21</sup>. Attachment of an isoprenyl group occurs at cysteine by a thioester bond. Subsequently, AAX is removed by proteolytic cleavage. Finally, the isoprenylated C-terminal cysteine is carboxymethylated. The A type lamins undergo a further proteolytic cleavage that results in the loss of this hydrophobically modified C-terminus. Thus, the A type lamins are easily solubilized during nuclear envelope breakdown while the B type lamins remain attached to the membrane vesicles.

### Interaction of lamin B with the inner nuclear membrane

Recent studies have shown that the interaction of lamins to other nuclear proteins may have a crucial role in their function. Using a solution binding assay, <sup>125</sup>I-lamin B was shown to bind lamin depleted avian erythrocyte nuclear membrane in a saturable and specific manner. One of the proteins with which lamin B interacted was identified as p58, a major integral membrane protein of the nuclear envelope<sup>22</sup>. It has been speculated that the p58 might serve as a receptor for lamin B. The observation that lamins A and C did not bind to p58 as efficiently as lamin B probably explains why lamin B is found attached to the nuclear membrane in all cell types. The lamin B receptor, p58, of turkey erythrocytes possesses a long and highly charged amino terminal domain protruding into the nucleus. It has 8 membrane spanning

segments and a hydrophilic carboxy-terminal region. In addition, its amino-terminal domain (205 a.a residues) contains several potential phosphorylation sites and DNA binding sequences<sup>23</sup>. During interphase, p58 is continuously phosphorylated while during mitosis, it is hyper-phosphorylated<sup>24</sup>. The p58 complex also contains a p58 kinase and 3 other polypeptides p34, p32 and p18. p34 is a homologue of human nuclear protein p32 which has been characterized as a partner of splicing factor SF2 in HeLa cells<sup>25</sup>. A recent report on the gene structure of p58 (ref. 26) shows that an intron separates two domains, one encoding a highly charged amino terminal domain and the other encoding the hydrophobic trans-membrane domain. In addition to the p58 receptor being the target of lamin B interaction (in the inner nuclear membrane), a class of lamin binding proteins have been recently identified called as 'LAPS' (Lamin associated polypeptides). They comprise chiefly of LAP 1A (75 kDa), 1B (68 kDa), 1C (55 kDa) and LAP 2 (53 kDa) (ref. 27). LAPs 1A and 1B were shown to bind to the paracrystals of lamins A, B and C, *in vitro*. LAP2, on the other hand, interacted solely with lamin B polymers as well as with the metaphase chromosomes. LAP1 C binds to both lamins A and C. The functions of these polypeptides, however, are not clear at present.

### Lamina structure and apoptosis

An important question that is gaining importance over the last few years is as to what determines the fate of a cell? For instance, a normal cell goes through the cell cycle comprising of G1, G2, S and M phase. A somatic cell of higher eukaryotes has to pass through a 'restriction point' before it is ready to divide at the G2/M transition. Several feedback control mechanisms enable a cell to complete various processes preceding cell division<sup>28</sup>. Embryonic cells on the other hand, go through the cell cycle without passing through the restriction point as cell division at this stage is rapid and unrestricted (programmed cell proliferation). During development, in many species, it is crucial for cells to divide and ultimately die to enable retention of only a certain type of cells to continue for later development. Cell death here is predetermined, or programmed (apoptosis), enabling appropriate tissue differentiation<sup>29</sup>, in contrast to unprogrammed or pathological cell death, which is different in cell morphology to the former.

A classical case of the suicide programme has been demonstrated in *C. elegans*. Of the one thousand ninety somatic cells formed during development of an adult hermaphrodite, one hundred and thirty one die, each with morphological features resembling apoptosis. Genetic analyses have identified two genes (*ced3* and *ced4*) that must function in dying cells. Mutations in these genes lead to survival of the worms and they develop into superficially normal worms<sup>30</sup>. *Ced3* codes for a

protein with many phosphorylation sites. *Ced9* is another gene identified in this organism that is responsible for preventing cell suicide. Interestingly, this nematode possesses a single lamin (C lam) belonging to the B type lamins<sup>31</sup>. However, it lacks the SPTR sequence in front of the coil 1A domain which constitutes the major phosphorylation site by cdc2 kinase. It remains to be seen whether the lack of this phosphorylation site in C lam is in anyway related to the suicide program prevalent in this organism.

Many hormone-dependent tissues are also shown to undergo programmed cell death upon hormone withdrawal<sup>32</sup>. In an interesting study, when MDA-MB 468 human breast cancer cells were made to overexpress the EGF receptors, their growth was inhibited by EGF<sup>33</sup>. It also resulted in fragmentation of DNA and an appearance of apoptotic nuclear morphology. An increased expression of *C-myc*, *C-fos* and *C-Jun* was also noticed. In addition, there was a noticeable proteolytic cleavage of poly(ADP ribose) polymerase and lamins.

As mentioned earlier, lamins are modified through post-translational modifications like phosphorylation and isoprenylation. Therefore, what is the nature of these modifications of lamins in cells undergoing apoptosis? This question was addressed in a recent article wherein confluent cultures of embryonic fibroblast cell line were made to undergo either mitosis or apoptosis depending on the serum concentration<sup>34</sup>. In mitotic cells, lamina breakdown is accompanied by lamina solubilization while in apoptotic cells, only a small percentage of lamins were solubilized at the initial stages. Subsequently, chromatin condensation was shown to be accompanied by degradation of lamins A and B to a 46 kDa fragment. However, there was no significant increase in the activation of p34cdc2 kinase at any time after the onset of apoptosis. Therefore, it appears that chromatin condensation during apoptosis is due to the rapid proteolysis of lamins and clearly did not involve p34cdc2 kinase. It remains to be seen whether  $\beta$ II protein kinase C has any function in this disassembly or not. Degradation of nuclear lamins has also been observed in physiological cell death mediated by cytotoxic T lymphocytes<sup>35</sup>. Isoprenylation of lamins also seems to play an important role in apoptosis. For example, it was recently shown that inhibition of mevalonate biosynthesis in HL-60 cells by lovastatin resulted in changes in nuclear morphology characteristic of apoptotic cells, including the DNA fragmentation pattern<sup>36</sup>.

### Lamins and uncontrolled cell proliferation

The expression of lamins in unprogrammed cell proliferation has been reported in small lung carcinomas<sup>37</sup>. There was a high expression of  $\beta$ -tubulin, heat shock proteins and lamin B. The study indicated that B type

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lamin could be an early marker for commitment to cell proliferation. The status of lamins under these conditions indicated that phosphorylation of these proteins could ultimately result from cell proliferation signals. Protein kinase C, with its numerous isotypes are involved in cell proliferation and differentiation in human leukemic cells<sup>38</sup>. For example, in human promyelocytic HL-60 leukemic cells,  $\beta$ II protein kinase C is selectively translocated to the nucleus in response to proliferation stimuli. As discussed earlier it has been shown that  $\beta$ II protein kinase C phosphorylates nuclear envelope bound lamin B. 10–20 times faster than  $\alpha$  protein kinase C and

200 times faster than p34 cdc kinase. Therefore, phosphorylation of lamin B may be a crucial event to transmit the proliferation signal.

A lot of evidence has accumulated indicating that *de novo* cholesterologenesis plays an important role in cell growth and proliferation<sup>39</sup>. A clear correlation exists between HMG CoA reductase activity and cell proliferation. Adult tissues that were stimulated to divide had increased levels of HMG CoA reductase activity and an accelerated cholesterol biosynthesis. This marked increase in the HMG CoA reductase activity was consistently observed at or just before peaks of DNA synthesis

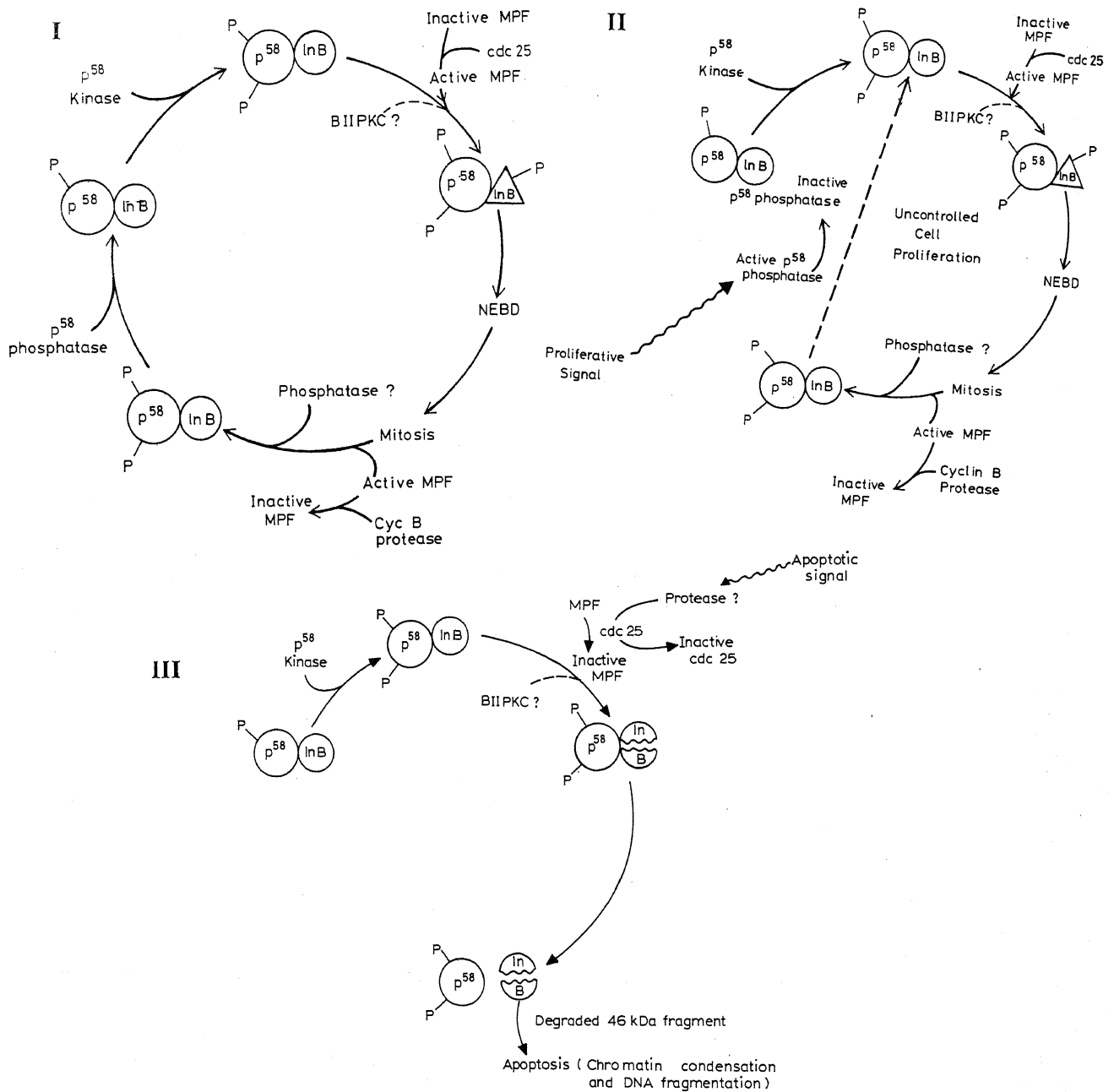


Figure 2. Case I, Cell undergoing mitosis; Case II, Signal for cell proliferation; Case III, Signal for cellular apoptosis.

in BHK-21 cell cultures. When HMG CoA reductase activity was suppressed by a competitive inhibitor, compactin, the normal S-phase burst of DNA synthesis was specifically and totally prevented. Addition of mevalonate resulted in reversal of this inhibition. However, addition of cholesterol-rich lipoproteins could not reverse this inhibition. Therefore, mevalonate or a product of mevalonate seems to be essential for the initiation of DNA synthesis and progression of the cell cycle. Thus, mevalonate after conversion into isoprene units, is incorporated into a number of cellular polypeptides including p21 ras and lamins. In recent years several reports have appeared on the anticarcinogenic effects of isoprenoid constituents present in food like fruits and vegetables<sup>40</sup>. One of the explanations for such an anticarcinogenic activity has been that these isoprenoids could induce hepatic phase II detoxifying processes, leading to the regression of tumours. Another possibility is that an inhibitor 'mevinolin' present in these food sources depletes the cells of products of the mevalonate pathway. Consequently, proteins like lamins and p21ras remain unmodified preventing cell proliferation.

The above discussion suggests that the lamins may act as 'conductors' of a master signal involved in the control circuitry of cellular fate. The entire lamina network in association with the nuclear envelope components, may act as a receiver of major signals which govern whether the cell should divide normally passing through all stages of the cell cycle or lose its control over the restriction point and divide rapidly, or die ultimately. We would like to consider the following events occurring at the nuclear lamina during different physiological states of the cell. It is highly likely that the lamina-p58 complex comprises of at least 3 other polypeptides which may include a p58 kinase, a p58 phosphatase and a protease. Under normal conditions in mitotically dividing cells, towards the end of G2-phase, p58 kinase phosphorylates the p58 receptor, which now becomes hyperphosphorylated. Upon activation of the MPF, the lamin B (which is bound to the hyperphosphorylated p58 receptor) gets phosphorylated at a critical state. This could lead to an altered conformation in lamin B resulting in the solubilization of the complex and ultimately leading to nuclear envelope breakdown. This process leads to open mitosis. At the end of telophase, MPF gets inactivated by the activation of a cyclin B protease. Lamin B gets dephosphorylated, leading to relaxation of the altered lamin B conformation. Ultimately, p58 phosphatase dephosphorylates p58 and the cells resume interphase (Figure 2, Case I).

A signal for the cell to proliferate may cause the inactivation of a member of this p58 complex, probably the p58 phosphatase. This results in p58 remaining in a hyperphosphorylated form (necessary for mitosis). The cell then enters a closed cycle of numerous mitotic phases, possibly due to loss of restriction point (triggered by

p58 dephosphorylation) thus resulting in uncontrolled cell proliferation (Figure 2, Case II).

An apoptotic signal, on the other hand, may trigger the following sequence of events. The signal may trigger the protease component in the p58 complex, leading to the inactivation of cdc25 phosphatase. As a consequence, cdc-25 fails to dephosphorylate Tyrosine 15 of MPF, leaving it in an inactivated state. This leads to lamin B remaining in a dephosphorylated state. In these circumstances, lamin B may become susceptible to a specific protease, resulting in the degradation of lamin B to the 46 kDa fragment. Such a cascade of events may set off the process of apoptosis (Figure 2, Case III). A chromatin-binding domain has been identified in lamin B recently<sup>41</sup> and the degradation of lamin B to its 46 kDa fragment may facilitate the condensation of chromatin normally observed during apoptosis.

### Concluding remarks

Thus, it appears that the lamina structure and, in particular, the interaction of lamin B with the nuclear envelope associated polypeptides plays a key role in directing the cells to undergo either uncontrolled cellular proliferation like in a cancer cell or apoptosis.

1. Glass, J. R. and Gerace, L., *J. Cell Biol.*, 1990, **111**, 1047-1057.
2. Fischer, D. E., Chaudary, N. and Blobel, G., *Proc. Natl. Acad. Sci. USA*, 1986, **83**, 6450-6454.
3. Weber, K., Plessman, U. and Traub, P., *FEBS Lett.*, 1990, **261**, 361-364.
4. McKeon, F. D., Kirschner, M. W. and Caput, D., *Nature*, 1986, **319**, 463-468.
5. Stewart, C. and Burke, B., *Cell*, 1987, **51**, 383-392.
6. Krohne, G. and Benavente, R., *Exp. Cell Res.*, 1986, **162**, 1-10.
7. Rober, R. A., Weber, K. and Osborne, M., *Development*, 1989, **105**, 365-378.
8. Stick, R. and Schwarz, H., *Cell Differ.*, 1982, **11**, 235-243.
9. Behal, A., Kulkarni, P. and Rao, M. R. S., *J. Biol. Chem.*, 1987, **262**, 10898-10902.
10. Sudhakar, L. and Rao, M. R. S., *J. Biol. Chem.*, 1990, **265**, 22526-22532.
11. Sudhakar, L., Sivakumar, N., Behal, A. and Rao, M. R. S., *Exp. Cell Res.*, 1991, **198**, 78-84.
12. Furukawa, L. and Hotta, Y., *EMBO J.*, 1993, **12**, 97-106.
13. Manjula, K., Karande, A. and Rao, M. R. S., *Cell Struct. Funct.*, 1994, **19**, 207-218.
14. Nigg, E., *Curr. Opin. Cell Biol.*, 1992, **4**, 105-109.
15. Gerace, L. and Blobel, G., *Cell*, 1980, **19**, 277-287.
16. Ward, G. F. and Kirschner, M. W., *Cell*, 1990, **61**, 561-577.
17. Hocevar, B. A., Burns, D. J. and Fields, A. P., *J. Biol. Chem.*, 1993, **268**, 7545-7552.
18. Goss, U. L., Hocevar, B. A., Thompson, J., Stratton, C. A., Burns, D. J. and Fields, A. P., *J. Biol. Chem.*, 1994, **269**, 19074-19080.
19. Peter, M., Sanghera, J. S., Pelech, S. L. and Nigg, E. A., *Eur. J. Biochem.*, 1992, **205**, 287-294.
20. Hennekes, H., Peter, M., Weber, K. and Nigg, E. A., *J. Cell Biol.*, 1993, **120**, 1293-1304.
21. Moores, S. L., Schaber, M. D., Mosser, S. D., Rands, E., O'Hara, M. B., Garsky, V. M., Marshall, M. S., Pompliano, D. L. and Gibbs, J. B., *J. Biol. Chem.*, 1991, **266**, 14603-14610.

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22. Worman, H. J., Yuan, J., Blobel, G. and Georgatos, S. D., *Proc. Natl. Acad. Sci. USA*, 1988, **85**, 8531-8534.
23. Worman, H. J., Evans, C. D. and Blobel, G., *J. Cell Biol.*, 1990, **111**, 1535-1542.
24. Appelbaum, J., Blobel, G. and Georgatos, S. D., *J. Biol. Chem.*, 1990, **265**, 4181-4184.
25. Krainer, A. R., Mayeda, A., Kozak, D. and Binns, G., *Cell*, 1991, **66**, 383-394.
26. Schuler, E., Lin, F. and Worman, H. J., *Mol. Biol. Cell*, 1993, **4**, 308 (a) (abstract).
27. Georgatos, S. D., Meir, J. and Simos, G., *Curr. Opin. Cell Biol.*, 1994, **6**, 347-353.
28. Hartwell, L. H. and Weinert, T. A. *Science*, 1989, **246**, 629-634.
29. Raff, M. C., *Nature*, 1992, **356**, 397-400.
30. Hedgecock, E. M., Sulston, J. E. and Thomson, J. N., *Science*, 1983, **220**, 1277-1279.
31. Reimer, D., Dodemont, H. and Weber, K., *Eur. J. Cell Biol.*, 1994, **62**, 214-223.
32. Wylie, A. H., *Arch. Toxicol. Suppl.*, 1987, **11**, 3-10.
33. Armstrong, D. K., Kaufmann, S. H., Ottaviano, Y. L., Furuya, Y., Bickley, J. A., Isaacs, J. T. and Davidson, N. E., *Cancer Res.*, 1994, **54**, 5280-5283.
34. Oberhammer, F. A., Hochegger, K., Froschl, G. and Tiefenbacher, R., *J. Cell Biol.*, 1994, **126**, 827-837.
35. Ucker, D. S., Obermiller, P. S., Eckhart, W., Appgar, J. R., Berger, N. A. and Meyers, J., *Mol. Cell Biol.*, 1992, **12**, 3060-3069.
36. Perez-Sala, D. and Mollinedo, F., *BBRC*, 1994, **199**, 1209-1215.
37. Broers, J. L. and Ramaeckers, F. C., *Int. J. Cancer Suppl. (US)*, 1994, **8**, 134.
38. Murray, N. R., Burns, D. J. and Fields, A. P., *J. Biol. Chem.*, 1994, **269**, 21385-21390.
39. Quesney-Hunecus, V., Wiley, H. M. and Siperstein, M. D., *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 5056-5060.
40. Elson, C. E. and Yu, S. G., *J. Nutr. US*, 1994, **124**, 607-614.
41. Taniura, H., Glass, C. and Gerace, L., *J. Cell Biol.*, 1995, **131**, 33-44.

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