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Are B-type lamins essential in all mammalian cells?

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The B-type lamins are widely assumed to be essential for mammalian cells. In part, this assumption is based on a highly cited study that found that RNAi-mediated knockdown of lamin B1 or lamin B2 in HeLa cells arrested cell growth and led to apoptosis. Studies indicating that B-type lamins play roles in DNA replication, the formation of the mitotic spindle, chromatin organization and regulation of gene expression have fueled the notion that B-type lamins must be essential. But surprisingly, this idea had never been tested with genetic approaches. Earlier this year, a research group from UCLA reported the development of genetically modified mice that lack expression of both *Lmnb1* and *Lmnb2* in skin keratinocytes (a cell type that proliferates rapidly and participates in complex developmental programs). They reasoned that if lamins B1 and B2 were truly essential, then keratinocyte-specific lamin B1/lamin B2 knockout mice would exhibit severe pathology. Contrary to expectations, the skin and hair of lamin B1/lamin B2-deficient mice were quite normal, indicating that the B-type lamins are dispensable in some cell types. The same UCLA research group has gone on to show that lamin B1 and lamin B2 are critical for neuronal migration in the developing brain and for neuronal survival. The absence of either lamin B1 or lamin B2, or the absence of both B-type lamins, results in severe neurodevelopmental abnormalities.

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The main protein constituents of the nuclear lamina in mammals are lamins A, C, B1 and B2.[1,2] Lamins A and C are alternatively spliced isoforms of *LMNA*,[3] while lamins B1 and B2 are products of independent genes (*LMNB1* and *LMNB2*, respectively).[4-6] The B-type lamins are expressed in most cell types, starting early in development, while the A-type lamins are expressed mainly in differentiated cells and generally later in development.[7-9]

Over the past few years, the general perception has been that the B-type lamins play essential roles in mammalian cells, while A-type lamins are important for more specialized functions in differentiated cells. For example, in a recent review on nuclear lamins, Broers et al.[10] wrote, "expression of B-type lamins is essential for nuclear integrity, cell survival and normal development," adding that "B-type lamins are the fundamental building blocks of the nuclear lamina, while the A-type lamins have more specialized functions." Numerous studies underlie the notion that the B-type lamins are particularly important for cells. One highly cited study reported that RNAi-mediated knockdown of either *LMNB1* or *LMNB2* in HeLa cells blocked cell growth and induced apoptosis, whereas a knockdown of *LMNA* did not.[11] The authors concluded that lamins B1 and B2 are essential proteins in mammalian cells. Many other studies have provided indirect support for this idea. For example, by studying lamin B localization in CHO cells, Belmont et al.[12] concluded that the B-type lamins

are likely important for organizing heterochromatin at the nuclear rim. Other investigators have suggested that the B-type lamins have unique and vital roles in chromatin organization, DNA replication and gene transcription.[13-15] Another study indicated that the B-type lamins are crucial for the organization and function of the mitotic spindle,[16] while another reported that a knockdown of lamin B1 in HeLa cells compromised the formation of nucleoli.[17] Together, these disparate cell biology studies suggested that mammalian cells would be unlikely to thrive in the absence of B-type lamins.

Recent Insights from *Lmnb1* and *Lmnb2* Knockout Mice

Studies of conventional knockout mice have complemented the aforementioned cell biology studies. A *Lmna* knockout did not interfere with development but led to premature death from cardiomyopathy/muscular dystrophy by ~6 weeks of age,[9] implying that lamins A/C are not required for cell growth or development but are essential for the function of certain differentiated cell types. In contrast, *Lmnb1*- and *Lmnb2*-deficient mice do not survive beyond birth. *Lmnb1*-deficient mice were initially generated from a gene-trap ES cell clone that led to the production of a lamin B1–βgeo fusion protein.[18] *Lmnb1*-deficient fibroblasts grew in culture, but they exhibited misshapen nuclei, premature senescence, and aneuploidy.18 Lmnb1-deficient embryos were small and died soon after birth with immature lungs and abnormalities in the cranium and other bones. And, as discussed further in the last few paragraphs of this article, Coffinier and coworkers have shown that lamin B1 deficiency leads to severe neurodevelopmental abnormalities.

Coffinier et al.[19,20] also created *Lmnb2*-deficient mice and found that those mice also died at birth. *Lmnb2*-deficient embryos were virtually normal in size, and the only noteworthy pathological findings were severe neurodevelopmental defects involving the cerebral cortex and cerebellum. In contrast to lamin B1-deficient fibroblasts, *Lmnb2*-deficient fibroblasts grew normally in culture and were free of misshapen nuclei and chromosomal abnormalities.[20]

The fact that both the *Lmnb1*-deficient mice and *Lmnb2*-deficient mice survived through embryonic development—and that fibroblasts from these mice grew in culture—implied that neither lamin B1 nor lamin B2 is absolutely required for the viability of mammalian cells. But this conclusion was subject to caveats. First, in the case of *Lmnb1*, there was a formal possibility that the lamin B1–βgeo fusion protein retained partial function, permitting "lamin B1–deficient" cells to survive. Subsequent studies by Coffinier and coworkers have excluded that caveat as a serious consideration. Second, it seemed possible that the two B-type lamins might play redundant functions, and that the capacity of *Lmnb1*-deficient embryos and *Lmnb2*-deficient embryos to survive until birth reflected their capacity to produce one of the two B-type lamins. Functional redundancy of the two B-type lamins is not a farfetched possibility; lamins B1 and B2 are both intermediate filament proteins of the nuclear lamina, and their amino acid sequences are much more similar to each other than to the A-type lamins.[4] Also, both of the B-type lamins share a carboxyl-terminal farnesyl lipid anchor (unlike mature lamin A or lamin C).

The field of human genetics has yielded few insights into the functional relevance of the B-type lamins. Unlike *LMNA*, where hundreds of missense, nonsense and frameshift mutations have been identified and characterized,[21] no one has yet uncovered any clinically significant loss-of-function mutations in *LMNB1* or *LMNB2*. The only bona fide association of B-type lamins to human disease is *LMNB1* gene duplications in patients with adult-onset autosomal-dominant leukodystrophy.[22] The absence of loss-of-function mutations in *LMNB1* and *LMNB2* (at least so far) suggests several possibilities. One—which is consistent with the knockout mice results—is that the loss of lamin B1 or lamin B2 in humans is incompatible with postnatal life. A second possibility is that the two B-type lamins are functionally redundant and therefore the loss of one is fully compensated by the other. Still another possibility is that neither B-type lamin is crucial as long as *LMNA* is expressed at a respectable level.

Keratinocyte-Specific *Lmnb1* and *Lmnb2* Knockout Mice

Lingering uncertainties about the in vivo relevance of the B-type lamins prompted Yang et al.[23] to investigate the effects of inactivating both *Lmnb1* and *Lmnb2* in specific tissues. They generated conditional knockout alleles for both genes ($Lmnb1^{fl}$ and $Lmnb2^{fl}$) and then took advantage of a well-characterized keratin 14-*Cre* (*K14-Cre*) transgene[24,25] to create mice lacking both *Lmnb1* and *Lmnb2* in skin keratinocytes. They chose to examine skin keratinocytes for two reasons. First, the formation of a cornified epithelium and the appendages of the skin (e.g., hair, nails) involve complex developmental programs and they imagined that these programs would be compromised by the loss of the B-type lamins. Second, skin keratinocytes proliferate rapidly and they suspected that a deficiency of the B-type lamins would interfere with cell growth, particularly in light of the literature suggesting that B-type lamins are important for DNA replication and gene transcription.[13-15]

The newly created *Lmnb1* and *Lmnb2* conditional knockout alleles worked as planned, and the *K14-Cre* transgene led to high levels of recombination in keratinocytes.[23] But surprisingly, the keratinocyte-specific double knockout mice ($Lmnb1^{fl/fl}Lmnb2^{fl/fl}K14Cre$ mice, designated $Lmnb1^{\Delta/\Delta}Lmnb2^{\Delta/\Delta}$) survived development and appeared normal at birth.[23] The skin keratinocytes of $Lmnb1^{\Delta/\Delta}$ $Lmnb2^{\Delta/\Delta}$ mice express negligible levels of *Lmnb1* and *Lmnb2* transcripts, as judged by qRT-PCR (**Fig. 1A and B**) and lamin B1 and lamin B2 proteins were undetectable by protein gel blots (**Fig. 1C**). The absence of both B-type lamins was confirmed by immunocytochemistry in freshly isolated $Lmnb1^{\Delta/\Delta}Lmnb2^{\Delta/\Delta}$ keratinocytes (**Fig. 1D**) and by immunohistochemistry in skin biopsies from $Lmnb1^{\Delta/\Delta}$ $Lmnb2^{\Delta/\Delta}$ mice (**Fig. 1E**). Occasional misshapen nuclei, mainly cells with nuclear blebs, were observed in cultured $Lmnb1^{\Delta/\Delta}$ $Lmnb2^{\Delta/\Delta}$ keratinocytes (**Fig. 2**), but

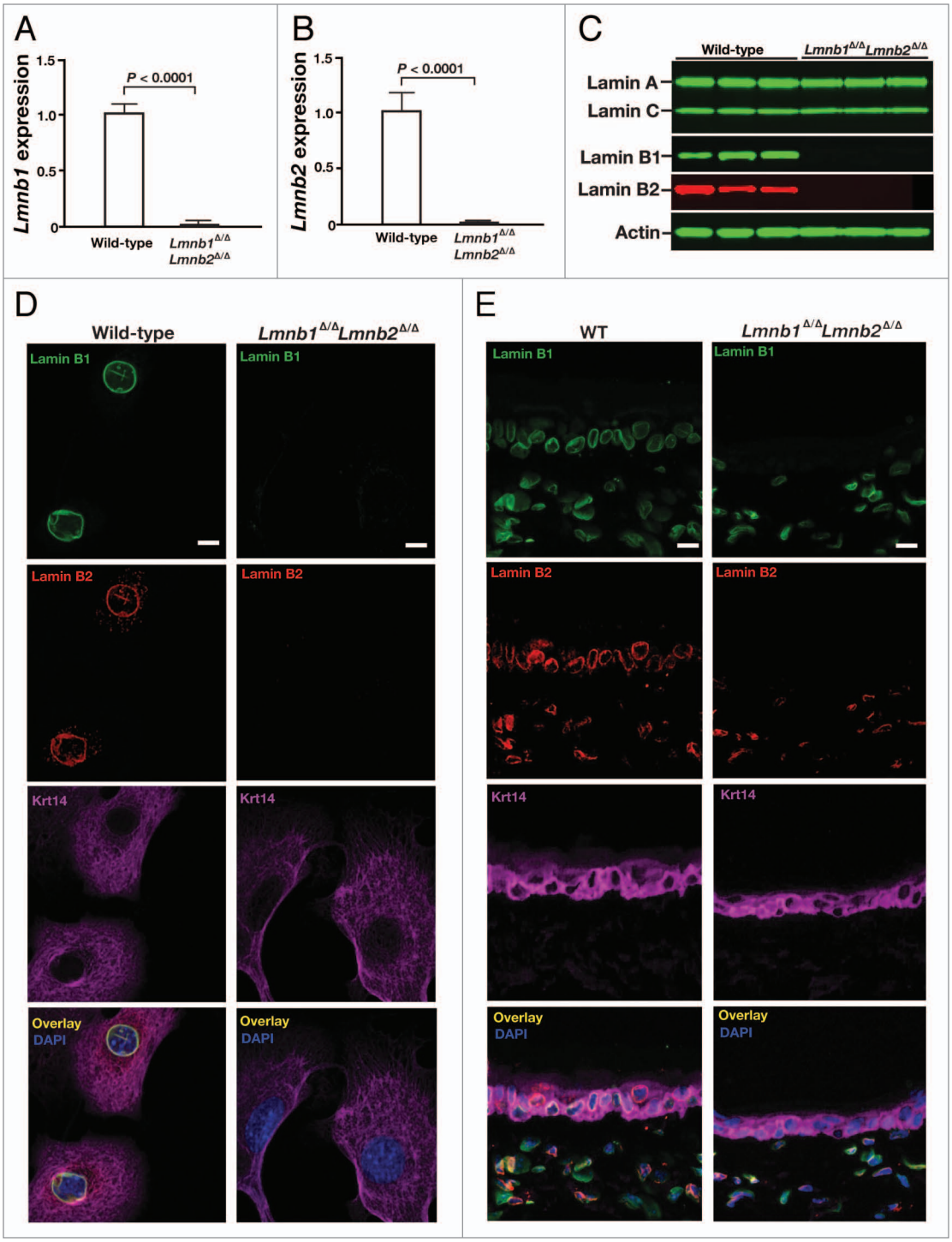

Figure 1. Inactivation of *Lmnb1* and *Lmnb2* in skin keratinocytes. (A, B) qRT-PCR studies of *Lmnb1* (A) and *Lmnb2* (B) expression in keratinocytes from wild-type and $Lmnb1^{fl/fl}Lmnb2^{fl/fl}K14Cre$ ($Lmnb1^{\Delta/\Delta}Lmnb2^{\Delta/\Delta}$) mice (n = 4/genotype). Data were normalized to β2-microglobulin and gene expression was compared with that in the $Lmnb1^{+/+}$ mice (which was set at 1.0). (C) protein gel blot analysis of lamin expression in keratinocytes isolated from wild-type and $Lmnb1^{\Delta/\Delta}Lmnb2^{\Delta/\Delta}$ mice (n = 3/group) with antibodies against lamin A/C, lamin B1, and lamin B2. Actin was used as a loading control. (D, E) Immunofluorescence microscopy of primary keratinocytes (D) and ear skin (E) from wild-type (WT) and $Lmnb1^{\Delta/\Delta}Lmnb2^{\Delta/\Delta}$ mice (4 mice/group were examined) with antibodies against lamin B1 (green), lamin B2 (red), and keratin 14 (magenta). DNA was visualized with DAPI (blue). Scale bar, 20 μm. Reproduced with permission from Yang et al.[23]

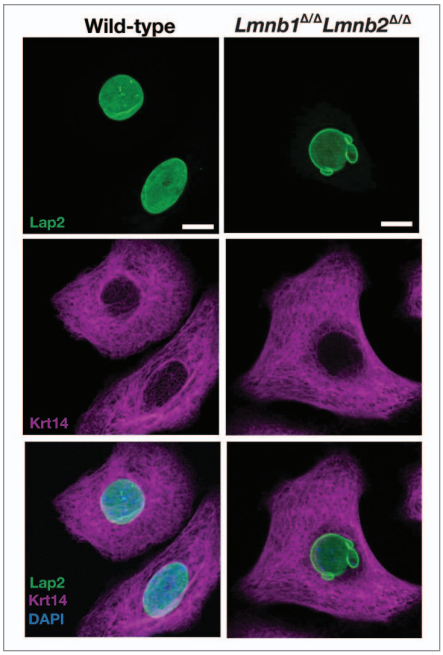

Figure 2. Immunohistochemical staining of keratinocytes from wild-type and *Lmnb1*$^{\Delta/\Delta}$ *Lmnb2*$^{\Delta/\Delta}$ mice with antibodies against Lap2 (green) and keratin 14 (Krt14; magenta), revealing an increased frequency of nuclear blebbing in *Lmnb1*$^{\Delta/\Delta}$*Lmnb2*$^{\Delta/\Delta}$ keratinocytes. DNA was visualized with DAPI (blue). Reproduced with permission from Yang et al.[23]

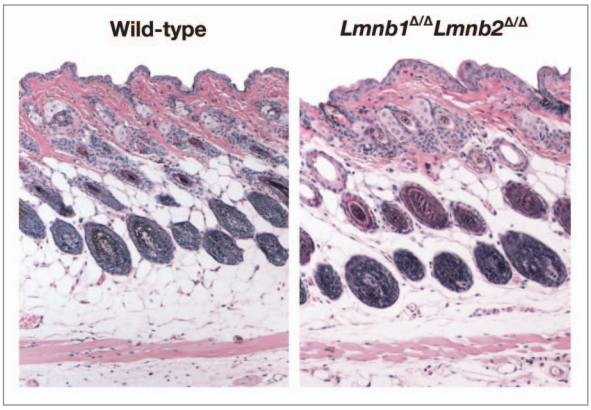

Figure 3. Hematoxylin and eosin staining on skin sections from 40-d-old wild-type and *Lmnb1*$^{\Delta/\Delta}$ *Lmnb2*$^{\Delta/\Delta}$ mice. Ten × magnification. Reproduced with permission from Yang et al.[23]

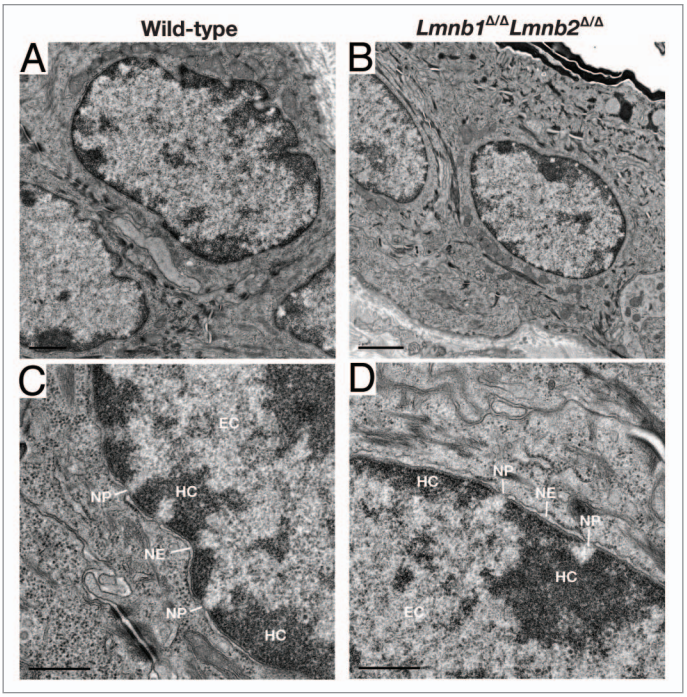

Figure 4. Electron micrographs of keratinocyte nuclei in the skin from wild-type and *Lmnb1*$^{\Delta/\Delta}$ *Lmnb2*$^{\Delta/\Delta}$ mice, showing heterochromatin (HC), euchromatin (EC), nuclear pores (NP), and nuclear envelope (NE). Magnification in A–B, × 22700. Scale bar, 1.0 µm. Magnification in C–D, × 74200. Scale bar, 0.5 µm. Reproduced with permission from Yang et al.[23]

nuclear shape abnormalities were never observed in skin biopsies, as judged by immunofluorescence microscopy.[23]

The absence of lamin B1 and lamin B2 in skin keratinocytes had no apparent deleterious consequences. *Lmnb1*$^{\Delta/\Delta}$ *Lmnb2*$^{\Delta/\Delta}$ mice survived for > 2 y and the skin, hair and nails were indistinguishable from those of wild-type mice.[23] The histology of the skin and hair was entirely normal, as judged by hematoxylin and eosin-stained histological sections (**Fig. 3**). The proliferation of keratinocytes in the skin of *Lmnb1*$^{\Delta/\Delta}$*Lmnb2*$^{\Delta/\Delta}$ mice was normal, as judged by BrdU incorporation, and the growth of cultured *Lmnb1*$^{\Delta/\Delta}$*Lmnb2*$^{\Delta/\Delta}$ keratinocytes was also normal.[23] Unlike lamin B1-deficient fibroblasts described by Vergnes et al.,[18] *Lmnb1*$^{\Delta/\Delta}$*Lmnb2*$^{\Delta/\Delta}$ keratinocytes did not exhibit aneuploidy. Interestingly, wild-type and *Lmnb1*$^{\Delta/\Delta}$*Lmnb2*$^{\Delta/\Delta}$ keratinocytes were indistinguishable by electron microscopy (**Fig. 4**) and in particular, the distribution of heterochromatin at the periphery of the cell nucleus was similar.

In parallel, Yang et al.[23] isolated embryonic fibroblasts lacking both lamin B1 and lamin B2 (by treating *Lmnb1*$^{fl/fl}$*Lmnb2*$^{fl/fl}$ fibroblasts with *Cre*-adenovirus), immortalized the fibroblasts by serial passaging, and uncovered no defects in growth or chromosome number. The complete absence of *Lmnb1* and *Lmnb2* expression did not result in upregulation of *Lmna* expression. Those observations strongly support the dispensability of B-type lamins in skin keratinocytes and fibroblasts and challenged the notion that B-type lamins have unique and essential roles in DNA replication, cell proliferation, or gene expression.[13-15]

Knocking Out Both *Lmnb1* and *Lmnb2* in Hepatocytes

We considered the possibility that the dispensability of B-type lamins in

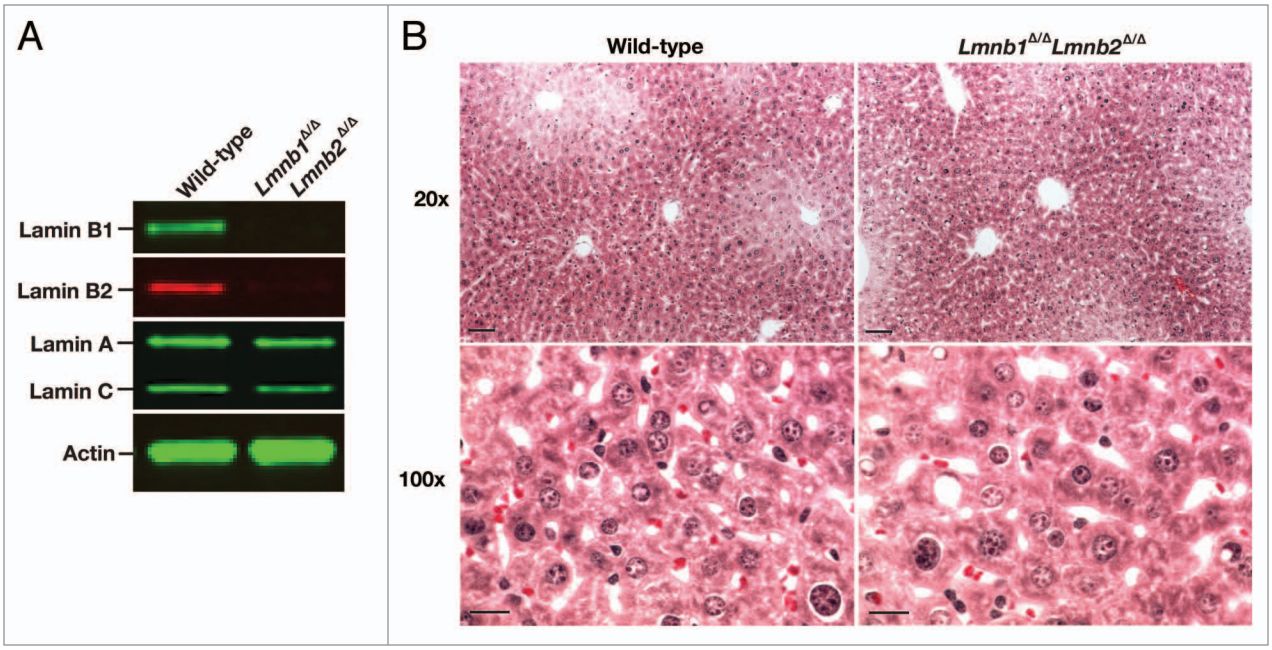

Figure 5. Inactivation of *Lmnb1* and *Lmnb2* in hepatocytes. (A) Protein gel blot analysis of lamin expression in hepatocytes isolated from wild-type (WT) and Hep-*Lmnb1*$^{\Delta/\Delta}$*Lmnb2*$^{\Delta/\Delta}$ mice (n = 3/group) with antibodies against lamin A/C, lamin B1 and lamin B2. Actin was used as a loading control. (B) Hematoxylin and eosin–stained liver sections from 6-mo-old wild-type and Hep-*Lmnb1*$^{\Delta/\Delta}$*Lmnb2*$^{\Delta/\Delta}$ mice. Upper panel: 20 × magnification. Scale bar: 30 μm. Lower panel: 100 × magnification. Scale bar: 10 μm.

keratinocytes might be a peculiarity of that cell type. Therefore, we took advantage of an albumin-Cre transgenic line[26] and created mice lacking both lamin B1 and lamin B2 in liver hepatocytes (*Lmnb1*$^{fl/fl}$*Lmnb2*$^{fl/fl}$*AlbCre*, or Hep-*Lmnb1*$^{\Delta/\Delta}$*Lmnb2*$^{\Delta/\Delta}$). Once again, Cre-mediated recombination was efficient; levels of *Lmnb1* and *Lmnb2* transcripts were negligible in Hep-*Lmnb1*$^{\Delta/\Delta}$*Lmnb2*$^{\Delta/\Delta}$ hepatocytes and lamin B1 and lamin B2 proteins were virtually undetectable (**Fig. 5A**). The Hep-*Lmnb1*$^{\Delta\Delta}$*Lmnb2*$^{\Delta/\Delta}$ mice exhibited normal vitality and liver function tests were entirely normal (**Table 1**). Liver histology was normal in Hep-*Lmnb1*$^{\Delta/\Delta}$*Lmnb2*$^{\Delta/\Delta}$ mice, as judged by hematoxylin and eosin-stained sections (**Fig. 5B**). Misshapen nuclei were not observed in frozen sections of liver from Hep-*Lmnb1*$^{\Delta/\Delta}$*Lmnb2*$^{\Delta/\Delta}$ mice, although we did observe an increased frequency of nuclear blebs in cultured Hep-*Lmnb1*$^{\Delta/\Delta}$*Lmnb2*$^{\Delta/\Delta}$ hepatocytes (**Fig. 6**).

The fact that nuclear blebs were more frequent in isolated *Lmnb1*$^{\Delta/\Delta}$*Lmnb2*$^{\Delta/\Delta}$ keratinocytes and hepatocytes implies that the B-type lamins play a role in the structural integrity of the nuclear lamina. We were not surprised, however, by the failure to detect misshapen nuclei in the tissues of the mutant mice. Earlier, our group found severe nuclear shape abnormalities in fibroblasts from mice that synthesize exclusively mature lamin A ("mature lamin A-only" or *Lmna*$^{LAO/LAO}$ mice); however, we could not identify any abnormalities in the cell nuclei in tissues of *Lmna*$^{LAO/LAO}$ mice.[27] We suspect that growing cells on plastic surfaces, where the nuclei tend to be pancake-shaped, makes it easier to detect subtle changes in the integrity of the nuclear envelope.

Table 1. Evaluation of liver function in wild-type and Hep-Lmnb1$^{\Delta/\Delta}$Lmnb2$^{\Delta/\Delta}$ mice

Serum Chemistry	Wild-type	Hep-Lmnb1$^{\Delta/\Delta}$Lmnb2$^{\Delta/\Delta}$	Normal
ALT (U/L)	137 ± 96	80 ± 57	7–227
ALP	83 ± 6	92 ± 12	13–291
AST (U/L)	169 ± 120	134 ± 67	37–329
CHOL (mg/dl)	80 ± 9	73 ± 19	34–129
GLU (mg/dl)	222 ± 36	242 ± 52	46–279
ALB (g/dl)	2.35 ± 0.21	2.3 ± 0.28	2.5–5.4
DBILI (mg/dl)	0.20 ± 0.04	0.24 ± 0.09	0–0.3
TBILI (mg/dl)	0.67 ± 0.15	0.3 ± 0.07	0.1–1.1

ALT, alanine transaminase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; CHOL, cholesterol; GLU, glucose; ALB, albumin; DBILI, direct bilirubin; TBILI, total bilirubin

Interpretation of Experiments with Tissue-Specific *Lmnb1/Lmnb2* Knockout Mice

Keratinocytes and hepatocytes were free of significant pathology in the absence of the B-type lamins, suggesting that these proteins are dispensable in those cell types. Given the host of publications pointing to many key functions of the B-type lamins in the cell nucleus, how should these knockout studies be

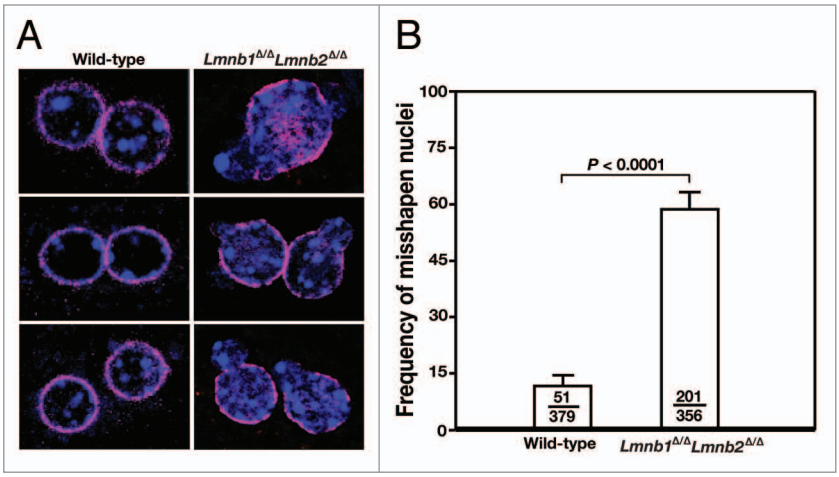

Figure 6. Increased frequency of nuclear blebbing in Hep-*Lmnb1*$^{\Delta/\Delta}$*Lmnb2*$^{\Delta/\Delta}$ hepatocytes. (A) Immunohistochemical staining of hepatocytes from wild-type and Lmnb1Δ/ΔLmnb2Δ/Δ mice with antibodies against lamin A (magenta); DNA was visualized with DAPI (blue). (B) Frequency of misshapen nuclei in wild-type and Hep-*Lmnb1*$^{\Delta/\Delta}$*Lmnb2*$^{\Delta/\Delta}$ hepatocytes (n = 3/genotype tested). Bars indicate the frequency of misshapen nuclei; the number of cells with nuclear blebs and the total number of cells examined are provided within each bar. Hep-*Lmnb1*$^{\Delta/\Delta}$*Lmnb2*$^{\Delta/\Delta}$ hepatocytes had more nuclear blebs ($p < 0.0001$, chi-square test).

interpreted? Can one reconcile the earlier cell culture studies with the absence of phenotypes in tissue-specific knockout mice? Some might be tempted to conclude that the B-type lamins have no roles in DNA replication, gene transcription, organization of the mitotic spindle, etc. and that the earlier reports were simply incorrect, but we would advise against jumping to this sort of conclusion. First, the studies by Yang et al.[23] only assessed cell viability and gross phenotypes and more subtle studies of nuclear functions are yet to be performed. One could also argue that the significance of the mouse experiments were limited because no efforts were made to "stress the system" (e.g., no wound healing or liver regeneration studies were performed). This type of criticism is reasonable, but on the other hand, it is noteworthy that both the keratin 14-*Cre* and albumin-*Cre* transgenes are expressed early in development (by E14.5–15.5).[26,28] Thus, deficiencies of the B-type lamins appear to have little effect on the vitality of the liver or skin during periods of robust differentiation and cell growth. Also, the studies by Yang et al.[23] examined only two differentiated cell types and it is entirely possible that unique functions of the B-type lamins would be more obvious in other tissues. Third and most importantly, the B-type lamins have been conserved through vertebrate evolution, and we seriously doubt that this would have occurred in the absence of unique and important functions. At this point, more research is needed to identify and define those functions.

It is important to emphasize that keratinocytes and hepatocytes—the cell types where the B-type lamins appear dispensable—represent differentiated cells that express not only the B-type lamins but also lamins A and C. It seems possible that the A-type lamins possess redundant functional capacities and are capable of carrying out all of the crucial "lamin functions" in *Lmnb1*/*Lmnb2*-deficient keratinocytes and hepatocytes. Interestingly, the consequences of lamin B1 and lamin B2 deficiencies are quite striking in at least one tissue where lamin A/C expression levels are minimal. Coffinier and coworkers have shown that lamin B1 and lamin B2 deficiencies lead to severe defects in the developing brain,[20,30] a tissue where lamin A/C expression is low and is activated late in development.[7]

In considering results with conditional knockout alleles, one should always worry about "leaky alleles" and the possibility that recombined alleles produce proteins with partial function.[29] In the case of the *Lmnb1* and *Lmnb2* conditional knockout alleles, our combination of qRT-PCR, protein gel blot and immunohistochemistry studies provided little evidence of persistent protein expression. For both alleles, the recombination event excises exon 2 and yields a frameshift; neither allele resulted in the production of β-galactosidase fusion proteins. In the case of *Lmnb1*, exon 1 encodes 121 amino acids; in the case of *Lmnb2*, exon 1 encodes 66 amino acids. Any residual "exon 1 truncated proteins" produced by the recombined alleles would lack important protein domains, including the vast majority of the rod domain, the carboxyl-terminal globular domain, the nuclear localization signal and the carboxyl-terminal *CaaX* motif. We doubt that significant amounts of exon 1 truncated proteins are produced. When we measured *Lmnb1* and *Lmnb2* transcripts in *Lmnb1*$^{\Delta/\Delta}$*Lmnb2*$^{\Delta/\Delta}$ keratinocytes by qRT-PCR with exon 1 primers, we found only trace levels, likely due to rapid turnover of the mutant transcripts.

We anticipate that some cell biologists may be skeptical of the mouse knockout mouse experiments and they may even contend that the functions of the B-type lamins in DNA replication, the mitotic spindle formation, gene transcription, etc. are so crucial that nature has evolved back-up systems to deal with their absence. Over the next few years, the nuclear lamin field will undoubtedly need to sort out those types of arguments with additional experiments. In any case, genetically modified mice—and cell lines derived from them—will remain important tools for defining both unique and redundant roles of the different nuclear lamins in the cell nucleus.

What Is the In Vivo Functional Relevance of B-Type Lamins?

The B-type lamins may be dispensable in the skin and liver, but they are clearly important in the brain.[19,20] In a 2010 paper in PNAS, Coffinier et al.[20] made a seminal observation regarding about the function of B-type lamins in mammals. They demonstrated, in a comprehensive fashion and with a variety of experimental techniques, that a deficiency of *Lmnb2* in

mice causes defective neuronal migration in the developing cerebral cortex, resulting in defective layering of cortical neurons. Layering of neurons depends on the timely migration of neurons from the ventricular zone, where they are born, to the proper layer in the cortical plate. Interestingly, migration of neurons to the cortical plate is utterly dependent on moving the cell nucleus forward in the direction of cell migration. The movement of the cell nucleus, which depends on a network of microtubules and cytoplasmic motors, likely exerts significant deformational forces on the cell nucleus. We suspect that the nucleus is unable to withstand those forces in the absence of lamin B2, which would explain defective migration of the neurons. We have suggested that the B-type lamins might be particularly well suited for ensuring the integrity of the nuclear envelope since they are anchored to the inner nuclear membrane by a farnesyl lipid.[21]

Given that lamin B2 plays an important role in brain development,[20] and given that the cranial vault in lamin B1 knockout mice is abnormal in size and shape,[18] Coffinier and coworkers[30] were suspicious that lamin B1 might also play an important role in brain development. Their suspicions were confirmed! In a 2011 Mol Biol Cell paper,[30] Coffinier and coworkers established, with multiple experimental approaches, that lamin B1 is critical for neuronal survival, neuronal migration, and proper layering of neurons in the brain. Interestingly, a deficiency of lamin B1 in neurons resulted in striking abnormalities in the shape of cortical neurons. Most cortical neurons in lamin B1–deficient mice contained a solitary nuclear bleb; also, many cortical neurons exhibited an asymmetric distribution of lamin B2. In contrast, lamin B2 deficiency led to increased numbers of neurons with elongated nuclei.

In addition to studies with conventional lamin B1- and lamin B2-knockout mice, Coffinier and coworkers[30] created conditional knockout alleles for both *Lmnb1* and *Lmnb2* and went on to breed forebrain-specific knockout mice. Both forebrain-specific *Lmnb1* knockout embryos and forebrain-specific *Lmnb2* knockout embryos had small forebrains with disordered layering of neurons and nuclear shape abnormalities, similar to the abnormalities in the conventional knockout embryos. In adult forebrain-specific knockout mice, brain pathology was even more striking than in the embryos. A far more severe phenotype, complete atrophy of the cortex, was observed in forebrain-specific *Lmnb1/Lmnb2* double knockout mice. No viable neurons were found in the forebrains of adult *Lmnb1/Lmnb2* double knockout mice, indicating that neuronal survival requires the expression of one of the two B-type lamins. The studies by Coffinier et al.[30] established, for the first time, that deficiencies in lamin B1 and lamin B2 lead to severe neurodevelopmental abnormalities, expanding the realm of disorders associated with lamin defects. Sooner or later, we suspect that *LMNB1* and *LMNB2* mutations will be identified in infants with severe neurodevelopmental abnormalities. Also, given that neuronal migration abnormalities have been implicated in milder neurological diseases, including epilepsy, it would not be particularly surprising if geneticists and neurologists ultimately uncovered *LMNB1* and/or *LMNB2* mutations in outpatients in neurology clinics.

Interestingly, *Lmna* expression is very low in the developing brain[30]—at a time period when *Lmnb1* and *Lmnb2* expression in the brain are so essential.[20,30] The fact that *Lmna* expression is low in the developing brain raises many questions, including: Why is *Lmna* expression low in the developing brain when it is robust in other tissues? Would the synthesis of lamin A/C in neurons interfere with neuronal migration? Would the synthesis of lamin A/C in developing neurons effectively substitute for the roles of lamin B2 and rescue *Lmnb2*-deficient mice from their neurodevelopment abnormalities? Are the farnesyl lipid anchors in lamins B1 and B2 crucial for the development of the brain? These are intriguing questions and we strongly suspect that efforts to address these questions will yield important insights into the unique and redundant functions of the nuclear lamins in mammalian development and cell biology.

Acknowledgments

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