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Reciprocal knock-in mice to investigate the functional redundancy of lamin B1 and lamin B2

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ABSTRACT Lamins B1 and B2 (B-type lamins) have very similar sequences and are expressed ubiquitously. In addition, both \textit{Lmnb1-} and \textit{Lmnb2-deficient} mice die soon after birth with neuronal layering abnormalities in the cerebral cortex, a consequence of defective neuronal migration. The similarities in amino acid sequences, expression patterns, and knockout phenotypes raise the question of whether the two proteins have redundant functions. To investigate this topic, we generated “reciprocal knock-in mice”—mice that make lamin B2 from the \textit{Lmnb1} locus (\textit{Lmnb1B2/B2}) and mice that make lamin B1 from the \textit{Lmnb2} locus (\textit{Lmnb2B1/B1}). \textit{Lmnb1B2/B2} mice produced increased amounts of lamin B2 but no lamin B1; they died soon after birth with neuronal layering abnormalities in the cerebral cortex. However, the defects in \textit{Lmnb1B2/B2} mice were less severe than those in \textit{Lmnb1-knockout} mice, indicating that increased amounts of lamin B2 partially ameliorate the abnormalities associated with lamin B1 deficiency. Similarly, increased amounts of lamin B1 in \textit{Lmnb2B1/B1} mice did not prevent the neurodevelopmental defects elicited by lamin B2 deficiency. We conclude that lamins B1 and B2 have unique roles in the developing brain and that increased production of one B-type lamin does not fully complement loss of the other.

INTRODUCTION

The nuclear lamina, an intermediate-filament meshwork beneath the inner nuclear membrane, provides a structural scaffold for the cell nucleus (Worman \textit{et al.}, 2009; Burke and Stewart, 2013). The main protein components of the nuclear lamina are lamins A, C, B1, and B2 (Gerace \textit{et al.}, 1984; Worman \textit{et al.}, 2009; Burke and Stewart, 2013). Lamins A and C (A-type lamins) are splice isoforms from the same gene (LMNA; Lin and Worman, 1993) and are not expressed until late in embryonic development (Rober \textit{et al.}, 1989; Coffinier \textit{et al.}, 2011; Burke and Stewart, 2013). \textit{Lmna-knockout} mice survive development but die between 2 and 6 wk of age with myopathy and cardiomyopathy (Sullivan \textit{et al.}, 1999). Lamins B1 and B2 (B-type lamins) are products of independent genes, \textit{LMNB1} and \textit{LMNB2} (Zewe \textit{et al.}, 1992; Biamonti \textit{et al.}, 1992; Lin and Worman, 1995; Maeno \textit{et al.}, 1995; Worman \textit{et al.}, 2009). Lamins B1 and B2 are \textit{c}60% identical at the amino acid level (Davies \textit{et al.}, 2011) and are expressed ubiquitously from the earliest stages of development. For years, dogma held that the B-type lamins played essential functions in the cell nucleus (e.g., DNA replication, formation of the mitotic spindle; Belmont \textit{et al.}, 1993; Moir \textit{et al.}, 1994; Harborth \textit{et al.}, 2001; Tsai \textit{et al.}, 2006; Malhas \textit{et al.}, 2007, 2009, 2010; Shimi \textit{et al.}, 2008; Tang \textit{et al.}, 2008; Martin \textit{et al.}, 2009), but recent studies with tissue-specific knockout mice cast doubt on that view—at least for certain cell types. For example, the absence of both lamins B1 and B2 in keratinocytes or hepatocytes does not lead to any obvious abnormalities (Yang \textit{et al.}, 2011a,b).

We previously showed that lamins B1 and B2 have important functions in the developing brain (Vergnes \textit{et al.}, 2004; Coffinier \textit{et al.}, 2010, 2011). \textit{Lmnb2-deficient} mice were nearly normal in size during development but died soon after birth with a neuronal layering defect in the cerebral cortex. Neuronal birthdating studies...
and did not have misshapen nuclei. Lmnb1-deficient mice (Vergnes et al., 2004) survived development but were small and died soon after birth with a neuronal layering defect in the cerebral cortex (Coffinier et al., 2011). The neurodevelopmental defects in Lmnb1-deficient mice were more severe than those in Lmnb2-deficient mice (Coffinier et al., 2010, 2011). Lmnb1-deficient fibroblasts had multiple nuclear blebs (Vergnes et al., 2004). Studies with forebrain-specific knock-out mice revealed that both lamins B1 and B2 are also important for survival of cortical neurons (Coffinier et al., 2011).

The fact that lamins B1 and B2 have similar sequences and expression patterns, along with the fact that Lmnb1- and Lmnb2-knockout mice have similar neurodevelopmental defects, naturally leads to the question of whether lamins B1 and B2 have redundant functions. The best way to assess functional redundancy in closely related proteins is to determine whether increased production of one protein can prevent the disease phenotypes associated with loss of the other (Wang et al., 1996; Geng et al., 1999; Schweda et al., 2009). In the present study, we adopted this approach. We generated two strains of reciprocal knock-in mice—one knock-in line producing lamin B2 from the Lmnb1 locus and another producing lamin B1 from the Lmnb2 locus. These new knock-in mouse models provided fresh, definitive insights regarding the functional redundancy of the B-type lamins in mammals.

RESULTS
Lmnb1^{B2/B2} mice

We used gene targeting to insert a lamin B2 cDNA (3287 base pairs) into the translational start site of exon 1 of Lmnb1 (generating the Lmnb1^{B2} allele); this allele was designed to eliminate Lmnb1 transcripts and produce lamin B2 from the Lmnb1 promoter (Figure 1A). Targeted embryonic stem (ES) cell clones were identified by long-range PCR (Figure 1, B and C) and real-time PCR (RT-PCR; Figure 1D). Sequencing of the Lmnb1^{B2} RT-PCR product identified the junction between the Lmnb1^{5′} untranslated region (UTR) and the Lmnb2 protein-coding sequences (Figure 1E).

Because Lmnb1^{B2/B2} mice produce lamin B2 transcripts from both Lmnb1^{B2} and Lmnb2, we predicted that lamin B2 expression in these mice would be greater than in wild-type mice. Indeed, Lmnb2 transcript levels in the cerebral cortex of Lmnb1^{B2/B2} mice were 2.61 ± 0.32-fold higher than in wild-type mice (Figure 2A). Similar findings were observed at the protein level; lamin B2 levels in the cerebral cortex of Lmnb1^{B2/B2} mice were 3.01 ± 0.16-fold higher than in wild-type mice (Figure 2, B and C).

FIGURE 1: Generation of the Lmnb1^{B2} allele, which yields lamin B2 from the Lmnb1 locus. (A) Map of the Lmnb1 locus and the targeting vector, which was designed to introduce a Lmnb2 cDNA into the translational start site in exon 1 of Lmnb1 (at an Ncol site). The Lmnb2 cDNA was modified to introduce a new EcoRV site and remove an existing SacI site (depicted by an asterisk), making it possible to distinguish Lmnb1^{B2} transcripts from those of the endogenous Lmnb2 locus. Exons are depicted as black boxes (E1 and E2); the noncoding region of exon 1 is in white. Black arrowheads indicate the loxP sites. The neo cassette is shown as a gray box; a diphtheria toxin (DTA) counterselection cassette is shown as a black box. The primers used for recombineering (GF, GR, LF, and LR) are indicated. The primers used for 5′- and 3′ long-range PCR (5′ LR-PCR) and 3′ long-range PCR (3′ LR-PCR) are indicated by arrows. (B) Screening of ES cell clones by 5′-LR-PCR and 3′-LR-PCR. A 5.8-kb fragment was amplified from the Lmnb1^{B2} allele; the identity of the fragment was confirmed by EcoRV digestion (yielding 5.4- and 0.4-kb fragments). (C) Screening of ES cell clones by 3′-long-range PCR. A 6-kb DNA fragment was amplified from the Lmnb1^{B2} allele; the identity of the fragment was confirmed by BamHI digestion (yielding 4.3- and 1.7-kb fragments). A nonspecific band is indicated by an asterisk. (D) EcoRV digestion of a 950-base pair Lmnb2 RT-PCR fragment (amplicon from exons 1–7 of Lmnb2) from Lmnb1^{B2/B2} and Lmnb1^{+/−} ES cells. Lmnb2 RT-PCR DNA fragments from the Lmnb1^{B2} allele (but not from the endogenous Lmnb2 allele) were cleaved by EcoRV (yielding an 850-base pair fragment). (E) DNA sequencing chromatogram of an RT-PCR fragment from the Lmnb1^{B2} allele showing the junction between the Lmnb1^{5′} UTR and Lmnb2 coding DNA sequences (CDS).
Lamin B1 was undetectable in mice (set at 1.0). Lamin B2 protein levels in the cerebral cortex were relative to actin (mean cerebral cortex biopsies of E18.5 in cyclophilin A and compared with the levels in quantitative RT-PCR. Transcript levels (mean cerebral cortex of E18.5 FIGURE 2: 1668 ever, the abnormalities in Lmnb1 Lmnb1 Lmnb1-Lmnb1 −/− mice (Figure 3). The body weights of embryonic day embryos. (B) Western blot of protein extracts from Lmnb1 −/− Lmnb1 B2/B2 Lmnb1 −/+ +/+ Lmnb1 Lmnb1 Lmnb1 Lmnb1 Lmnb1 B2/B2 Lmnb1 Lmnb1 Lmnb1 B2/B2 Lmnb1 Lmnb1 Lmnb1 Lmnb1 Lmnb1 Lmnb1 Lmnb1 B2/B2 immunohistochemistry with antibodies against Cux1 and Ctip2 (Supplemental Figure S1). Immunohistochemistry studies on the putamen of Lmnb1B2/B2 mice revealed higher-than-normal levels of lamin B2 expression and absent expression of lamin B1 (Figure 3D).

Lmnb2B1/B1 mice
We inserted a lamin B1 cDNA (2516 base pairs) at the translational start site within exon 1 of Lmnb2 (generating the Lmnb2B1 allele). The Lmnb2B1 allele was designed to eliminate Lmnb2 transcripts and drive the expression of lamin B1 (Figure 4A). Targeted E5 cell clones were identified by long-range PCR (Figure 4, B and C) and RT-PCR (Figure 4D). Sequencing of an RT-PCR product from the Lmnb2B1 allele revealed the junction between Lmnb2's 5'UTR and the Lmnb1 protein-coding sequences (Figure 4E).

In earlier studies, we reported that body weights in Lmnb2-knockout mice (Lmnb2B−/−) were normal, but their brains were slightly smaller than in wild-type mice (Coffinier et al., 2010). In the present studies, we found that the body weights of E18.5 Lmnb2B1/B1 embryos (1.18 ± 0.11 g; n = 20) were similar to those of wild-type mice (1.20 ± 0.14 g; n = 22; p = 0.65); however, the brain weights in Lmnb2B1/B1 embryos (0.06 ± 0.01 g) were 27.6 ± 0.15% lower than in wild-type embryos (0.09 ± 0.01 g, p < 0.001; Supplemental Table S1). Lmnb2 transcripts were absent in the cerebral cortex of Lmnb2B1/B1 embryos (p = 0.001), but Lmnb1 transcript levels in the cerebral cortex of Lmnb2B1/B1 embryos (n = 5) were 70 ± 7.1% higher than in wild-type mice (n = 4; p = 0.001; Figure 5A). Lamin B2 protein was absent in the cerebral cortex of E18.5 Lmnb2B1/B1 embryos, but levels of lamin B1 protein were slightly increased (Figure 5, B and C).

Like Lmnb2-knockout mice (Coffinier et al., 2010), Lmnb2B1/B1 mice died soon after birth. Hematoxylin and eosin–stained sections of the cerebral cortex of Lmnb2B1/B1 embryos were essentially normal in size (Figure 6A), but there was a neuronal layering defect in the cerebral cortex (Figure 6, B and C, and Supplemental Figure S1)—similar to the pathology in Lmnb2B−/− mice (Coffinier et al., 2010). Immunohistochemistry studies on the putamen confirmed an absence of lamin B2 in Lmnb2B1/B1 mice (Figure 6D).

Interccrosses of mice harboring both knock-in alleles
We generated mice carrying both knock-in alleles (Lmnb1B2/B2/Lmnb2B1/B1) and then intercrossed those mice. No viable Lmnb1B2/B2/Lmnb2B1/B1 mice were observed by the age of weaning among 207 offspring (p < 0.001 by the chi-squared statistic). Subsequent studies revealed that Lmnb1B2/B2/Lmnb2B1/B1 mice survived development but died soon after birth. As expected, both lamins B1 and B2 were present in the cerebral cortex of Lmnb1B2/B2/Lmnb2B1/B1 mice, but the steady-state levels of lamin B1 were only 16.4% of those in wild-type mice (Figure 7, B and C). The levels of lamin B2 in Lmnb1B2/B2/Lmnb2B1/B1 mice tended to be somewhat lower than in
Lmnb1 and Lmnb2 are not redundant

Body weights of E18.5 Lmnb1+/− B2/B2 mice (0.66 ± 0.11 g; n = 5) were similar to those of Lmnb1−/− B2/B2 embryos (0.86 ± 0.12 g, n = 10; p = 0.98). However, the brain weights of Lmnb1+/− B2/B2 embryos (0.06 ± 0.01 g) were 17.9 ± 0.04% higher than in Lmnb1−/− B2/B2 embryos (0.05 ± 0.01 g; p = 0.002) (Figure 7D and Supplemental Table S1). In addition, the pathology in the cerebral cortex of Lmnb1+/− B2/B2 embryos was less severe than in Lmnb1−/− B2/B2 embryos (i.e., a higher neuronal density in the cerebral cortex of Lmnb1+/− B2/B2 embryos; Figure 7, E and F).

In the intercrosses of Lmnb1+/− B2/B2 embryos, we never observed viable Lmnb1+/− B2/B2 offspring, presumably because of low amounts of lamin B1 production (even lower than in Lmnb1−/− B2/B2 embryos). In contrast, Lmnb1+/− B2/B2 offspring were born at the expected Mendelian frequency and remained healthy and fertile during >1 yr of observation (Figure 8A). Brain specimens from these mice appeared normal, and microscopic analysis of the cerebral cortex revealed no abnormalities (Figure 8, B and D). The level of lamin B1 expression in Lmnb1+/− B2/B2 embryos was greater than half-normal (Figure 8C), reflecting lamin B1 production from two Lmnb1 alleles and one wild-type Lmnb1 allele. The level of lamin B2 expression in Lmnb1+/− B2/B2 embryos was half-normal, reflecting lamin B2 synthesis from a single Lmnb1 allele (Figure 8C).

**DISCUSSION**

Lamins B1 and B2 are ubiquitously expressed proteins with a high degree of sequence similarity (Worman et al., 2009; Davies et al., 2011; Burke and Stewart, 2013), and a deficiency of either protein leads to defective neuronal migration in the cerebral cortex (Coffinier et al., 2010, 2011; Young et al., 2012). These observations naturally elicit a simple question: Are lamins B1 and B2 functionally interchangeable? In the present study, we addressed this question by creating reciprocal knock-in mice (i.e., mice that produce lamin B1 from the Lmnb2 locus, and mice that produce lamin B2 from the Lmnb1 locus). Both knock-in mice died soon after birth with neuronal layering abnormalities in the cerebral cortex, demonstrating that increased production of one B-type lamin cannot prevent the developmental defects associated with loss of the other. However, we did find evidence that increased production of one protein led to partial amelioration of disease phenotypes. For example, the decrease in body weight and the neurodevelopmental defects in Lmnb1+/− B2/B2 mice (which produced twofold to threefold more lamin B2 than wild-type mice) were milder than those in Lmnb1−/− mice, implying that the supplemental amounts of lamin B2 reduced the disease phenotypes associated with the deficiency of lamin B1. In the case of Lmnb2−/− mice, the increase in lamin B1 expression was more modest, and the neurodevelopmental abnormalities were similar to those in Lmnb2−/− mice (Coffinier et al., 2010). However, the pathology in the cerebral cortex of Lmnb1+/− B2/B2 Lmnb2−/− embryos was less severe than in Lmnb1+/− B2/B2 embryos, implying that the modest amounts of lamin B1 from the Lmnb2−/− allele reduced the severity of the abnormalities elicited by homozygosity for the Lmnb1−/− allele.

The observation that neither B-type lamin prevents the neurodevelopmental abnormalities elicited by the loss of the other B-type lamin demonstrates that the two proteins play unique and important roles in brain development. With this lesson firmly established, one can, in hindsight, view earlier observations as being consistent with unique functions for the two proteins. For example, Coffinier et al. (2011) showed that deficiencies in lamins B1 and B2 yield distinct morphological abnormalities in cortical neurons. Lmnb2 deficiency resulted in markedly elongated nuclei but did not affect the distribution of lamin B1 along the nuclear rim, whereas Lmnb1 deficiency led to solitary nuclear blebs and a

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**FIGURE 3:** Phenotypes of Lmnb1+/− B2/B2 mice. (A) Photographs of E18.5 Lmnb1+/− B2/B2, and Lmnb1+/− embryos and the brains from these mice. Scale bar, 2 mm. (B) Hematoxylin and eosin–stained sagittal sections of brains from E18.5 embryos. Scale bar, 500 μm. (C) Higher-magnification images of the boxed areas in B, showing abnormal layering of cortical neurons in Lmnb1+/− B2/B2 mice. Scale bar, 100 μm. (D) Immunofluorescence microscopy of the putamen in E18.5 Lmnb1+/− and Lmnb1+/− B2/− embryos with antibodies against lamin B1 (green) and lamin B2 (red). Merged images show DAPI (blue) staining. Scale bar, 50 μm.

wild-type mice, but this difference was not statistically significant (p = 0.16; Figure 7C)
FIGURE 4: Generation of the Lmnb2B1 allele, which yields lamin B1 from the Lmnb2 locus. (A) Map of the Lmnb2 locus and the targeting vector, which was designed to introduce a Lmnb1 cDNA into the translational start site in exon 1 of Lmnb2 (at an Ncol site). A novel BgIII site was introduced into the Lmnb1 cDNA and an existing NotI site (depicted by an asterisk) was eliminated, making it possible to identify Lmnb1 transcripts originating from the Lmnb2B1 and Lmnb1 alleles. Exons are depicted as black boxes (E1 and E2); the noncoding region of exon 1 is in white. Black arrowheads depict the loxP sites. The neo cassette is shown as a gray box; a diphtheria toxin (DTA) counterselection cassette is shown as a black box. The primers used for recombineering (GF, GR, LF, and LR) are indicated. The primers used for 5′ long-range PCR (5′ LR-PCR) and 3′ long-range PCR (3′ LR-PCR) are indicated by arrows. (B) Screening of ES cell clones by 5′ LR-PCR. A 7.6-kb fragment was amplified from the Lmnb2B1 allele; the identity of the fragment was confirmed by BgIII digestion (yielding fragments of 3.0, 2.2, 1.4, and 1 kb). No product was amplified from wild-type DNA (Lmnb2+/+). (C) Screening of ES cell clones by 3′ LR-PCR. A 5.77-kb fragment was amplified from the Lmnb2B1 allele; the identity of the fragment was confirmed with BgIII digestion (yielding fragments of 3.80, 1.64, 0.22, and 0.11 kb (the 0.11-kb fragment ran off the gel and is not seen in this photograph). (D) BgIII digestion of a 706–base pair Lmnb1 RT-PCR fragment (amplicon from Lmnb2’s 5′ UTR to exon 4 of Lmnb1) from Lmnb2B1/ES cells. The RT-PCR fragment from the Lmnb2B1 allele was cleaved by BgIII. (E) DNA sequencing chromatogram of an RT-PCR fragment from the Lmnb2B1 allele showing the junction between the Lmnb2 5′ UTR and the Lmnb1 coding sequences (CDS).
interactions) for the two B-type lamins in neurons. Another observation in favor of unique functions for the two proteins is the finding that the farnesyl lipid anchor is extremely important for lamin B1 function but dispensable for lamin B2 (Jung
et al., 2013). Knock-in mice expressing a nonfarnesylated version of lamin B1 had neuronal migration defects and a unique nuclear abnormality in neurons (the nuclear lamina was pulled away from the bulk of the chromosomal DNA; Jung
et al., 2013). In contrast, knock-in mice expressing a nonfarnesylated version of lamin B2 were healthy and free of pathology (Jung
et al., 2013).
suspect that the low amounts of lamin B1 expression in these mice were incompatible with normal brain development and survival. However, because all of the lamin B1 in Lmnb1<sup>B2/B2</sup> Lmnb2<sup>B1/B1</sup> mice exhibited neurodevelopmental abnormalities and died soon after birth. The amount of lamin B1 in the brain of these mice was only ∼16% of that in wild-type mice, and we

**FIGURE 7:** Characterization of Lmnb<sup>B2/B2</sup>Lmnb2<sup>B1/B1</sup> mice. (A) Levels of lamin transcripts in Lmnb<sup>+/+</sup>, Lmnb<sup>B2/B2</sup>, Lmnb<sup>B2/B2</sup>Lmnb2<sup>B1/B1</sup>, and Lmnb<sup>−/−</sup> embryos. Quantitative RT-PCR analysis of Lmnb1 and Lmnb2 transcript levels in the cerebral cortex of E18.5 embryos. Transcript levels (mean ± SD) were normalized to cyclophilin A and compared with the levels in wild-type mice (set at 1.0). Lmnb1 transcript levels in Lmnb<sup>B2/B2</sup>Lmnb2<sup>B1/B1</sup> mice (n = 5) were 23.9% of those in wild-type mice (n = 4); Lmnb2 transcript levels in Lmnb<sup>B2/B2</sup>Lmnb2<sup>B1/B1</sup> mice were 51.1% higher than those in wild-type mice; Lmnb2 transcript levels in Lmnb<sup>B2/B2</sup> mice (n = 6) were 171% higher than those in wild-type mice. (B) Western blot of protein extracts from the cerebral cortex of E18.5 Lmnb<sup>B2/B2</sup>, Lmnb<sup>B2/B2</sup>Lmnb2<sup>B1/B1</sup>, and Lmnb<sup>−/−</sup> embryos and wild-type embryos. (C) Quantification of lamin B1 and lamin B2 protein levels in the Western blot shown in B relative to actin (mean ± SD) and compared with wild-type controls (set at 1.0). Lamin B1 was undetectable in Lmnb<sup>B2/B2</sup> embryos. Lamin B1 levels in Lmnb<sup>B2/B2</sup>Lmnb2<sup>B1/B1</sup> embryos were only 16.4% of those in wild-type mice. (D) Photographs of E18.5 wild-type, Lmnb<sup>B2/B2</sup>, and Lmnb<sup>B2/B2</sup>Lmnb2<sup>B1/B1</sup> embryos along with the brains from the same mice. Scale bar, 2 mm. (E) Hematoxylin and eosin–stained sagittal sections of brains from E18.5 embryos. Scale bar, 500 μm. (F) Higher-magnification images of the boxed areas in E. Scale bar, 100 μm.
B-type lamin proteins are not redundant

The absence of pathology in these mice demonstrates that lamin B2 functions normally when it is produced under the control of Lmnb1 gene-regulatory elements. Thus any differences in the spatiotemporal expression pattern conferred by the Lmnb1 and Lmnb2 loci are not critical for lamin B2. In hindsight, the survival of Lmnb1+/−Lmnb2+/− mice was not particularly surprising because these mice produce at least half-normal amounts of both lamins B1 and B2. Earlier studies showed that Lmnb1+/−Lmnb2−/− mice, which produce half-normal amounts of both B-type lamins, survive development, are fertile, and are free of neurodevelopmental abnormalities (Kim et al., 2011; Coffinier and Young, unpublished data; present study).

The present studies with reciprocal knock-in mice demonstrate that lamins B1 and B2 have unique and vital roles in the developing brain. Increased lamin B1 production does not prevent the disease phenotypes elicited by the loss of lamin B2, and increased lamin B2 synthesis does not prevent the defects associated with lamin B1 deficiency. Our studies also suggest that the amount of lamin B1 expression in the developing brain matters. The −16%–normal levels of lamin B1 expression in Lmnb2+/− mice and Lmnb1−/−Lmnb2+/− mice were not sufficient to prevent reduced body weight and neurodevelopmental abnormalities. In contrast, half-normal levels of lamin B1 production in Lmnb2−/−Lmnb1+/− mice were quite sufficient to prevent neurodevelopmental defects.

**MATERIALS AND METHODS**

Mice expressing a lamin B2 cDNA from the Lmnb1 locus and mice expressing a lamin B1 cDNA from the Lmnb2 locus

We used homologous recombination to introduce a full-length Lmnb2 cDNA (beginning at the ATG translational start site) into the translational start site in exon 1 of Lmnb1 (thereby creating a new Lmnb1 allele, Lmnb1B2). We also introduced a Lmnb1 cDNA (beginning at the ATG translational start site) into the translational start site in exon 1 of the Lmnb2 gene (thereby creating a new Lmnb2 allele, Lmnb2B1). The Lmnb2 and Lmnb1 cDNAs were from IMAGE clones (IMAGE:5695459 and IMAGE:6816118, respectively). Each cDNA contained two neutral restriction site changes, making it possible to distinguish transcripts from the targeted allele from transcripts originating from the endogenous gene. For the Lmnb2 cDNA, a new EcoRV site was introduced by changing cytosine 168 to adenine with primers 5′-CTC TTC CTT CTC GGA TAT CCG GAG CAG CAA C-3′ (and a complementary primer); a S5′ site was removed by changing guanine 852 to adenine with primer 5′-GGC CTC CTT GAG TTC CTC-3′ (and a complementary primer). Neither change altered the lamin B2 amino acid sequence. For the Lmnb1 cDNA, a new BglII site was introduced into the lamin B1 cDNA by changing thymine 381 to adenine with primer 5′-GGG CTC CTT CAC TGA GAT CTG ATT CCT TCT TGG CAT-3′ (and a complementary primer); a NotI site was removed by changing cytosine-219 to adenine with primer 5′-GTG AGC TCG CTT GCG CCG ACC TC-3′ (and a complementary primer). Neither change altered the lamin B2 amino acid sequence.

The gene-targeting vectors were generated by BAC recombining. The Lmnb1 cDNA was introduced into BAC clone CH38-14G10 (containing Lmnb2), and the Lmnb2 cDNA was introduced into BAC clone CH38-24P17 (containing Lmnb1). The cDNAs were introduced in-frame at the translational start site (at an Ncol site). The remainder of the vector was constructed by recombingineering with primers 5′-AAA CAT TGA TTA CTA CGG AAG TGG CGC AAG GAC ATT AAT G-3′ and 5′-GGT CGT ACG TCG CCA TCA GTT CAG ACG GGC CCA AGC TCC AGA TGA TC AAG CGG AGC ACG CAG A-3′ (to introduce the loxp-P-Neo-loxp cassette into Lmnb1B2) and primers 5′-CAA ATA AAA CGC TGT TTT
After verifying that the ES cells were euploid, two independently sequenced to verify that no additional mutations were introduced. Fragments from targeted ES cells were sequenced to confirm that the expected 950-base pair DNA fragment was cleaved by Lmnb2 primer pairs 5'-CTC CTG TT-3' and 5'-GCC ACC CCT GGT CTT TA-3', and the Lmnb2 allele was identified by amplifying a 256-base pair product with primer 5'-GTC TGA CGC CAT CAG TT-3' and the same reverse primer.

All mice were fed a chow diet and housed in a virus-free barrier facility with a 12-h light/dark cycle. The UCLA Animal Research Committee approved all animal protocols.

**Knockout mice**

The Lmnb1-knockout mice (Lmnb1 ΔΔ) used in this study were generated by breeding mice homozygous for a Lmnb1 conditional knockout allele (Lmnb1 ΔΔ; Yang et al., 2011a) with mice harboring an Ella-Cre transgene (Bergo et al., 2002). The Cre-mediated recombination event deleted exon 2 of Lmnb1, resulting in a null allele. Unlike the Lmnb1-knockout mice described earlier (Vergnes et al., 2004), the Lmnb1 ΔΔ used here did not produce an Lmnb1-lacZ fusion protein.

**Western blots**

Mouse tissues were prepared as described (Dechat et al., 1998; Fong et al., 2004; Jung et al., 2012). Snap-frozen mouse tissues were pulverized with a chilled metal mortar and pestle, resuspended in ice-cold phosphate-buffered saline, and homogenized with a glass tissue grinder as previously described (Jung et al., 2013). The cell pellets were resuspended in urea buffer, sonicated, and centrifuged to remove cell debris. SDS-PAGE was performed on a 4–12% gradient polyacrylamide Bis-Tris gel, transferred to nitrocellulose membranes, and incubated with the following antibodies: a goat polyclonal antibody against lamin A/C (sc-6215; Santa Cruz Biotechnology, Santa Cruz, CA), a goat polyclonal antibody against lamin B1 (sc-6217; Santa Cruz Biotechnology), a mouse monoclonal antibody against lamin B2 (33-2100; Invitrogen), and a goat polyclonal antibody against actin (sc-1616; Santa Cruz Biotechnology). Binding of primary antibodies was assessed with infrared dye-conjugated secondary antibodies (Rockland Immunocytremics, Boyertown, PA) and quantified with an Odyssey infrared scanner (Li-Cor Biosciences, Lincoln, NE).

**Quantitative RT-PCR**

Snap-frozen mouse tissues were homogenized in TRI reagent (Molecular Research Center, Cincinnati, OH); total RNA was extracted and treated with DNase I (Life Technologies, Ambion). RNA was then reverse transcribed with random primers, oligo(dT), and SuperScript III (Invitrogen). Quantitative PCRs were performed on a 7900 Fast Real-Time PCR system (Life Technologies, Applied Biosystems) with SYBR Green PCR Master Mix (Bioline, Taunton, MA). Transcript levels were determined by the comparative cycle threshold method and normalized to levels of cyclophilin A.

**Histology and immunofluorescence microscopy**

Mouse tissues were fixed in 10% Formalin (Evergreen, Los Angeles, CA), embedded in paraffin, sectioned (5-μm thick), and stained with hematoxylin and eosin. For immunohistochemical staining, mouse tissues were embedded in Optimum Cutting Temperature compound (Sakura Finetek, Torrance, CA) and cut into 8-μm-thick sections using a cryostat. Sections were fixed in ice-cold methanol, rinsed with acetone, washed with 0.1% Tween-20 in Tris-buffered saline, and incubated with M.O.M. Mouse Ig Blocking Reagent (Vector Laboratories, Burlingame, CA). The following primary antibodies were used: a goat polyclonal antibody against lamin B1 (sc-6217; Santa Cruz Biotechnology), a mouse monoclonal antibody against...
lamin B2 (33-2100; Invitrogen), a rat monoclonal antibody against Ctip2 (ab18465; Abcam, Cambridge, MA), and a rabbit polyclonal antibody against CDP (sc-13024, Santa Cruz Biotechnology). Alexa Fluor–labeled donkey antibodies against goat, mouse, rat, or rabbit immunoglobulin G (Invitrogen) were used to detect binding of primary antibodies. DNA was stained with 4′,6-diamidino-2-phenylindole (DAPI).

Light microscopy images were obtained with a Leica MZ6 dissecting microscope (Plan 0.5x objective, air) or a Nikon Eclipse E600 microscope (Plan Fluor 2x/0.1 numerical aperture [NA] or 10x/0.2 NA objective, air) with a Digital Sight DS-Fi2 camera (Nikon, Tokyo, Japan). The images were captured with Leica Application Suite imaging software and NIS-Elements F4.00.00 (Nikon), respectively. Confocal fluorescence microscopy was performed with a Zeiss LSM700 laser-scanning microscope with a Plan Apochromat 20x/0.80 NA objective (air).

Statistical analyses

Statistical analyses were performed with Excel for Mac 2011 (Microsoft, Redmond, WA). Differences in expression levels of lamins B1 and B2 and differences in body weights and brain weights were analyzed by a two-tailed Student’s t test. Chi-squared tests were performed with Excel for Mac 2011.

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REFERENCES

Belmont AS, Zhai Y, Thilenius A (1993). Lamin B distribution and association with peripheral chromatin revealed by optical sectioning and electron microscopy tomography. J Cell Biol 123, 1671–1685.

Berger MO et al. (2002). Zmpste24 deficiency in mice causes spontaneous bone fractures, muscle weakness, and a prelamin A processing defect. Proc Natl Acad Sci USA 99, 13049–13054.

Biamonti G et al. (1992). The gene for a novel human lamin maps at a highly conserved locus of chromosome 19 which replicates at the onset of S-phase. Mol Cell Biol 12, 3499–3506.

Burke B, Stewart CL (2013). The nuclear lamins: flexibility in function. Nat Rev Mol Cell Biol 14, 13–24.

Coffinier C, Chang SY, Nobumori C, Tu Y, Farber EA, Toth JI, Fong LG, Young SG (2010). Abnormal development of the cerebral cortex and cerebellum in the setting of lamin B2 deficiency. Proc Natl Acad Sci USA 107, 5076–5081.

Coffinier C et al. (2011). Deficiencies in lamin B1 and lamin B2 cause neurodevelopmental defects and distinct nuclear shape abnormalities in neurons. Mol Biol Cell 22, 4683–4693.

Davies B, Coffinier C, Yang S, Jung H, Fong L, Young S (2011). Posttranslational processing of nuclear lamins. In: The Enzymes, ed. F Tamanoi, CA Moir RD, Montag-Lowy M, Goldman RD (1994). Dynamic properties of nuclear lamins: lamin B is associated with sites of DNA replication. J Cell Biol 125, 1201–1212.

Rober RA, Weber K, Osborn M (1989). Differential timing of nuclear lamin A/C expression in the various organs of the mouse embryo and the young animal: a developmental study. Development 105, 365–378.

Schweda F, Kurtz L, de Wit C, Janssen-Bienhold U, Kurtz A, Wagner C (2009). Substitution of connexin40 with connexin45 prevents hyperkeratosis and attenuates hypertension. Kidney Int 75, 482–489.

Shimi T et al. (2008). The A- and B-type nuclear lamin networks: microdomains involved in chromatin organization and transcription. Genes Dev 22, 3409–3421.

Sullivan T, Escalante-Alcalde D, Bhatt H, Anver M, Bhat N, Nagashima K, Stewart CL, Burke B (1999). Loss of A-type lamin expression compromises nuclear envelope integrity leading to muscular dystrophy. J Cell Biol 147, 913–919.

Tang CW, Maya-Mendoza A, Martin C, Zeng K, Chen S, Feret D, Wilson SA, Jackson DA (2008). The integrity of a lamin-B1-dependent nucleoskeleton is a fundamental determinant of RNA synthesis in human cells. J Cell Sci 121, 1014–1024.

Tsai MY, Wang S, Heidinger JM, Shumaker DK, Adam SA, Goldman RD, Zheng Y (2006). A mitotic lamin B matrix induced by RanGTP required for spindle assembly. Science 311, 1887–1893.

Verneges L, Peterfy M, Bergo MO, Young SG, Reue K (2004). Lamin B1 is required for mouse development and nuclear integrity. Proc Natl Acad Sci USA 101, 10428–10433.

Wang Y, Schnegelsberg PN, Dausman J, Jaenic R (1996). Functional redundancy of the muscle-specific transcription factors Myf5 and myogenin. Nature 379, 823–825.

Worman HJ, Fong LG, Muchir A, Young SG (2009). Laminopathies and the long strange trip from basic cell biology to therapy. J Clin Invest 119, 1825–1836.

Yang SH, Chang SY, Yin L, Tu Y, Hu Y, Yoshinaga Y, de Jong PJ, Fong LG, Young SG (2011a). An absence of both lamin B1 and lamin B2 in keratinocytes has no effect on cell proliferation or the development of skin and hair. Hum Mol Genet 20, 3537–3544.

Yang SH, Jung HJ, Coffinier C, Fong LG, Young SG (2011b). Are B-type lamins essential in all mammalian cells? Nucleus 2, 562–569.

Young SG, Jung HJ, Coffinier C, Fong LG (2012). Understanding the roles of nuclear A- and B-type lamins in brain development. J Biol Chem 287, 16103–16110.

Zewe M, Höger TH, Fink T, Lichter P, Krohne G, Franke WW (1995). Gene structure and chromosomal localization of the murine lamin B2 gene. Eur J Cell Biol 56, 342–350.