Dependence of Diffusional Mobility of Integral Inner Nuclear Membrane Proteins on A-Type Lamins†

Cecilia Östlund,‡ Teresa Sullivan,§ Colin L. Stewart,§ and Howard J. Worman*,‡

Departments of Medicine and of Anatomy and Cell Biology, College of Physicians and Surgeons, Columbia University, Room 10-509, 630 West 168th Street, New York, New York 10032, and Cancer and Developmental Biology Laboratory, Center for Cancer Research, National Cancer Institute at Frederick, Frederick, Maryland 21702

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ABSTRACT: Integral proteins of the nuclear envelope inner membrane have been proposed to reach their sites by diffusion after their co-translational insertion in the rough endoplasmic reticulum. They are then retained in the inner nuclear membrane by binding to nuclear structures. One such structure is the nuclear lamina, an intermediate filament meshwork composed of A-type and B-type lamin proteins. Emerin, MAN1, and LBR are three integral inner nuclear membrane proteins. We expressed these proteins fused to green fluorescent protein in embryonic fibroblasts from wild-type mice and *Lmna* −/− mice, which lack A-type lamins. We then studied the diffusional mobilities of emerin, MAN1, and LBR using fluorescence recovery after photobleaching. We show that emerin and MAN1, but not LBR, are more mobile in the inner nuclear membrane of cells from *Lmna* −/− mice than in cells from wild-type mice. In cells from *Lmna* −/− mice expressing exogenous lamin A, the protein mobilities were similar to those in cells from wild-type mice. This supports a model where emerin and MAN1 are at least partly retained in the inner nuclear membrane by binding to A-type lamins, while LBR depends on other binding partners for its retention.

Integral membrane proteins synthesized on endoplasmic reticulum (ER)[1]-bound ribosomes are partially translocated through intramembrane channels and incorporated into the membrane with topologies determined by their primary structures (*1*, *2*). Many of the integral proteins synthesized on the ER membrane reach the plasma membrane by vesicular transport in the secretory pathway (*3*−*5*). Others contain specific sequences retaining them in, or targeting them to, intermediate secretory compartments or organelles such as the ER itself, pre-Golgi vesicles, Golgi apparatus, lysosomes, and endosomes (*5*, *6*).

Some integral membrane proteins synthesized on the rough ER are targeted to the membranes of the nuclear envelope, which are divided into three morphologically distinct but interconnected domains: outer, pore, and inner membranes. As the membrane domains of the nuclear envelope are continuous with each other and the rough ER, integral proteins synthesized on ER-bound ribosomes can potentially reach all of the nuclear membrane domains by lateral diffusion in the interconnected proteolipid bilayers. The results of most targeting studies of integral nuclear membrane proteins are consistent with a diffusion-retention model (*7*−*12*). In this model, proteins diffuse laterally after co-translational insertion into the rough ER membrane. They are then retained and immobilized at their destinations by binding to other proteins or structures.

The outer nuclear membrane has ribosomes on its cytoplasmic surface and is similar in composition to the rough ER, with which it is directly continuous (*13*, *14*). A few integral proteins may, however, be specifically localized to the outer nuclear membrane and excluded from the bulk ER by associating with luminal domains of integral proteins of the inner nuclear membrane in the perinuclear space (*15*). The pore membranes connect the inner and outer membranes at numerous points and are associated with the nuclear pore complexes. Unique integral proteins have been localized to the pore membranes (*16*−*18*). They are most likely structural components of the nuclear pore complexes. These proteins are targeted to the pore membrane either by sequences in their nucleocytoplasmic domains, which presumably bind to other pore complex proteins, or by sequences in the transmembrane domains, which are likely to laterally associate with resident transmembrane domains in the pore membrane (*19*, *20*).

Most of the integral proteins of the inner nuclear membrane contain targeting and retention signals in the parts of the proteins directed toward the nucleoplasmic side of the nuclear membrane (*7*, *8*, *10*, *11*, *21*). These domains likely contain regions that bind to nuclear proteins, such as the nuclear lamins, which form a meshwork of intermediate filaments associated with the inner nuclear membrane on its nucleoplasmic face (*22*−*25*). Two general types of lamins have been identified in somatic cells, A-type and B-type. The somatic cell A-type lamins, lamin A, lamin C, and lamin Adelta10, are alternative splice isoforms encoded by the *LMNA* gene (*26*, *27*). Two different genes encode the somatic cell B-type lamins, lamin B1 and lamin B2 (*28*, *29*). While

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* Corresponding author. E-mail, hjw14@columbia.edu; phone, 212-305-8156; fax, 212-305-6443.

‡ Columbia University.

§ National Cancer Institute at Frederick.

[1] Abbreviations: ER, endoplasmic reticulum; FITC, fluorescein isothiocyanate; FRAP, fluorescence recovery after photobleaching; GFP, green fluorescent protein; MEF, mouse embryonic fibroblast; PBS, phosphate-buffered saline; RFP, red fluorescent protein; TRITC, tetramethyl rhodamine isothiocyanate.

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B-type lamins are expressed in most cell types, A-type lamin expression is developmentally regulated (*30*). Because the lamina is a discontinuous structure (*31*), the inner nuclear membrane proteins may also be able to directly associate with the chromatin at certain points. Approximately 80 different integral proteins are localized to the inner nuclear membrane in interphase cells (*32*). Several of these proteins have been shown to bind components of the lamina and chromatin (*33*−*45*).

Studies of several inner nuclear membrane proteins have shown that they diffuse more rapidly in the ER than in the inner nuclear membrane, giving support to the diffusion-retention model (*9*−*12*). To determine the contribution of A-type lamins in retention of integral proteins in inner nuclear membrane, we studied the targeting and diffusion of three inner nuclear membrane proteins: emerin (*46*, *47*), MAN1 (*48*), and LBR (*34*, *35*). Emerin is known to bind A-type lamins (*41*, *42*, *44*) and requires these proteins for efficient retention in inner nuclear membrane (*49*−*51*). MAN1 co-fractionates with (*52*), and binds to (*45*), lamins. LBR has very weak affinity for A-type lamins (*34*) and binds B-type lamins (*34*, *38*), heterochromatin proteins (*39*, *40*) and DNA (*38*, *43*).

EXPERIMENTAL PROCEDURES

Plasmid Construction. Plasmids encoding nucleoplasmic inner nuclear membrane targeted domains of LBR (amino acids 1−238) and MAN1 (amino acids 1−538) and full-length emerin, fused via their C-termini to the F64L, S65T, H231L variant of green fluorescent protein (GFP), have been previously described (*9*−*11*). RFP-C1, a vector encoding monomeric red fluorescent protein (RFP; *53*) inserted in the place of GFP in an EGFP-C1 vector (Clontech Laboratories, Inc., Mountain View, CA) was a gift from Dr. Eugene Marcantonio, Columbia University, New York. Lamin A-RFP was constructed by inserting lamin A cDNA generated by PCR with a *Xho*I restriction site engineered at the 5′-end and a *Bam*HI restriction site at the 3′-end into these restriction sites in the RFP-C1 vector. All cloning procedures were performed according to standard methods (*54*). The resulting cDNA was then sequenced using an ABI 3100 capillary sequencer (Applied Biosystems, Foster City, CA).

Cell Culture and Transfection. Immortalized mouse embryonic fibroblasts (MEFs) from wild-type and *Lmna* −/− mice (*49*) were grown in Dulbecco's modified Eagle medium containing 10% fetal bovine serum at 37 °C and 5% CO_2. For fluorescence recovery after photobleaching (FRAP), cells were transfected using Lipofectamine PLUS (Invitrogen, Carlsbad, CA), following the manufacturer's instructions. Cells were overlaid with the lipid−DNA complexes for approximately 23 h, the first 5 h of which were in serum-free OPTI-MEM media (Invitrogen). The cells were transfected in dishes and split to chambered coverglasses approximately 29 h post-transfection. They were then grown for an additional 16−40 h before the photobleaching experiments.

Immunofluorescence Microscopy. Cells were grown in chamberslides for approximately 24 h before being prepared for immunofluorescence microscopy. They were washed three times with phosphate-buffered saline (PBS) and then fixed with methanol for 6 min at −20 °C. The cells were permeabilized with 0.5% Triton X-100 in PBS for 2 min at room temperature, washed three times with 0.1% Tween-20 in PBS (solution A), and incubated with the primary antibodies diluted in PBS containing 0.1% Tween-20 and 2% bovine serum albumin (solution B) for 1 h at room temperature. The primary antibodies used were mouse monoclonal anti-emerin antibody (Novocastra, Newcastle upon Tyne, U.K.) at a dilution of 1:30, rabbit polyclonal anti-lamin B1 antibody (*55*) at a dilution of 1:2000, rabbit polyclonal anti-MAN1 antibody 3509 (*56*; a gift from Dr. Kunxin Luo, University of California, Berkeley) at a dilution of 1:200, and guinea pig polyclonal anti-LBR antibody (*57*; a gift from Dr. Harald Herrmann, German Cancer Research Center, Heidelberg, Germany) at a dilution of 1:1000. After four washes with solution A, the cells were incubated with the secondary antibodies diluted 1:200 as described for the primary antibodies. Secondary antibodies used were lissamine rhodamine B-conjugated goat anti-rabbit IgG, fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG, and tetramethyl rhodamine isothiocyanate (TRITC)-conjugated donkey anti-guinea pig IgG. The cells were then washed four times with solution A and three times with PBS. The slides were dipped in methanol and air-dried, and coverslips were mounted using SlowFade Light Anti-fade Kit (Molecular Probes, Eugene, OR). Immunofluorescence microscopy was performed on a Zeiss Axiovert 200 M microscope attached to a Zeiss LSM 510 confocal laser scanning system (Carl Zeiss, Inc., Thornwood, NY).

Fluorescence Recovery after Photobleaching Experiments. FRAP experiments were performed on a Zeiss Axiovert 200 M microscope attached to a Zeiss LSM 510 confocal laser scanning system using the 488 nm line of a 30 mW argon laser in conjunction with a 40 × 0.9 NA objective. The bleached area was photobleached at full laser power (100% transmission) for 25 iterations, and recovery of photobleaching was monitored by scanning at low power (5% transmission) in 2 s intervals. The average intensity of the fluorescence signal was measured in the region of interest using NIH Image J software (http://rsb.info.nih.gov/ij/). It was then normalized to the change in total fluorescence as $I_{rel} = T_0 I_t / T_t I_0$ where T_0 is total cellular intensity during prebleach; T_t, total cellular intensity at time point t; I_0, the average intensity in the bleach-region during prebleach; and I_t, the average intensity in the bleach-region at time point t (*58*). The normalized fluorescence was then plotted against time after bleach.

As the immobile fraction (the difference between the fluorescence intensity in the bleached area prebleach and the intensity at infinity after bleach) was different for the different proteins and cell types, we used a modified time of half-recovery value ($t1/2$), where $t1/2$ is the time after bleach required for the fluorescence levels to reach the median between levels immediately after bleach and prebleach, rather than using the median between prebleach levels and steady-state levels. To determine $t1/2$, we used a modification of the method described by Harrington et al. (*59*). We plotted $\ln(1 - i_t)$ versus time after bleach, where i_t is the average normalized fluorescence intensity in the bleach-region at time t and 1 the average normalized fluorescence intensity in the bleach-region prebleach. The curves were fitted using MacCurveFit 1.5 (http://www.krs.com.au/mcf.html), and $t1/2$ was calculated as $t1/2 = \ln 2(-1/\text{slope})$. Data from the first 31 s after bleach were used in all experiments.

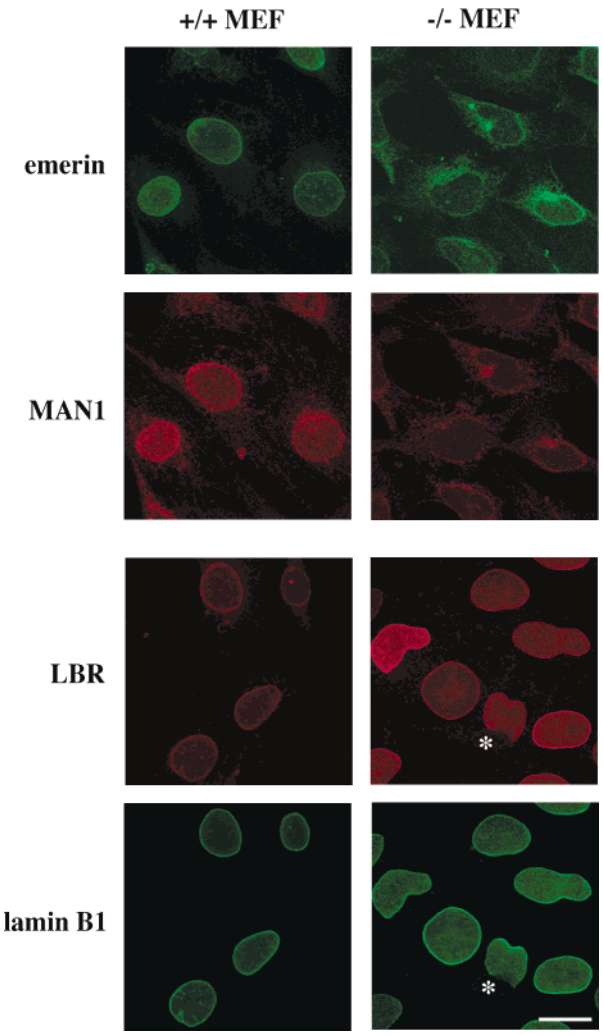

FIGURE 1: Emerin and MAN1 are partly mislocalized to the ER in MEFs lacking A-type lamins, while LBR and lamin B1 are not. Panels show laser scanning confocal immunofluorescence microscopy images of immortalized wild-type MEFs (left panels) or MEFs lacking A-type lamins (right panels). Cells shown in the first and second rows were double-labeled with monoclonal antibodies against emerin recognized by FITC-conjugated secondary antibodies (first row) and polyclonal antibodies against MAN1 recognized by lissamine rhodamine B-conjugated secondary antibodies (second row). Cells shown in the third and fourth rows were double-labeled with polyclonal antibodies against LBR recognized by TRITC-conjugated secondary antibodies (third row) and polyclonal antibodies against lamin B1 recognized by FITC-conjugated secondary antibodies (fourth row). Asterisks show a cell where both LBR and lamin B1 are excluded from an area of the nuclear envelope in MEFs lacking A-type lamins. Bar: 10 μm.

RESULTS

Emerin and MAN1 Are Partly Mislocalized to the ER in MEFs Lacking A-Type Lamins, While LBR Is Not. Previous work has shown that emerin is mislocalized to the ER in embryonic fibroblasts that lack A-type lamins from *Lmna* −/− mice (*49*). Several other proteins, such as B-type lamins, are still exclusively localized to the nuclear envelope, although they exhibit abnormalities such as exclusion from one pole of the nucleus in a subset of the cells. Immunofluorescence microscopy studies on immortalized MEFs from wild-type and *Lmna* −/− mice (Figure 1, rows 1 and 4) confirmed previous data (*49*) on emerin and lamin B1. We also studied the localization of MAN1 and LBR. MAN1 was, similarly to emerin, partly mislocalized to the ER in cells from *Lmna* −/− mice (Figure 1, row 2). The MAN1 antibodies available to us exhibited some nonspecific cytoplasmic background, also seen in wild-type MEFs, which complicated our analysis of the protein localization. However, in a "blind" study where two experienced persons looked at randomly taken confocal micrographs, they identified MAN1 as partly mislocalized to the ER in *Lmna* −/− and as retained in the nuclear envelope in wild-type cells in 100% of the cases (six micrographs of each cell type). LBR did not mislocalize to the ER in *Lmna* −/− MEFs, although some cells exhibited abnormalities as previously described for lamin B1 (*49*) (Figure 1, row 3).

Emerin and MAN1 Are More Mobile in the Nuclear Envelope in MEFs Lacking A-Type Lamins Than in Wild-Type MEFs, While LBR Is Not. Previous studies have shown that emerin-GFP, MAN1-GFP, and LBR-GFP are less mobile in the nuclear envelope than in the ER (*9*−*11*). While the majority of LBR-GFP is essentially immobilized within the nuclear envelope (*9*), emerin-GFP and MAN1-GFP can diffuse slowly, compared to the ER, within the nuclear envelope (*10*, *11*). On the basis of our results shown in Figure 1, and previous studies showing that A-type lamins bind emerin and MAN1 (*41*, *42*, *44*, *45*), we hypothesized that these proteins would be more mobile in the nuclear envelope of cells lacking A-type lamins than in wild-type cells. To investigate the diffusional mobility of emerin, MAN1, and LBR, we performed FRAP. In these experiments, GFP-tagged proteins in an area in the nuclear envelope or ER of transiently transfected MEFs were irreversibly bleached using an argon laser at high power. The fluorescence recovery in the bleached area, corresponding to the influx of unbleached molecules from other areas, was then monitored. Figure 2 shows the results from FRAP experiments on emerin-GFP. Figure 2A shows fluorescence recovery in representative cells after photobleaching. As has previously been noted (*10*), emerin-GFP was not restricted to the nuclear envelope in cells overexpressing the proteins but was also present in the ER. There was also often some protein accumulation in an area most likely representing the Golgi apparatus. Although an exclusive localization of protein to the nuclear envelope was more frequent in wild-type MEFs than in *Lmna* −/− MEFs, wild-type MEFs also often exhibited some fluorescence in the ER. There was also often a clear accumulation of fusion protein in the nuclear envelope in *Lmna* −/− MEFs. Only cells with rim fluorescence and a moderate protein expression level were used in these experiments. Fluorescence in cells expressing emerin-GFP recovered more rapidly and to a higher degree in the nuclear envelope of MEFs lacking A-type lamins than in wild-type cells (Figure 2B). In the ER, the recovery rates were very similar in the two cell types and higher than in the nuclear envelope experiments (Figure 2C).

FRAP experiments were performed as described for emerin-GFP on cells expressing MAN1-GFP. Similar fluorescence patterns were seen as those for emerin (Figure 3A). The difference in nuclear envelope recovery rates between wild-type and *Lmna* −/− cells was, however, less pronounced for MAN1-GFP (Figure 3B,C).

We also performed FRAP experiments on wild-type and *Lmna* −/− cells transiently transfected to express LBR-GFP (Figure 4). This protein localized almost exclusively to the nuclear rim, with very little fluorescence in the ER in cells

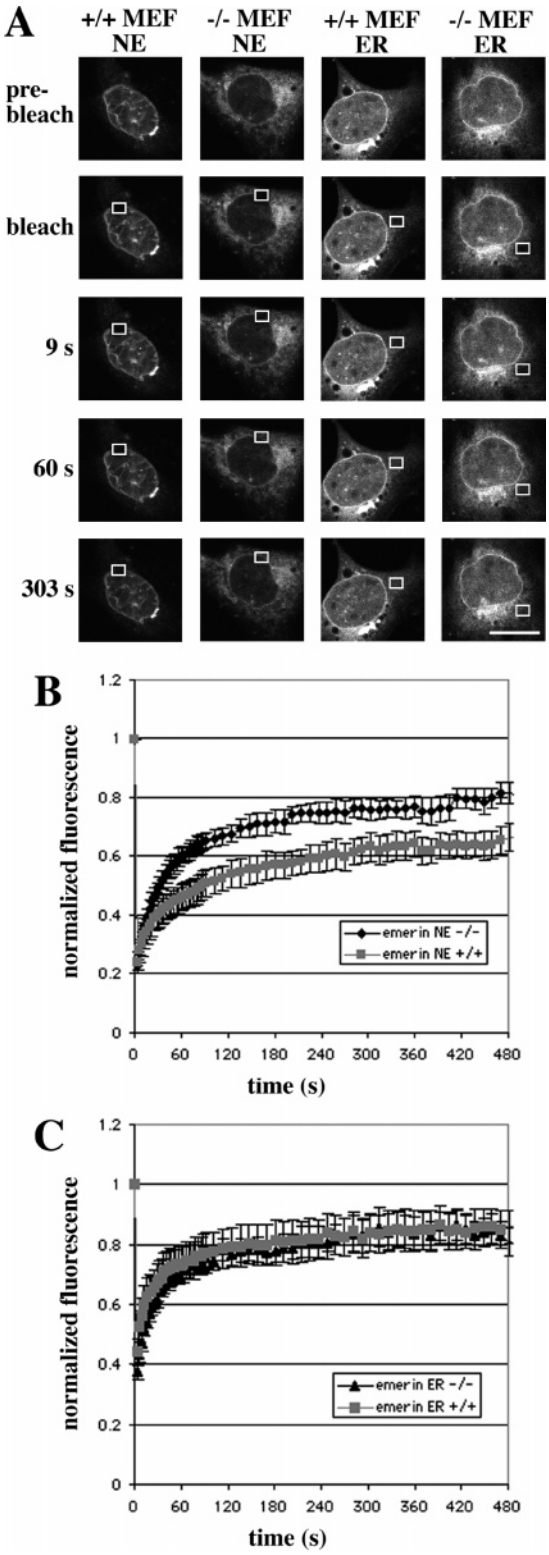

FIGURE 2: The mobility of emerin-GFP in the nuclear envelope (NE) is higher in MEFs lacking A-type lamins (-/- MEF) than in wild-type MEFs (+/+ MEF), while the mobility in the ER is the same in both cell types. (A) FRAP measurements of mobility of emerin-GFP showing fluorescence recovery in representative, transiently transfected MEFs. The boxed regions were bleached, and fluorescence recovery is shown 9, 60, and 303 s after bleaching. Bar: 10 μm. (B and C) Quantitative experiments showing normalized fluorescence recovery after photobleaching regions of the nuclear envelope (B) or ER (C), where 1 is the fluorescence level before bleaching. The fluorescence intensity in the bleached region was measured and expressed as relative recovery (see Experimental Procedures). Error bars indicate SEM, $n = 7$.

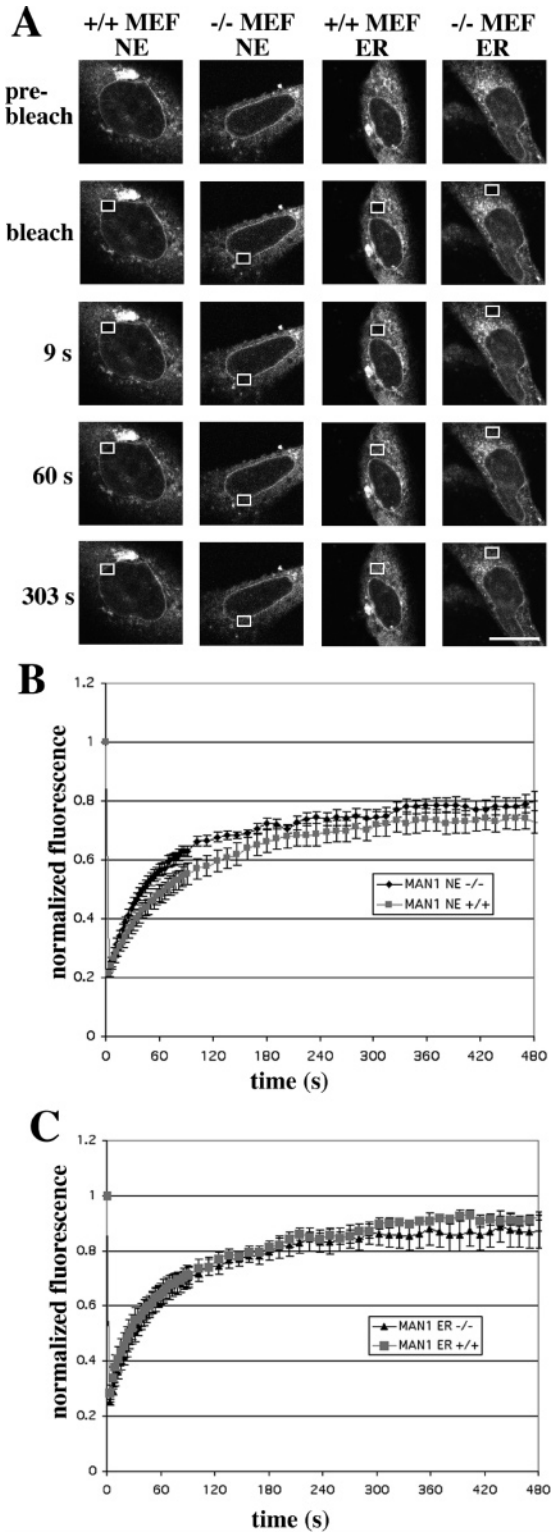

FIGURE 3: The mobility of MAN1-GFP in the nuclear envelope (NE) is higher in MEFs lacking A-type lamins (-/- MEF) than in wild-type MEFs (+/+ MEF), while the mobility in the ER is the same in both cell types. (A) FRAP measurements of mobility of MAN1-GFP showing recovery of fluorescence in representative, transiently transfected MEFs. The fluorescence in the boxed regions was bleached, and recovery is shown after 9, 60, and 303 s after bleaching. Bar: 10 μm. (B and C) Quantitative experiments showing normalized fluorescence recovery after photobleaching regions of the nuclear envelope (B) or ER (C), where 1 is the fluorescence level before bleaching. The fluorescence intensity in the bleached region was measured and expressed as relative recovery (see Experimental Procedures). Error bars indicate SEM, $n = 13$ for nuclear envelope experiments, $n = 8$ for ER experiments.

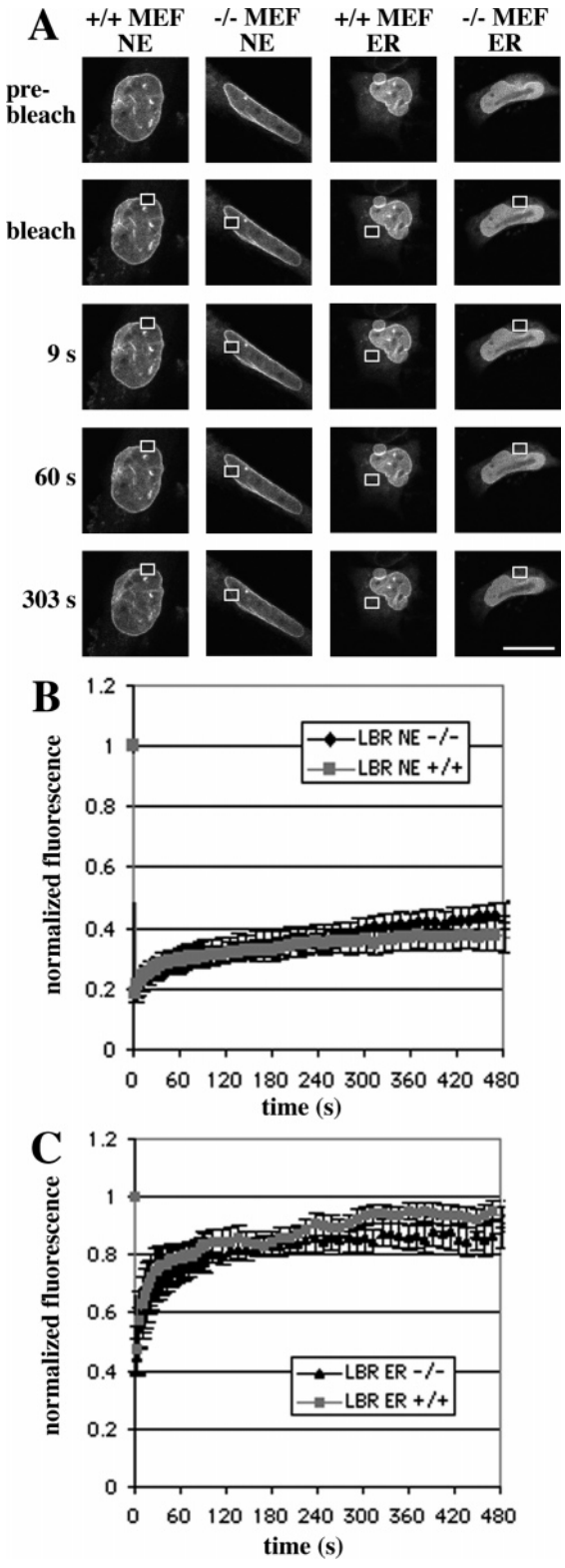

FIGURE 4: The mobility of LBR-GFP is the same in MEFs lacking A-type lamins (-/- MEF) and in wild-type MEFs (+/+ MEF), both in the nuclear envelope (NE) and in the ER. (A) FRAP studies of mobility of LBR-GFP showing fluorescence recovery in representative transiently transfected MEFs. The fluorescence in the boxed regions was bleached, and recovery is shown after 9, 60, and 303 s after bleaching. Bar: 10 μm. (B and C) Quantitative experiments showing normalized fluorescence recovery after photobleaching regions of the nuclear envelope (B) or ER (C), where 1 is the fluorescence level before bleaching. The fluorescence intensity in the bleached region was measured and expressed as relative recovery (see Experimental Procedures). Error bars indicate SEM, $n = 7$ for nuclear envelope experiments, $n = 6$ for ER experiments.

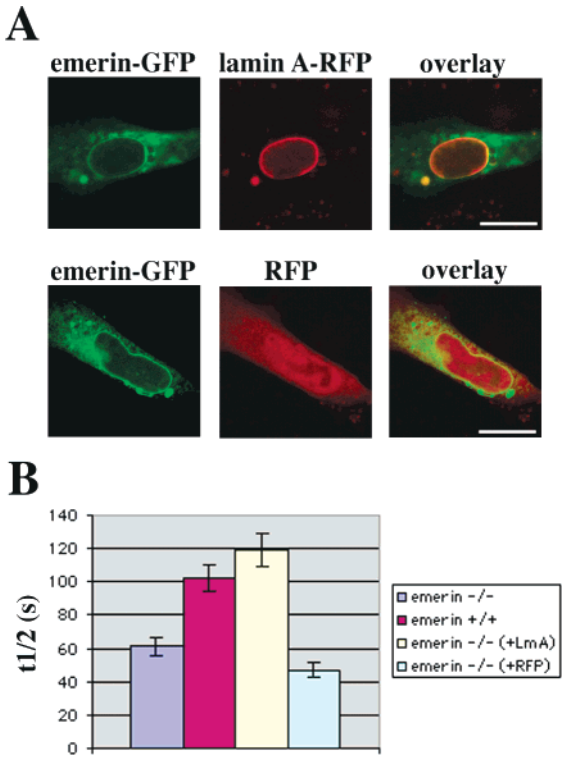

FIGURE 5: The mobility of emerin-GFP in the nuclear envelope of MEFs lacking endogenous A-type lamins expressing emerin-GFP and lamin A-RFP is similar to the mobility in wild-type MEFs. (A) Panels show laser scanning confocal microscopy images of representative MEFs lacking endogenous A-type lamins cotransfected with plasmids encoding emerin-GFP and lamin A-RFP (upper panels) or emerin-GFP and RFP (lower panels). Bars: 10 μm. (B) Diagram showing the time after bleaching ($t1/2$) required for fluorescence from emerin-GFP in regions of the nuclear envelope bleached as in Figure 2 to recover to the median between the prebleach value and the value immediately after bleaching. Columns show $t1/2$ in MEFs lacking A-type lamins (-/-, blue), wild-type MEFs (+/+, red), and MEFs lacking endogenous A-type lamins expressing either lamin A-RFP (-/- (+LmA), yellow) or RFP alone (-/- (+RFP), green). Error bars indicate SEM, $n = 17$ for MEFs lacking A-type lamins and wild-type MEFs, $n = 19$ for MEFs expressing lamin A-RFP, and $n = 14$ for MEFs expressing RFP. *T*-test showed significant differences in $t1/2$ between +/+ cells and -/- cells ($p = 0.00013$) and between -/- cells and -/- (+LmA) cells ($p = 2.5 \times 10^{-5}$). There were no significant differences between $t1/2$ from +/+ cells and -/- (+LmA) cells ($p = 0.20$) or -/- cells and -/- (+RFP) cells ($p = 0.057$).

with or without A-type lamins. However, some fluorescence was seen in the ER of cells expressing the protein at high levels (Figure 4A). There were no differences in the fluorescence recovery rates between wild-type MEFs and *Lmna* −/− MEFs (Figure 4B,C). The recovery rates in the nuclear envelope were much lower than those seen for emerin or MAN1, while the rates in the ER were similar for all three proteins.

Expression of Recombinant Lamin A Decreases the Mobility of Emerin-GFP and MAN1-GFP in the Nuclear Envelope of MEFs without Endogenous A-Type Lamins. To verify that the increased diffusional mobility of emerin in the nuclear envelope of *Lmna* −/− MEFs was due to the lack of A-type lamins, we cotransfected *Lmna* −/− MEFs with plasmids encoding lamin A-RFP and emerin-GFP (Figure 5A) and performed FRAP as described above. As a control, we also cotransfected cells with a plasmid encoding RFP. Lamin A-RFP localized to the nuclear rim, similarly to endogenous

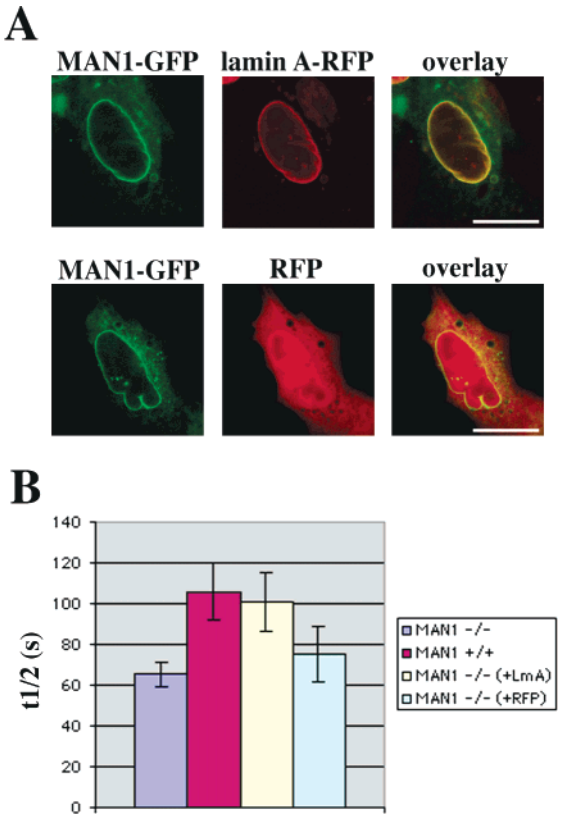

FIGURE 6: The mobility of MAN1-GFP in the nuclear envelope of MEFs lacking endogenous A-type lamins expressing MAN1-GFP and lamin A-RFP is similar to the mobility in wild-type MEFs. (A) Panels show laser scanning confocal microscopy images of representative MEFs lacking endogenous A-type lamins cotransfected with plasmids encoding MAN1-GFP and lamin A-RFP (upper panels) or MAN1-GFP and RFP (lower panels). Bars: 10 μm. (B) Diagram showing the time required after bleaching (t1/2) for fluorescence from MAN1-GFP in regions of the nuclear envelope bleached as in Figure 3 to recover to the median between the prebleach value and the value immediately after bleaching. Columns show t1/2 in MEFs lacking A-type lamins (-/-, blue), wild-type MEFs (+/+, red), and MEFs lacking endogenous A-type lamins expressing either lamin A-RFP (-/- (+LmA), yellow) or RFP (-/- (+RFP), green). Error bars indicate SEM, $n = 28$ for MEFs lacking A-type lamins and wild-type MEFs, $n = 25$ for MEFs expressing lamin A-RFP, and $n = 11$ for MEFs expressing RFP. T-test showed significant differences in t1/2 from +/+ cells and -/- cells ($p = 0.010$) and from -/- cells and -/- (+LmA) cells ($p = 0.022$). There were no significant differences between t1/2 from +/+ cells and -/- (+LmA) cells ($p = 0.81$) or -/- cells and -/- (+RFP) cells ($p = 0.45$).

lamin A, while RFP alone was present in both nuclei and cytoplasm (Figure 5A). To compare the initial recovery rates between the different experiments, we calculated the time after bleach required for the fluorescence levels to reach the median between levels immediately after bleach and prebleach levels (t1/2; see Experimental Procedures for further details). The t1/2 values for emerin-GFP fluorescence were significantly different between wild-type MEFs and *Lmna* −/− MEFs (Figure 5B). The t1/2 values for emerin-GFP fluorescence in *Lmna* −/− MEFs expressing emerin-GFP and lamin A-RFP were similar to those in wild-type MEFs and significantly different from those in *Lmna* −/− MEFs not expressing lamin A-RFP (Figure 5B). The t1/2 values in cells expressing emerin-GFP and RFP were similar to those in *Lmna* −/− MEFs with no expressed lamin A-RFP.

We also performed FRAP experiments on cells cotransfected with plasmids encoding MAN1-GFP and lamin A-RFP or RFP (Figure 6A) and calculated t1/2 values as described above for cells expressing emerin-GFP and lamin A-RFP or RFP (Figure 6B). Similar results were seen. The t1/2 values for MAN1-GFP fluorescence were significantly different between wild-type MEFs and *Lmna* −/− MEFs. The t1/2 values for MAN1-GFP fluorescence in *Lmna* −/− MEFs expressing MAN1-GFP and lamin A-RFP were similar to those in wild-type MEFs and significantly different from those in *Lmna* −/− MEFs not expressing lamin A-RFP. The t1/2 values in cells expressing MAN1-GFP and RFP were similar to those in *Lmna* −/− MEFs with no expressed lamin A-RFP. In conclusion, for both emerin-GFP and MAN1-GFP, there is a statistically significant difference between t1/2 in wild-type MEFs and *Lmna* −/− MEFs, and this difference is abolished when lamin A-RFP is expressed in the *Lmna* −/− cells.

DISCUSSION

Both emerin and MAN1 have been shown to bind to A-type lamins (*41*, *42*, *44*, *45*), and emerin mislocalizes to the ER in *Lmna* −/− MEFs (*49*). We have shown that emerin-GFP and MAN1-GFP are significantly more mobile in the nuclear envelope of MEFs lacking A-type lamins than in wild-type fibroblasts. This provides additional support for the "diffusion and retention" model, which has been proposed for the targeting of integral membrane proteins to the inner nuclear membrane (*7*−*12*). The results also support the hypothesis that A-type lamins are partly responsible for anchoring emerin and MAN1 in the inner nuclear membrane after their post-translational diffusion from the ER membranes. Both emerin and MAN1 also bind to several other proteins, among them B-type lamins (*41*, *45*) and the chromatin protein barrier-to-integration factor (*45*, *60*). Interactions with such proteins in cells lacking A-type lamins can explain why emerin and MAN1 are still more mobile in the ER than in the inner nuclear membrane of *Lmna* −/− MEFs. Retention by interaction with barrier-to-integration factor does, however, seem less likely as this protein diffuses more rapidly at the nuclear envelope than emerin and MAN1 (*61*). We did not detect any difference in the mobility of LBR in the nuclear envelope between cells with or without A-type lamins. This is consistent with findings that LBR has very weak affinity for A-type lamins (*34*) and is likely retained in the inner nuclear membrane by binding other partners, such as B-type lamins (*34*, *38*), heterochromatin proteins (*39*, *40*), and DNA (*38*, *43*).

Mutations in A-type lamins, emerin, MAN1, and LBR cause human diseases. Mutations in *LMNA*, encoding lamins A and C, cause a wide range of human disorders affecting several different tissues (*62*). Examples are limb-girdle muscular dystrophy and autosomal dominant Emery-Dreifuss muscular dystrophy, both affecting cardiac and skeletal muscle, lipodystrophy syndromes sometimes combined with abnormalities in skin and bone, peripheral neuropathy, and premature aging syndromes. Mutations in emerin, in most cases leading to a loss of the protein, cause X-linked Emery-Dreifuss muscular dystrophy (*63*). MAN1 mutations cause osteopoikilosis, Buschke-Ollendorff syndrome, and nonsporadic melorheostosis, diseases affecting mainly bone and skin

(*64*). Heterozygous mutations in the LBR gene cause Pelger-Huët anomaly, a benign alteration in neutrophil nuclear morphology (*65*), while homozygous mutations cause fatal HEM/Greenberg skeletal dysplasia (*66*). The pathogenic mechanisms of most of these diseases are not understood. FRAP studies on transfected cells have shown that some lamin A mutants that cause disease are more mobile in the nuclear envelope than wild-type lamin A (*67*, *68*). Emerin is also partly mislocalized to the ER in some cells transfected with plasmids encoding certain lamin A and C mutants (*69*−*71*). However, emerin appears to be exclusively localized to the nuclear envelope in cells from human subjects heterozygous for dominant A-type lamin mutations examined by immunofluorescence microscopy (*72*−*74*). Emerin is also localized exclusively to the nuclear envelope in mice, both heterozygous and homozygous for the *Lmna*$^{H222P/H222P}$ mutation, which in humans causes autosomal-dominant Emery-Dreifuss muscular dystrophy (*75*).

Subtle changes in the localization or dynamics of emerin and MAN1, secondary to mutations in A-type lamins and not detected by light microscopy, could affect the functions of these proteins. The function of emerin is poorly understood, but it interacts with several nuclear proteins, including transcription factors, and has been suggested to anchor these to the inner nuclear membrane (*76*). Emerin also appears to be involved in transcriptional regulation of mechanosensitive genes in response to strain (*77*). Smad proteins are intracellular mediators of transforming growth factor β, bone morphogenic proteins, and activin signaling (*78*). MAN1 binds to Smads and antagonizes these signaling pathways (*56*, *64*, *79*−*81*), potentially by sequestering the Smad proteins at the inner nuclear membrane and competing with other Smad-binding proteins for formation of transcription activator complexes. Alterations in A-type lamins may therefore affect the mobility of MAN1 in the inner nuclear membrane, as suggested by our studies, in turn affecting Smad sequestration.

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