The apparent absence of lamin B1 and emerin in many tissue nuclei is due to epitope masking

The apparent absence of lamin B1 and emerin in many tissue nuclei is due to epitope masking

Darran Tunnah[1] , Caroline A. Sewry[1] , David Vaux[2] , Eric C. Schirmer[3] & Glenn E. Morris[1,*]

[1]Centre for Inherited Neuromuscular Disease, LMARC Building, Robert Jones and Agnes Hunt Orthopaedic Hospital, Oswestry, SY10 7AG, UK [2]Sir William Dunn School of Pathology, South Parks Road, Oxford, OX1 3RE, UK [3]Wellcome Trust Centre for Cell Biology, University of Edinburgh, Edinburgh, EH9 3JR, UK

*Author for correspondence:

G.E. Morris

Centre for Inherited Neuromuscular Disease,

LMARC Building,

Robert Jones and Agnes Hunt Orthopaedic Hospital,

Oswestry, SY10 7AG, UK

Phone: 44-1691-404155

FAX: 44-1691-404170

Email: glenn.morris@rjah.nhs.uk

Summary

Immunolocalization studies have concluded that the nuclear membrane protein, emerin, is absent from many cell types and that lamin B1 is absent from adult heart and skeletal muscle. We now show that epitope masking in the nucleus is often responsible for failure to detect emerin and lamins in human, rat and pig tissues. Human heart cardiomyocyte nuclei were negative for lamin B1 using a commercial mAb, but were positive using two other lamin B1 antibodies, mAb8D1 and pAbB1-cbs. Rat hippocampal neuronal nuclei were immunostained by mAb8D1, but not pAbB1-cbs, while the commercial antibody stained only a subset. These data suggest that different regions of the lamin B1 molecule are masked in different tissues. Similarly, pig spleen had fewer emerin-positive nuclei than lung (5% vs. 32%), although their emerin content was similar by Western blotting. As mAbs against six epitopes gave the same result, the whole emerin molecule is either masked or redistributed in a subset of cells. Our findings argue that immunostaining evidence can be misleading for expression of nuclear envelope proteins. Problems with lamin B1 immunostaining can be avoided by using mAb8D1, but use of antibodies recognizing different epitopes may reveal cell-specific protein interactions in the nucleus.

Introduction

The nuclear lamina is a dynamic, fibrous structure located beneath the inner nuclear membrane and is made up of the intermediate filament lamin proteins together with associated integral membrane proteins. Higher mammals have three different lamin genes, A/C, B1, and B2 that encode several different splice variants. There are two principle products encoded by the lamin A/C gene: lamins A and C. All lamins have a short head domain followed by a series of heptad repeats that form a 52 nm long rod in homotypic dimers. They differ principally in their large globular tail region. Lamin C differs only in the extreme carboxyl-terminal end of the tail, being for practical purposes a shorter form of lamin A (Lin & Worman, 1993). Emerin is a 254 amino acid, type II integral membrane protein that is anchored to the inner nuclear membrane by its hydrophobic tail (Manilal et al. 1996, Nagano et al. 1996) and interacts directly with lamins A and C (Clements et al. 2000). The emerin-lamin A/C interaction is of particular interest because mutations in either protein cause different variants of Emery-Dreifuss muscular dystrophy (EDMD) (Bione et al. 1994, Bonne et al. 1999, reviewed by Morris, 2001). The interaction occurs between the globular tail region common to both lamins A and C (Wilkinson et al. 2003) and a central region (aminoacids 70–164) of the emerin molecule (Lee et al. 2001). A largely-overlapping region of emerin (amino-acids 107– 175) is required for its localisation to the nuclear rim (Tsuchiya et al. 1999, Ostlund et al. 1999), suggesting involvement of lamin A/C in the localisation process, and this was confirmed by the relocation of emerin to the ER in embryonic fibroblasts from the lamin A/C knockout mouse (Sullivan et al. 1999).

Both lamins and emerin have a wide range of binding partners, some of which may compete for the same binding sites. Thus the interaction of emerin with A-type

lamins is complicated by the presence of syne proteins (Apel et al. 2000, also called nesprins, Zhang et al. 2001), very large nuclear membrane proteins that separately have been shown to bind both emerin and lamin A (Mislow et al. 2002) and may serve as an additional targeting and functional site for emerin at the nuclear envelope. Actin also interacts directly with both nesprin (ANC-1; Starr & Han, 2002) and emerin (Holaska et al. 2004), adding yet more possible tethering and functional mechanisms. While the specific binding sites have not been mapped for many partners of emerin, its interaction with the chromatin protein BAF (Furukawa, 1999) has been mapped to a domain in emerin that is shared by several other nuclear membrane proteins, termed the 'LEM' domain (Lee et al. 2001). The interactions of emerin may prove to be exponentially more complicated as it contains sequences that will bind to nuclear transcription factors or splicing factors (reviewed by Zastrow et al. 2004). Lamins also interact directly with chromatin and with a number of other integral membrane proteins, including lamina-associated proteins (LAPs), MAN1 and the lamin B receptor (Gruenbaum et al. 2003, Reviewed in Hutchinson & Worman, 2004).

Although both mRNA (Su et al. 2002) and protein (Worman et al. 1988, Manilal et al. 1996) studies of whole tissues indicate that emerin and lamin B1 are nearly ubiquitous, nuclei in some cell types are not stained by immunofluorescence microscopy using antibodies to these proteins (Nagano et al., 1996, Broers et al. 1997). In skeletal muscle and heart, for example, the nuclei of contractile cells were immunostained with emerin antibodies but not with a lamin B1 monoclonal antibody, while interstitial cells were positive for the lamin B1 mAb and negative for emerin (Manilal et al. 1999a). This seemed of particular interest because heart and skeletal muscle are the primarily affected tissues in Emery-Dreifuss muscular dystrophy. The

mutual exclusivity between emerin and lamin B1, however, was also seen in kidney tissue (Manilal et al. 1999b, see also Broers et al. 1997 for other tissues). As this mutual exclusivity could have implications for a functional mechanism of these proteins, we sought to ensure that it was not due to differential antibody accessibility, or epitope masking, especially in view of the known masking of nucleoplasmic lamins by chromatin (Hozak et al. 1995). In the present study, we show that different lamin B1 epitopes are masked in different cell types and that masking is a possibility for the apparent absence of the whole emerin molecule in some cell types. Changes at a late stage in cell and tissue differentiation, in chromatin or in the composition of heteroligomeric protein complexes, are possible explanations for the observations.

Materials and methods

Antibodies

Monoclonal antibodies against lamin B1 have been described previously (clone 8D1; Maske et al. 2003) or were obtained from Chemicon Europe (Chandlers Ford, UK) (clone 119D5-F1; Cat No. MAB3213). A polyclonal anti-peptide serum against lamin B1 (pAbB1-cbs) has also been described previously (Schirmer et al. 2001). The lamin A/C mAb (131C3; cat MAB3538) was obtained from Chemicon or from Dr. Yves Raymond (Institut du Cancer, Hoˆ pital Notre-Dame, Montreal). A panel of monoclonal antibodies against human emerin has been described previously (Manilal et al. 1996) and mapped to specific epitopes in the emerin amino-acid sequence (Manilal et al. 1999a). A rabbit antiserum raised against full-length recombinant human emerin has also been described (Holt et al. 2003).

Recombinant proteins

The full length human lamin B1 coding sequence (1– 586) and a fragment corresponding to residues 356–586 were cloned into pET28a (Novagen) with a 6x -His epitope tag for purification. The proteins were expressed in BL21-(DE3) cells by induction with 0.3 mM isopropyl-1-thio-ß-D-galactopyranoside at A595 0.7 for 3 h at 37 °C, collected by centrifugation, and lysed by sonication in 25 mM HEPES pH 8.0, 0.1 mM $MgCl_2$, 3 mM ß-mercaptoethanol containing 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 µg/ml aprotinin, 1 µM leupeptin, and 1 µM pepstatin. The pellets from a 20 min centrifugation at 27,000x g were washed with 1% Triton X-100 and resuspended in 20 mM HEPES pH 8.0, 8 M urea, 3 mM ß-mercaptoethanol. For further purification, this was incubated with nickel resin (Qiagen) for interaction of

the 6x-His tag from the pET28a vector and proteins were eluted with the same buffer containing 200 mM imidazole. Proteins were then dialyzed into 20 mM Tris–HCl pH 8.0, 8 M urea, 2 mM DTT, 1 mM EDTA with protease inhibitors for storage.

Immunofluorescence microscopy

Five micron frozen tissue sections were air-dried and stored at -80 °C. Sections were incubated for 30 min at room temperature with antibodies diluted to the recommended concentrations (1:100 for rabbit antisera, 1:4 to 1:100 for mAbs) in PBS (150 mM NaCl, 25 mM sodium phosphate, pH 7.4). Slides were washed with PBS and incubated for 30 min with FITC- or TRITC-conjugated horse anti-(mouse Ig) diluted 1:50 (Vector Laboratories, Peterborough, UK). For double labelling, primary antibodies were added sequentially, followed by FITC goat anti-(rabbit Ig) and TRITC horse anti- (mouse Ig) together. After mounting in Hydromount (National Diagnostics, Hull, UK), images were captured with a Leica DMRB epifluorescence photomicroscope using a 40x PL Fluotar objective.

Western blotting

Tissue samples were homogenized and boiled for 2 min in 4–8 volumes of extraction buffer (2% sodium dodecyl sulphate- SDS, 5% 2-mercaptoethanol, 62.5 mM Tris–HCl, pH 6.8) and centrifuged for 1 h at 100,000 xg. The supernatants were subjected to electrophoresis on 10% polyacrylamide gels and then transferred to nitrocellulose membrane by electrophoresis at 100 mA for 16 h in 25 mM Tris, 192 mM glycine. The membranes were blocked in 3% skimmed milk powder in incubation buffer (IB: 0.05% Triton X-100 in PBS) for 1 h before incubation with mAb (1/100 dilution of culture supernatant) for 1 h at 20 °C. After washing in IB, blots were incubated with

1:1000 peroxidase-labeled rabbit anti- (mouse Ig) (DAKOpatts, washed again with PBS and developed with Supersignal West Pico chemiluminescent system according to the manufacturer’s instructions (Pierce, Rockford, USA)

Results

Epitope masking of lamin B1

Adult human cardiomyocytes did not immunoreact with the lamin B1 monoclonal antibody 119D5-F1 in situ in an earlier study (Manilal et al. 1999a). To determine if lamin B1 was truly absent from these cells or the epitope recognised by the antibody was masked, we have reinvestigated this problem using a wider panel of antibodies. One of these, pAbB1-cbs, is a rabbit polyclonal antiserum raised against a lamin B1-specific peptide (amino-acids 391–428; Schirmer et al. 2001) corresponding to the comparative location of the biochemically- defined chromatin-binding region of lamin A (Taniura et al. 1995). This was subsequently shown to be one of two separate chromatin-binding sites in the globular tail region of lamin B1 (Goldberg et al. 1999). The second was a monoclonal antibody, mAb8D1, that recognises the farnesylated carboxy-terminus of lamin B1, yet does not cross-react with the farnesylated carboxy-terminus of lamin B2 (Maske et al. 2003). The original mAb, 119D5-F1, recognises an epitope in the carboxy-terminal two-thirds of the molecule after a caspase cleavage site at Asp-231 (Weaver et al. 1996). The epitope positions of all three lamin B1 antibodies are illustrated graphically in Figure 1a. Figure 1c shows that 119D5-F1 recognises a lamin B1 fragment of amino-acids 1–356. This maps the 119D5-F1 epitope to within the region 231–356 of rod domain (Figure 1a).

The earlier conclusion that mAb 119D5-F1 (red) does not stain large cardiomyocyte nuclei in the heart is confirmed in Figure 2c. The proper function of the antibody is affirmed by strong staining of the nucleus in a smaller interstitial cell. Sections were lightly-counterstained for the muscle plasma membrane marker,

dystrophin (also red), to show the outline of cardiomyocytes clearly. In contrast, mAb8D1 (red; Figure 2a) and pAbB1-cbs (green; Figure 2b) did stain cardiomyocyte nuclei, as well as interstitial cell nuclei. This suggests that the 119D5-F1 epitope on lamin B1 is specifically masked in cardiomyocytes.

In adult rat hippocampus, all neuronal cell nuclei were positive with mAb8D1 (Figure 2d), but they were all negative with pAbB1-cbs (Figure 2e). The antibody was functional in rat as shown by its staining of rat kidney nuclei (Figure 2e-insert). The selectivity of staining by 119D5-F1 was striking, with only the inner neurons of the hippocampus significantly stained (Figure 2f). Whether this relates to functional differences that are known between dorsal and ventral neurons within subregions of the hippocampus is unclear (Moser & Moser, 1998).

Epitope masking of emerin

Nagano et al. (1996) showed that rabbit antisera against emerin failed to stain the nuclear rim in several specific tissues and cell types, while other nuclei were brightly stained. Once again, this could be due either to absence of emerin or to epitope masking. It is difficult to resolve this issue by western blotting because no tissues are homogeneously ‘emerin-negative’ and homogeneous, cultured cells are invariably emerin-positive.

In Figure 3 we have compared adult pig lung and spleen for levels and distribution of lamin A/C and emerin (counts of at least 250 nuclei). On immunostained sections, over 50% of all nuclei were positive with lamin A/C mAb in both lung (144/270 = 53%) and spleen (183/288 = 64%). With emerin mAbs, 32% (86/270) of lung nuclei were positive but only 5% (15/ 288) of spleen nuclei. If the

poor immunostaining in spleen nuclei were due to absence of emerin, one would expect a greatly-reduced ratio of emerin to lamin A/C on western blots (materials for sections and western blots were taken from the same pieces of pig tissue). Quantitation of the western blots in Figure 3b shows that the ratio of emerin to lamin A/C is the same, or very similar, in lung and spleen (Table 1). The simplest explanation of these data is that emerin is masked (inaccessible to antibodies) in most spleen nuclei (and in a smaller proportion of lung nuclei). Similar immunostaining results were obtained with different emerin mAbs against at least six different epitopes (MANEM1, 4, 5, 6, 7, 14 and 16: Manilal et al. 1999a) spread throughout the emerin sequence (Figure 1b). This suggests that the whole emerin molecule is masked, unlike the differential masking of epitopes observed with lamin B1 antibodies. A third possibility is that emerin is not masked but is relocated away from the nuclear rim in the ‘emerin-negative’ cells. Absence of lamin A/C is known to cause relocation of emerin into the peripheral ER (Sullivan et al. 1999, Holt et al. 2003) but, although most nuclei that are lamin A/C-negative were also emerin-negative, the absence of lamin A/C staining did not invariably cause absence of emerin staining (Figure 3a and data not shown). The relationship between lamin A/ C and emerin staining, however, deserves closer attention by studies using a number of different lamin A/C epitopes. Preliminary attempts to ‘unmask’ emerin epitopes by treatment of tissue sections with DNAseI have not been successful (data not shown), though this approach would be worth pursuing more systematically for both emerin and lamins, alongside positive controls.

Discussion

We have shown that absence of nuclear rim staining with antibodies against emerin or lamins can be due to epitope masking, rather than absence of the protein antigen from the nuclear rim.

Epitope masking can occur as a result of conformational changes in the antigen, post-translational modification (such as phosphorylation) or interaction with other macromolecules that physically block the epitope. While the possibility that lamins vary in conformation in different stages of differentiation or cell division seems unlikely in view of their polymeric filament structure in the lamina (Stuurman et al. 1998), lamins are nonetheless highly promiscuous proteins that may have many interaction partners capable of blocking access to epitopes (Zastrow et al. 2004). The loss of staining in the hippocampus with pAbB1-cbs is interesting as this antibody recognises a chromatin binding site of lamin B1. An important phosphorylation site in lamin A associated with cell division was identified at the end of the equivalent region within the peptide used to generate pAbB1-cbs (Heald & McKeon, 1990). However, one would not expect this site to be phosphorylated in postmitotic neuronal nuclei in the hippocampus (Figure 2e). Furthermore, since this is a polyclonal antiserum against a 38 amino-acid peptide, it seems unlikely that all component antibodies in the serum recognise the same modification-sensitive epitope. Unlike other masking mechanisms, masking due to post-translational modification would occur on western blots as well as in immunofluorescence microscopy. mAb8D1 recognises a farnesylated epitope at the lamin B1 carboxy-terminus and it is interesting that this epitope was invariably expressed in all nuclei studied. Modification seems even less likely in the case of emerin, since the whole molecule is masked and several of the

mapped epitopes in this study contain no phosphorylation sites. The lamin phosphorylation changes that occur during the cell cycle do not cause epitope masking, since cultured cell nuclei are invariably positive for emerin and all lamins, even in quiescent cells (data not shown). Indeed, it is the fact that masking only occurs in tissues, and not in cultured cells, that makes the process difficult to study.

There is good evidence that chromatin can mask lamin B epitopes, especially in the interior of the nucleus (Hozak et al. 1995) and heterochromatin often accumulates at the nuclear periphery in human skeletal and cardiac muscle nuclei. Gene-poor chromosomal domains are also found preferentially close to the nuclear rim (Croft et al. 1999). Both lamins and emerin interact with chromatin, directly or indirectly. Ausma et al. (1996) noted that lamin A/C staining of cardiomyocytes was related to dispersal of the heterochromatin in the nuclei. Furthermore, in both the lamin A/C knockout mouse and Emery-Dreifuss muscular dystrophy patients with emerin or lamin A/C mutations, there is evidence for disrupted attachment of chromatin to the nuclear rim (Ognibene et al. 1999, Sewry et al. 2001, Fidzianska & Hausmanowa-Petrusewicz, 2003). We might hypothesise, therefore, that interaction with chromatin is responsible for epitope masking in emerin and lamins. In such a model, changes in masking in different cell types might be produced by the extensive chromatin remodelling that occurs during differentiation. This explanation is supported by the fact that one of the antibodies, pAbB1-cbs, is directed against a known chromatin-binding region of lamin B1 (Schirmer et al. 2001). However, the possibility that nuclear proteins cause the masking cannot be ruled out, especially in view of the large number of interactions of the emerin- lamin A/C complex recently identified (Zastrow et al. 2004). Some of these proteins, notably synes or nesprins, are expressed at high levels in cardiac and skeletal muscle tissues (Zhang et al. 2001). It

is relevant that many of the nuclei in adult lung and spleen that were negative with emerin antibody were also negative for a lamin A/C epitope (Figure 3a), in view of the direct interaction between these two proteins (Clements et al. 2000). We have no direct evidence that the lamin A/C epitope is also masked (rather than absent), like lamin B1. The absence of emerin staining at the nuclear rim does not appear to be due to ‘absence’ of lamin A/C, since emerin staining is absent in many nuclei that are negative for lamin A/C (Figure 3).

This study raises important general issues about the interpretation of immunostaining experiments. Absence of staining by antibody clearly does not always demonstrate absence of the antigen. Sometimes, as in the case of lamin B1, this can be overcome by the use of several antibodies against different epitopes, but this does not solve the problem in the case of emerin. Normally, a high affinity mAb would be capable of displacing interacting proteins from an antigen by mass action. Chemical cross-linking would prevent such displacement and may partially explain why many monoclonal antibodies do not recognise formalin-fixed antigens in situ. Interaction of an antigen with a large number of different proteins, as well as chromatin and the inner nuclear membrane, may have similar effects to chemical cross-linking

Acknowledgements

Emerin and lamin studies in the Morris laboratory were supported by a British Heart Foundation grant (PG97142). DT was supported by a grant from the Clwyd Institute of Health Studies. We thank Yves Raymond (Hospital Notre-Dame, Montreal) for mAbs against A-type lamins.

References

Apel ED, Lewis RM, Grady RM, Sanes JR (2000) Syne-1, a dystrophin- and Klarsicht-related protein associated with synaptic nuclei at the neuromuscular junction. J Biol Chem 275: 31986– 31995.

Ausma J, van Eys GJ, Broers JL, Thone F, Flameng W, Ramaekers FC, Burgers M (1996) Nuclear lamin expression in chronic hibernating myocardium in man. J Mol Cell Cardiol 28: 1297–1305.

Bione S, Maestrini E, Rivella S, Mancini M, Regis S, Romeo G, Toniolo D (1994) Identification of a novel X-linked gene responsible for Emery-Dreifuss muscular dystrophy. Nat Genet 8: 323–327.

Bonne G, DiBarletta MR, Varnous S, Becane HM, Hammouda EH, Merlini L, Muntoni F, Greenberg CR, Gary F, Urtizberea JA, Duboc D, Fardeau M, Toniolo D, Schwartz K (1999) Mutations in the gene encoding lamin A/C cause autosomal dominant Emery- Dreifuss muscular dystrophy. Nat Genet 21: 285–288.

Broers JL, Machiels BM, Kuijpers HJ, Smedts F, van den Kieboom R, Raymond Y, Ramaekers FC (1997) A- and B-type lamins are differentially expressed in normal human tissues. Histochem Cell Biol 107: 505–517.

Clements L, Manilal S, Love DR, Morris GE (2000) Direct interaction between emerin and lamin A. Biochem Biophys Res Commun 267: 709–714.

Croft JA, Bridger JM, Boyle S, Perry P, Teague P, Bickmore WA (1999) Differences in the localization and morphology of chromosomes in the human nucleus. J Cell Biol 145: 1119–1131.

Fidzianska A, Hausmanowa-Petrusewicz I (2003) Architectural abnormalities in muscle nuclei. Ultrastructural differences between Xlinked and autosomal dominant forms of EDMD. J Neurol Sci. 210: 47–51.

Furukawa K (1999) LAP2 binding protein 1 (L2BP1/BAF) is a candidate mediator of LAP2-chromatin interaction. J Cell Sci 112: 2485–2492.

Goldberg M, Harel A, Brandeis M, Rechsteiner T, Richmond TJ, Weiss AM, Gruenbaum Y (1999) The tail domain of lamin Dm0 binds histones H2A and H2B. Proc Natl Acad Sci USA. 96: 2852– 2857.

Gruenbaum Y, Goldman RD, Meyuhas R, Mills E, Margalit A, Fridkin A, Dayani Y, Prokocimer M, Enosh A (2003) The nuclear lamina and its functions in the nucleus. Int Rev Cytol. 226: 1–62.

Heald R, McKeon F (1990) Mutations of phosphorylation sites in lamin A that prevent nuclear lamina disassembly in mitosis. Cell 61: 579–589.

Holaska JM, Kowalski AK, Wilson KL (2004) Emerin caps the pointed end of actin filaments: Evidence for an actin cortical network at the nuclear inner membrane. PLoS Biol. 2: E231.

Holt I, Ostlund C, Stewart CL, Nguyen thi Man, Worman HJ, Morris GE (2003) Effect of pathogenic mis-sense mutations in lamin A on its interaction with emerin in vivo. J Cell Sci 116: 3027–3035.

Hozak P, Sasseville AM, Raymond Y, Cook PR (1995) Lamin proteins form an internal nucleoskeleton as well as a peripheral lamina in human cells. J Cell Sci 108: 635–644.

Hutchison CJ, Worman HJ (2004) A-type lamins: guardians of the soma?. Nat Cell Biol 6: 1062–1067.

Lee KK, Haraguchi T, Lee RS, Koujin T, Hiraoka Y, Wilson KL (2001) Distinct functional domains in emerin bind lamin A and DNA-bridging protein BAF. J Cell Sci 114: 4567–4573.

Lin F, Worman HJ (1993) Structural organization of the human gene encoding nuclear lamin A and nuclear lamin C. J Biol Chem 268: 16321–16326.

Manilal S, Nguyen thi Man, Sewry CA, Morris GE (1996) Emery- Dreifuss muscular dystrophy protein, emerin, is a nuclear membrane protein. Hum Mol Genet 5: 801–808.

Manilal S, Sewry CA, Pereboev A, Nguyen thi Man, Gobbi P, Hawkes S, Love DR, Morris GE (1999a) Distribution of emerin and lamins in the heart and implications for Emery-Dreifuss muscular dystrophy. Hum Mol Genet 8: 353–359.

Manilal S, Sewry CA, Morris GE (1999b) Emerin is associated with lamin A/C in one subset of kidney cell nuclei while lamin B1 is present in the emerin-negative nuclei. Amer J Hum Genet 65(Suppl. S): 2715.

Maske CP, Hollinshead MS, Higbee NC, Bergo MO, Young SG, Vaux DJ (2003) A carboxyl-terminal interaction of lamin B1 is dependent on the CAAX endoprotease Rce1 and carboxymethylation. J Cell Biol 162: 1223–1232.

Mislow JM, Holaska JM, Kim MS, Lee KK, Segura-Totten M, Wilson KL, McNally EM (2002) Nesprin-1alpha self-associates and binds directly to emerin and lamin A in vitro. FEBS Lett 525: 135– 140.

Morris GE (2001) The role of the nuclear envelope in Emery-Dreifuss muscular dystrophy. Trends Mol Med 17: 572–577.

Moser MB, Moser EI (1998) Functional differentiation in the hippocampus. Hippocampus 8: 608–619.

Nagano A, Koga R, Ogawa M, Kurano Y, Kawada J, Okada R, Hayashi YK, Tsukahara T, Arahata K (1996) Emerin deficiency atthe nuclear membrane in patients with Emery-Dreifuss muscular dystrophy. Nat Genet 12: 254–259.

Nguyen thi Man, Cartwright AJ, Morris GE, Love DR, Bloomfield JF, Davies KE (1990) Monoclonal antibodies against defined regions of the muscular dystrophy protein, dystrophin. FEBS Lett 262: 237–240.

Ognibene A, Sabatelli P, Petrini S, Squarzoni S, Riccio M, Santi S, Villanova M, Palmeri S, Merlini L, Maraldi NM (1999) Nuclear changes in a case of X-linked Emery-Dreifuss muscular dystrophy. Muscle Nerve 22: 864–869.

Ostlund C, Ellenberg J, Hallberg E, Lippincott-Schwartz J, Worman HJ (1999) Intracellular trafficking of emerin, the Emery-Dreifuss muscular dystrophy protein. J Cell Sci 112: 1709–1719.

Schirmer EC, Guan T, Gerace L (2001) Involvement of the lamin rod domain in heterotypic lamin interactions important for nuclear organization. J Cell Biol 153: 479–489.

Sewry CA, Brown SC, Mercuri E, Bonne G, Feng L, Camici G, Morris GE, Muntoni F (2001) Skeletal muscle pathology in autosomal dominant Emery-Dreifuss muscular dystrophy with lamin A/C mutations. Neuropathol Appl Neurobiol 27: 281–290.

Starr DA, Han M (2002) Role of ANC-1 in tethering nuclei to the actin cytoskeleton. Science 298: 406–409.

Stuurman N, Heins S, Aebi U (1998) Nuclear lamins: their structure, assembly, and interactions. J Struct Biol 122: 42–66.

Su AI, Cooke MP, Ching KA, Hakak Y, Walker JR, Wiltshire T, Orth AP, Vega RG, Sapinoso LM, Moqrich A, Patapoutian A, Hampton GM, Schultz PG, Hogenesch JB (2002) Large-scale analysis of the human and mouse transcriptomes. Proc Natl Acad Sci USA 99: 4465–4470.

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: 913–920.

Taniura H, Glass C, Gerace L (1995) A chromatin binding site in the tail domain of nuclear lamins that interacts with core histones. J Cell Biol 131: 33–44.

Tsuchiya Y, Hase A, Ogawa M, Yorifuji H, Arahata K (1999) Distinct regions specify the nuclear membrane targeting of emerin, the responsible protein for Emery-Dreifuss muscular dystrophy. Eur J Biochem 259: 859–865.

Weaver VM, Carson CE, Walker PR, Chaly N, Lach B, Raymond Y, Brown DL, Sikorska M (1996) Degradation of nuclear matrix and DNA cleavage in apoptotic thymocytes. J Cell Sci 109: 45–56.

Wilkinson FL, Holaska JM, Zhang Z, Sharma A, Manilal S, Holt I, Stamm S, Wilson KL, Morris GE. (2003) Emerin interacts in vitro with the splicing-associated factor YT521-B. Eur J Biochem 270: 2459–2466.

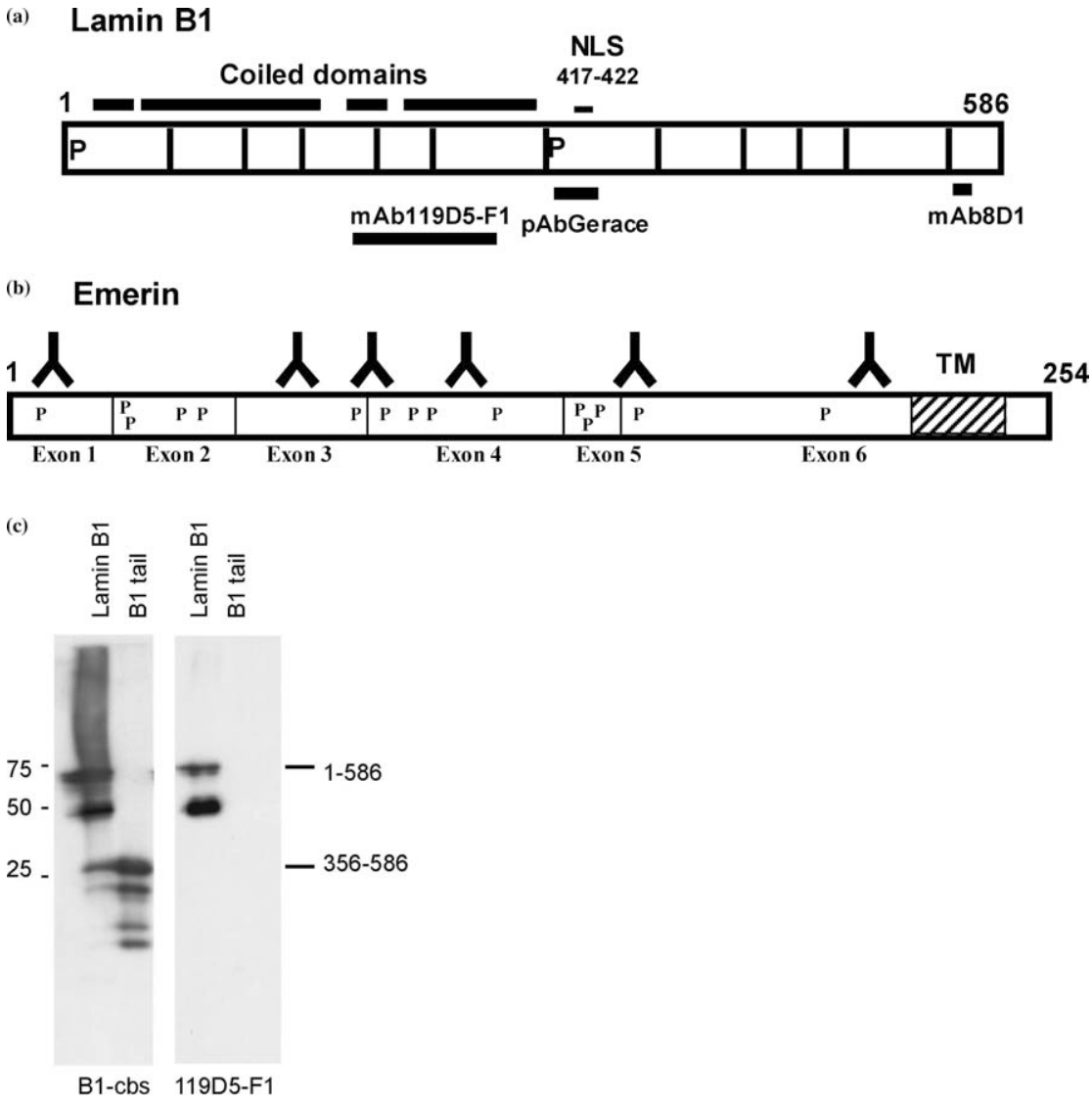
Worman HJ, Lazaridis I, Georgatos SD (1988) Nuclear lamina heterogeneity in mammalian cells. Differential expression of the major lamins and variations in lamin B phosphorylation.. J Biol Chem 263: 12135–12141.

Zastrow MS, Vlcek S, Wilson KL (2004) Proteins that bind A-type lamins: integrating isolated clues. J Cell Sci 117: 979–987.

Zhang Q, Skepper JN, Yang F, Davies JD, Hegyi L, Roberts RG, Weissberg PL, Ellis JA, Shanahan CM (2001) Nesprins: a novel family of spectrin-repeat-containing proteins that localize to the nuclear membrane in multiple tissues. J Cell Sci 114: 4485–4498.

Figure Legends

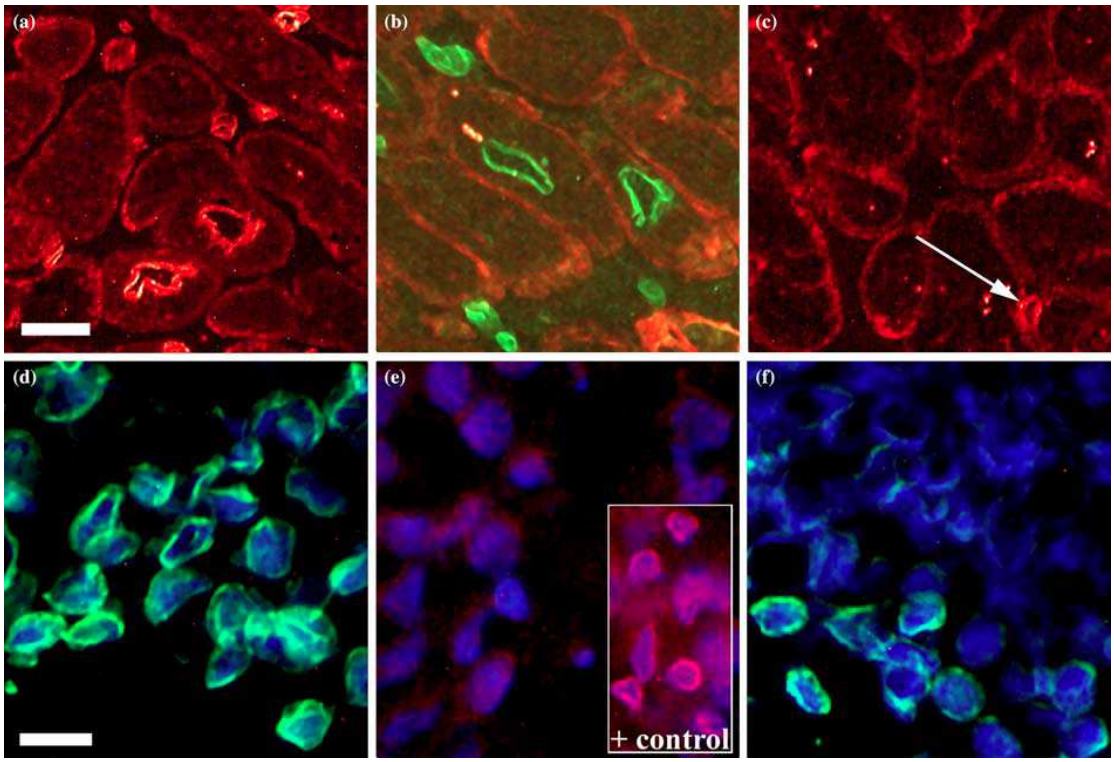
Figure 1.



Antibody binding sites on lamin B1 and emerin. (a)Lamin B1. Coiled-coil regions and the nuclear localization sequence (NLS) are shown above the diagram and the three epitope positions below. (b)Emerin. Regions encoded by the six exons are shown and TM is the transmembrane sequence. All putative phosphorylation sites (P) are shown. Epitopes recognised by emerin mabs are shown above the diagram (inverted Y; Manilal et al. 1999b). (c)Western blot of recombinant lamin B1 fragments for epitope mapping. Both full length lamin B1 and a fragment including the entire C-terminus

and five heptads of the rod domain reacted with pAbB1-cbs, but only the full-length lamin B1 reacted with 119D5-F1. As previous mapping studies had determined the epitope to be between the middle of the rod and the end of the molecule, this further maps it to the latter half of the rod.

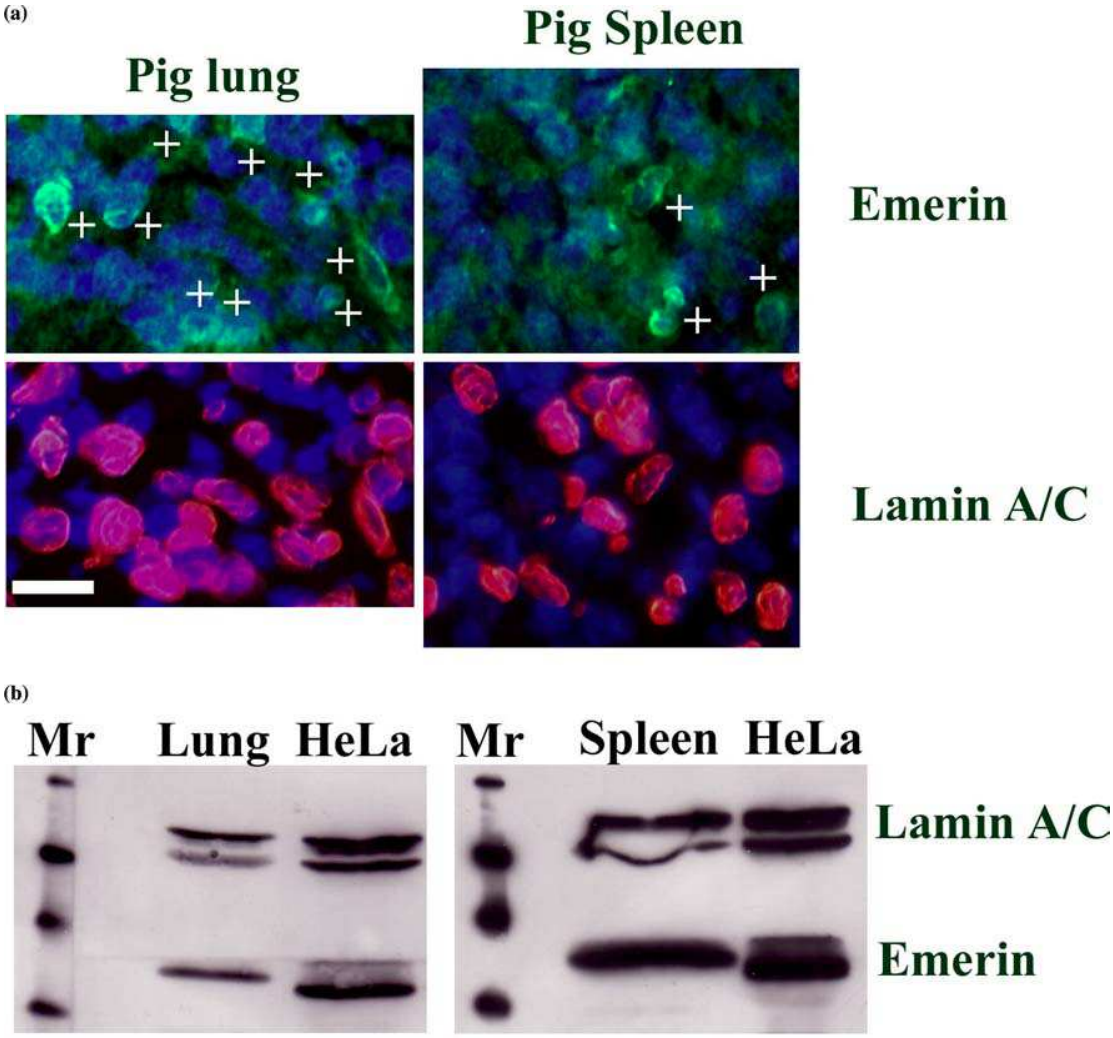
Figure 2.



Epitope masking of lamin B1 in human heart and rat hippocampus. Cardiomyocytes in human heart sections (a, b, c) were distinguished by a light counterstain for dystrophin (weak red) by co-incubating lamin antibodies with 1:1000 MANDYS1 mAb (Nguyen thi Man et al. 1990); this stains the plasmalemma of large cardiomyocytes only. (a) 8D1 monoclonal anti-lamin B1 and TRITC-conjugated anti-(mouse Ig), (b) pAbB1- cbs anti-lamin B1 and FITC-conjugated anti-(rabbit Ig) or (c) 119D5-F1 monoclonal anti-lamin B1 and TRITC-conjugated anti-(mouse Ig). The white arrow in (e) shows an interstitial cell nucleus that is stained by 119D5-F1 (positive control). Nuclei are not visible in all cardiomyocytes because of these large and elongated cells extend far beyond the image frame. Scale bar = 20 microns. Rat hippocampal sections (d, e, f) were immunostained with (a) 8D1 monoclonal anti-lamin B1 and FITC-conjugated anti-(mouse Ig), (b) pAbB1-cbs anti-lamin B1 and TRITCconjugated anti-(rabbit Ig) or (c) 119D5-F1 monoclonal anti-lamin B1 and FITC-conjugated anti-(mouse Ig). All were counterstained for nuclei with DAPI

(blue). The inset in (e) is a rat kidney section to show that pAbB1-cbs can recognise rat lamin B1 when it is accessible. All sections were through the CA1 region of the hippocampus and the nuclei are neuronal. The positive nuclei in (f) are on the ventral, or inner, side of the septotemporal axis. Scale bar = 20 microns

Figure 3.



Evidence for masking of emerin in pig tissues. Double-labelling of pig lung and spleen sections with rabbit anti-emerin serum and monoclonal anti-lamin A/C, followed by FITC anti-rabbit Ig (green) and TRITC anti-mouse Ig (red). The emerin antiserum for double-label was not affinity-purified and gives some background cytoplasmic staining in tissues that is not observed with mAbs. Nuclei were counterstained with DAPI (blue). White 'plus-signs' show emerin-positive nuclei. All emerin-positive nuclei were lamin A/C-positive but many more nuclei were positive only for lamin A/C. The percentage of antibody-positive nuclei in over 250 nuclei counted was determined (see text). Scale bar = 20 microns. (b)Although the ratio of emerin to lamin A/C appears lower in spleen than in lung by immunostaining (a), the

ratios are similar by Western blotting in which proteins are unfolded, allowing access to all epitopes based on the linear sequence. The two pig tissues were run alongside a human HeLa cell extract and biotinylated protein Mr markers (Sigma SDS-6B). The blots were cut in half and reacted with either lamin A/C mAb (upper half) or anti-emerin serum (lower half). Note that pig emerin migrates more slowly on SDS-PAGE than human emerin. The mass ratios of emerin:lamin A/C determined by microdensitometry of the western blot are shown in Table 1 and are not significantly different from each other.

Table 1. The ratio of emerin to lamin A/C is the same in pig lung and spleen as in HeLa cells.

Tissue/cell type	HeLa	Lung
Ratio emerin/lamin A/C	1.35±0.13[4]	1.38±0.31[4]
Tissue/cell type	HeLa	Spleen
Ratio emerin/lamin A/C	1.13±0.13 [4]	1.40±0.25[4]

Data were obtained by densitometry of western blots of the type shown in Figure 3 and the mean of four determinations is shown. The ratios do not reflect the molecular stoichiometry because of possible differences in antibody avidity.