

Both emerin and lamin C depend on lamin A for localization at the nuclear envelope

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SUMMARY

Physical interactions between lamins and emerin were investigated by co-immunoprecipitation of *in vitro* translated proteins. Emerin interacted with *in vitro* translated lamins A, B₁ and C in co-immunoprecipitation reactions. Competition reactions revealed a clear preference for interactions between emerin and lamin C. Structural associations between lamins and emerin were investigated in four human cell lines displaying abnormal expression and/or localisation of lamins A and C. In each cell line absence of lamins A and C from the nuclear envelope (NE) was correlated with mis-localisation of endogenous and exogenous emerin to the ER. In two cell lines that did not express lamin A but did express lamin C, lamin C as well as emerin was mis-localised. When GFP-lamin A was expressed in SW13 cells (which normally

express only very low levels of endogenous lamin A and mis-localise endogenous emerin and lamin C), all three proteins became associated with the NE. When GFP-lamin C was expressed in SW13 cells neither the endogenous nor the exogenous lamin C was localised to the NE and emerin remained in the ER. Finally, lamins A and C were selectively eliminated from the NE of HeLa cells using a dominant negative mutant of lamin B₁. Elimination of these lamins from the lamina led to the accumulation of emerin as aggregates within the ER. Our data suggest that lamin A is essential for anchorage of emerin to the inner nuclear membrane and of lamin C to the lamina.

Key words: Lamins, Emerin, Nuclear envelope, Nuclear lamina, Emery-Dreifuss muscular dystrophy

INTRODUCTION

The nuclear envelope (NE) forms the boundary between the nucleus and the cytoplasm and physically separates transcription and translation. The nuclear lamina, together with the inner (INM) and outer nuclear membranes (ONM) and the nuclear pore complex are the major components of the NE. The nuclear lamina is a flattened orthogonal network of filaments lying subjacent to the INM. The filaments of the lamina are composed of type V intermediate filament proteins termed lamins. Like all intermediate filament proteins, lamins possess a long central α -helical rod domain flanked by a short globular N-terminal head and a long C-terminal tail domain. Lamins apparently assemble into filaments through a series of hierarchical associations. These include the initial formation of dimers through parallel in register coiled-coil associations through the rod domain, followed by anti-parallel half-staggered lateral associations of dimers to form tetramers. Tetramers then make head-to-tail longitudinal associations to form protofilaments (see Goldberg et al., 1999; Stuurman et al., 1998).

The composition of the lamina varies according to cell type and stage of differentiation. Two broad classes of lamins are

expressed in vertebrates and these are referred to as A-type and B-type. These lamins vary in primary sequence and biochemical properties as well as in their expression patterns. B-type lamins are expressed in all germ cells and somatic cells although different B-type lamins are expressed in each (reviewed by Vaughan et al., 2000). Lamins B₁ and B₂ are the major B-type lamins expressed in mammalian somatic cells and these proteins are the products of separate genes (reviewed by Gant and Wilson, 1997). Lamins A, C, C2 and A Δ 10 comprise the A-type lamins and all are alternatively spliced products of a single gene. Lamin A and C are the most abundant A-type lamins and differ in that lamin C lacks a 90 amino acid C-terminal extension possessed by lamin A but has five unique amino acids at its C-terminus (reviewed by Quinlan et al., 1995). Both lamin A and lamin C are expressed only in differentiated cells and during mouse development appear at the time of organogenesis (Rober et al., 1989); however, they are dispensable for development since a lamin A/C knockout mouse survives to adulthood (Sullivan et al., 1999).

Recently, a number of different autosomal dominant diseases have been shown to be caused by mutations in the gene encoding lamins A and C (reviewed by Flier, 2000). These diseases include an autosomal dominant form of Emery-

Dreifuss muscular dystrophy (AD EDMD) (Bonne et al., 1999; Raffaele et al., 2000), dilated cardiomyopathy with conduction system disease (Fatkin et al., 1999) and a Dunnigan-type familial partial lipodystrophy (Shackleton et al., 2000; Cao and Hegele, 2000). It is currently unclear why these very different diseases arise through mutations in the same proteins. However, since a majority of disease phenotypes are caused by missense mutations that occur in different parts of the proteins (Flier, 2000; Raffaele et al., 2000), one possibility is that these lamins interact with a number of different nuclear proteins and that different mutations affect different lamin interactions.

Emerin was first identified by positional cloning of a gene on chromosome Xq28 that is mutated in individuals with X-EDMD. The emerin gene encodes a 254 amino acid type II integral membrane protein (Bione et al., 1994). Structural analysis predicts that emerin contains a transmembrane region at the C-terminus and a large hydrophilic N-terminal domain with multiple putative phosphorylation sites (Bione et al., 1994). In addition, emerin contains the LEM domain signature common to a number of integral membrane proteins of the inner nuclear membrane (reviewed by Hutchison et al., 2001). Emerin is a serine rich protein that migrates as a 34 kDa band on SDS-PAGE. It is principally located at the INM in almost every tissue (Nagano et al., 1996; Manilal et al., 1996). In cardiac muscle, emerin has also been located at intercalated disks (Cartegni et al., 1997), although this finding was not substantiated by later studies (Manilal et al., 1999). In skeletal muscle cells grown in culture, a fraction of emerin is located in the endoplasmic reticulum (ER) (Fairley et al., 1999). The majority of lesions in the emerin gene that cause EDMD are null mutations (Nagano et al., 1996; Manilal et al., 1997; Manilal et al., 1998; Mora et al., 1997; Ellis et al., 1998; Yates et al., 1999). However, some mutations result in the production of modified forms of emerin (Manilal et al., 1997; Mora et al., 1997; Ellis et al., 1998; Wulff et al., 1997; Yates et al., 1999). These mutations occur throughout the protein with no obvious hot-spots. Interestingly, some mutations in the N-terminal 'nucleoplasmic' domain cause mis-localisation of emerin either to cytoplasmic membranes or to the nucleoplasm (Fairley et al., 1999; Ellis et al., 1998). This finding is consistent with the observation that sequences within the N-terminal nucleoplasmic domain are necessary and sufficient to target emerin to the INM (Östlund et al., 1999).

A possible link between X-EDMD and AD EDMD is that A-type lamins form structural associations with emerin at the INM. To test this hypothesis we have investigated lamin-emerin interactions *in vivo* and *in vitro*. Our data suggest that the organisation of both lamin C in the lamina and of emerin at the INM is dependent upon lamin A.

MATERIALS AND METHODS

Antibody reagents

Monoclonal antibodies against lamins A/C (JoL2 and JoL5), lamin A (JoL4) and lamin B₂ have been described previously (Dyer et al., 1997). In addition, monoclonal antibodies against emerin (MANEM3 and MANEM5; Manilal et al., 1996) and LAP2 β (LAP17; Dechat et al., 1998) have been described previously. The polyclonal rabbit antibody against lamin C was raised to the last eight amino acids of lamin C, including an N-terminal lysine as a linker (KHHVSGSRR). The peptide was coupled to keyhole limpet haemocyanin through

primary amino groups using glutaraldehyde. The resulting protein-peptide conjugate was dialysed overnight at 4°C against PBS. The conjugate was then used to immunise a rabbit. Immune serum was screened by indirect immunofluorescence and then affinity purified against 10 mg of the lamin C peptide conjugated to CH Sepharose 4B as described (Harlow and Lane, 1988). The antibody recognises the lamin C-specific tail domain of recombinant lamin C, but no other part of recombinant lamin C or any other recombinant lamins on western blots. The antibody detects a single band migrating at 65 kDa in western blots of nuclei isolated from HeLa, human dermal fibroblasts and SW13 cells. In the majority of human cell lines and primary human fibroblasts the antibody stains the NE exclusively (for a complete characterisation of this antibody, see Venables et al., 2001). Polyclonal anti-emerin was from J. Ellis (Ellis et al., 1998). The anti-lamin B₁ specific antibody was purchased from Santa Cruz Biotechnology. Monoclonal anti-Ki67 was purchased from DAKO.

Expression and immunoprecipitation of recombinant lamins, NUP-153 and emerin

Plasmid pWW1 hLamin B₁ was constructed by subcloning an NcoI-BamHI fragment of human lamin B₁ cDNA (Pollard et al., 1990) into NcoI-BamHI cut T7 expression vector pWW1 (Whitfield et al., 1990). Cloning of cDNA encoding the first 188 amino acids of emerin from total human skeletal muscle cDNA into the expression plasmid pMW172, and full length emerin cDNA into plasmid pET17xb have already been described (Manilal et al., 1996; Manilal et al., 1999). Plasmids pET-1 hLamin A and pET-1 hLamin C are described elsewhere (Moir et al., 1990; Moir et al., 1991). Nup-153 cDNA (gift from Brian Burke, University of Calgary, Canada) was cloned into pRSET-A using *Xho*I and *Pvu*II. Recombinant lamins A, C, B₁, Nup-153, and the N-terminal 188 amino acids of emerin were expressed using the TNT^r Quick Coupled Transcription/Translation System (Promega) under recommended conditions. Reticulolysates containing expressed lamins and emerin were pooled and incubated at 4°C overnight. Pefabloc^r, leupeptin, pepstatin and aprotinin (Boehringer Mannheim) were added to a final concentration of 1 mM each. Reaction mixtures were pre-cleared using paramagnetic Dynabeads^r M-280 (DYNAL). Lamins A and C were recovered using mAbs JoL2, JoL4 or JoL5 (or the lamin A-specific JoL4) conjugated to Dynabeads^r according to a method previously described (Jenkins et al., 1993). Likewise, emerin was immunoprecipitated using mAbs MANEM3 and -5 (Manilal et al., 1996), and NUP-153 using mAb 414.

Cell culture and preparation of SW13/20

Human cervix carcinoma (HeLa) and Human adrenal cortex carcinoma (SW13) cells (gift from H. Herrmann, Heidelberg) were routinely maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS, v/v) and 1% penicillin/streptomycin (v/v) at 37°C in a humidified atmosphere containing 5% CO₂. Lymphoblastoid, lymphoma and lung carcinoma cells were maintained in RPMI 1640 (GIBCO BRL) containing non-essential amino acids, 15% FCS (v/v) and 1% penicillin/streptomycin. SW13 cells were transfected with pCDNA-EGFP-HLA, a plasmid containing human lamin A fused to EGFP (gift from L. Karnitz, Mayo Clinic), using LipofectinTM (GIBCO BRL), 15 μ g of DNA per 2.5 \times 10⁵ cells and conditions recommended for *Lipofectin* transfections. Stable clones were selected using 300 μ g/ml G418 (Calbiochem).

Cell fractionation

Cells were scraped from a 75 cm² flask using a rubber policeman then washed twice with phosphate-buffered saline (PBS) at 4°C. Cell lysis occurred after incubation in cytoskeletal buffer (10 mM PIPES, pH 6.8, 300 mM sucrose, 100 mM NaCl, 3 mM MgCl₂ and 1 mM EGTA) containing 0.5% Triton X-100 at 4°C for 5 minutes. Chromatin was removed by digestion with 200 units/ml RNase-free DNase in

digestion buffer (10 mM PIPES, pH 6.8, 300 mM sucrose, 50 mM NaCl, 3 mM MgCl₂, 1 mM EGTA, 5 units/ml RNase inhibitor (Boehringer Mannheim) containing 0.5% Triton X-100 at 30°C for 30 minutes. Nuclei were washed with extraction buffer (10 mM PIPES, pH 6.8, 250 mM ammonium sulphate, 300 mM sucrose, 3 mM MgCl₂, and 1 mM EGTA) twice for 5 minutes each time at 4°C. Nuclear matrix fractions were solubilised in 8 M urea. Nuclei were pelleted after each step by centrifugation at 1000 rpm for 5 minutes within a pre-chilled microcentrifuge. Pefabloc^r (2 mM), leupeptin (10 μM), pepstatin (1 μM), and aprotinin (0.5 μM) were included throughout.

Construction and expression of GST/GFP fusion proteins

The construction of plasmid pGEX-XLaminB1Δ2+ is described elsewhere (Ellis et al., 1997). This construct expresses amino acid residues 34-420 from *Xenopus* lamin B₁ as a GST fusion protein. GST-XLaminB1Δ2+ fusion protein was expressed then purified from *Escherichia coli* BL21 cells using a method described previously (Ellis et al., 1997). The pEGFP-XLaminB1Δ2+ plasmid was constructed by subcloning the *Sall*-*NotI* insert of pGEX-XLaminB1Δ2+ into pEGFP-C1 (Clontech) cut with *Sall* and *Bsp120L*. Plasmid pEGFP-emerin was constructed by sub-cloning full length emerin from pET17xb into pEGFP-C1 (Clontech) cut with *Bam*HI and *Hind*III. Plasmids pS65T-lamC and pS65T-lamA have been described previously (Broers et al., 1997). HeLa and SW13 cells grown on coverslips to 20% confluence were transfected with relevant plasmids (1-2 μg/coverslip) using the calcium-phosphate method (Graham and van der Eb, 1973). The cells were grown overnight and media replaced, with expression allowed to proceed for a further 36-48 hours.

Immunofluorescence microscopy

Lymphoblast/lymphoma cells were centrifuged onto coverslips at 300 rpm for 3 minutes prior to fixation. Cells grown on coverslips were washed twice in PBS and fixed in ice-cold 1:1 (v/v) methanol-acetone for 10 minutes. Nuclear matrices were prepared as described elsewhere (Dyer et al., 1997). Coverslips were washed 3 times, with 0.5% newborn calf serum (NCS, v/v) in PBS. Hybridoma cell culture supernatants (undiluted) containing mAbs Jol2, Jol4 and LN43 (gift from Birgit Lane, Dundee) were used to stain nuclear lamins A/C, A and B₂ respectively. Likewise, supernatants of mAbs MANEM3 and -5 were used to immunodetect emerin, and LAP17 (gift from Roland Foisner, Vienna) to detect LAP 2β. Goat anti-lamin B (Santa Cruz Biotechnology) and rabbit anti-ER (gift from Daniel Louvard, Paris) and anti-calreticulin (Calbiochem) were used at recommended dilutions to stain lamin B₁ and ER, respectively. Affinity purified rabbit anti-lamin C was used at a dilution of 1/50, as described previously (Venables et al., 2000). Primary antibodies were added for 1 hour at room temperature. Coverslips were washed three times with PBS, then incubated with appropriate Rhodamine (TRITC)-conjugated affinity-purified secondary antibodies (donkey anti-goat, goat anti-mouse, and goat anti-rabbit; Jackson Immunoresearch) for a further hour at room temperature. After several washes in PBS, coverslips were mounted face down in Mowiol (Calbiochem) containing 1 μg/ml DAPI. Immunostained samples were viewed using a Zeiss axiovert 10 microscope with a plan-APOCHROMAT 63×/1.40-oil immersion lens and equipped with a Digital Pixel Instruments 12-bit CCD camera. Images were captured using IP Lab Scientific Imaging Software (Scanalytics). Additionally, a Zeiss LSM 410 confocal laser scanning microscope was used (63×/1.40 oil immersion lens) for imaging of emerin and ER within SW13 cells.

Gel electrophoresis and immunoblotting

Recombinant proteins and nuclear matrix fractions were resolved on 10% SDS-PAGE and transferred to nitrocellulose according to established protocols (Jenkins et al., 1993). Nitrocellulose membranes were washed with blocking buffer (5% milk powder (w/v), 0.1% Tween-20 in PBS) for 1 hour at room temperature. Undiluted cell

culture supernatants containing mAbs Jol2, Jol4 and LN43 were used to detect lamins A/C, A and B₂, respectively. Goat anti-lamin B₁ (Santa Cruz Biotechnology) was used at 1/200. Affinity purified rabbit anti-lamin C was used at a dilution of 1/100. All primary antibodies were incubated with membranes for 30 minutes at room temperature. Membranes were rinsed with blocking buffer several times then incubated with appropriate HRP-conjugated secondary antibodies (rabbit anti-mouse, DAKO; goat anti-rabbit, BIO-RAD; donkey anti-goat, Jackson Immunoresearch) for 30 minutes at room temperature. ECL reagents (Amersham Life Science) were used for the immunological detection of proteins after membranes were rinsed in PBS.

RESULTS

Lamin-emerin interactions in vitro

We initially investigated interactions between emerin and lamins using co-immunoprecipitation assays in rabbit reticulocyte lysates. Since interactions between emerin and lamins have already been demonstrated (Fairley et al., 1999) we established these assays as competition experiments in order to determine the specificity of emerin-lamin complexes and the relative strengths of emerin interactions with specific lamin subtypes. Initially ³⁵S-met-labelled lamins A, B₁ and C, emerin or the nuclear pore protein Nup153 were produced in rabbit reticulocyte lysates. ³⁵S-emerin and ³⁵S-Nup153 were then mixed, together with equal amounts of ³⁵S-lamin A, ³⁵S-lamin B₁ or ³⁵S-lamin C (Fig. 1A). We have previously shown that in vitro translated Nup153 interacts with lamins but not emerin. Therefore Nup153 was used here as a control. Emerin was then immunoprecipitated from each mixed lysate using an equal mixture of the anti-emerin mAbs MANEM3 and MANEM5. The immunoprecipitates were resolved on SDS-PAGE and fluorography was performed in order to detect co-immunoprecipitation of the other proteins. All three lamins co-immunoprecipitated efficiently with emerin. However, although Nup153 was present at the same concentration in each mixed lysate, it was co-immunoprecipitated with emerin either at very low levels or not at all (Fig. 1B). Thus we confirmed that interactions between emerin and each lamin subtype occurred. Moreover, where lamins are capable of forming alternative complexes (e.g. with Nup153), these were not present in the MANEM3/5 immunoprecipitates. This may either be because emerin-lamin interactions out-compete lamin-Nup153 interactions or because alternative complexes are excluded from the immunoprecipitates. However, the absence of Nup153 from the complex indicates that emerin forms a strong and exclusive interaction with each lamin subtype in vitro. To investigate whether emerin displayed a preference for any of the lamins, competition experiments were established between individual lamin polypeptides. In these experiments, ³⁵S-emerin was mixed with equal amounts of ³⁵S-lamins A and C, ³⁵S-lamins A and B₁ or ³⁵S-lamins C and B₁. Again emerin was immunoprecipitated with MANEM3 and MANEM5 and resolved on SDS-PAGE. The starting mixtures and the immunoprecipitates are shown in Fig. 1C and D, respectively. When emerin was mixed with lamins A and C, significantly more lamin C compared with lamin A was recovered in MANEM immunoprecipitates. When emerin was mixed with lamins A and B₁ similar amounts of each lamin was recovered in MANEM immunoprecipitates (see Fig. 1E). Finally, when

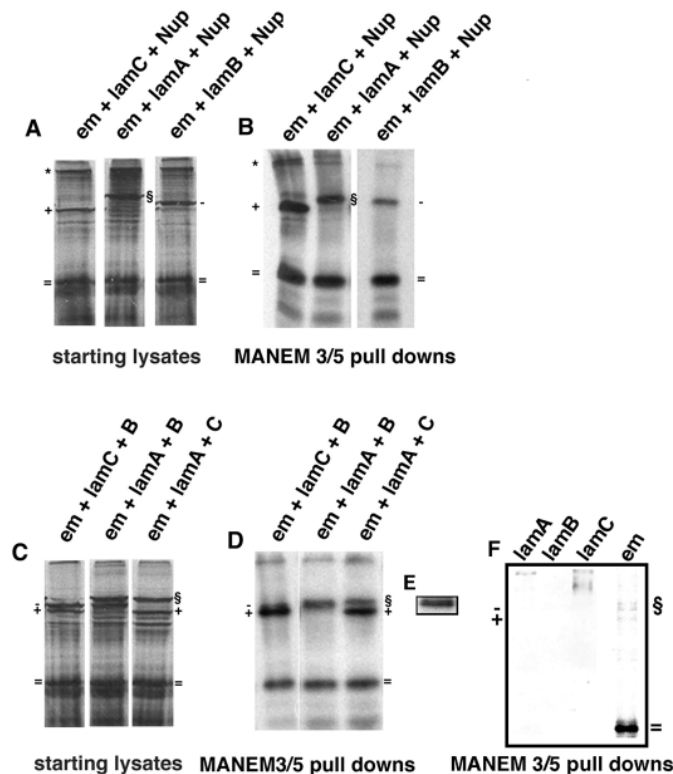


Fig. 1. Co-immunoprecipitation of in vitro translated lamins and emerlin. Human lamins A (lamA), B₁ (lamB), C (lamC), human Nup153 (Nup) and emerlin were translated as ³⁵S-met labelled proteins in rabbit reticulocyte lysates. Lysates were mixed in the following combinations to give approximately equal starting amounts of radiolabelled protein: (A,B) Emerlin+lamin C+Nup; emerlin+lamin A+Nup; emerlin+lamin B+Nup. (C,D) Emerlin+lamin C+lamin B; emerlin+lamin A+lamin B; emerlin+lamin A+lamin C. (F) Lamin A, lamin B₁, lamin C and emerlin translated separately and not mixed. E shows a lower exposure of a lamin A+lamin B+emerlin co-immunoprecipitation. The area corresponding to the lamin A and lamin B bands is presented. Two bands are clearly visible. Immunoprecipitations were performed with MANEM3 and -5 in combination (MANEM pull downs (A,C,E)). B and D show starting mixtures. Immunoprecipitates or samples of starting lysates were resolved on 8% SDS PAGE and fluorographed. § indicates the position of lamin A; - indicates the position of lamin B; + indicates the position of lamin C; = indicates the position of emerlin; * indicates the position of Nup153.

emerlin was mixed with lamins B₁ and C significantly more lamin C was recovered than lamin B₁. As a negative control, each lamin and emerlin was translated in isolation and immunoprecipitated with MANEM3 and -5 in combination. As expected, only emerlin was found in immunoprecipitates (Fig. 1F). Since emerlin appeared to display a preference for binding to lamin C, densitometry was performed on fluorographs and the intensity of each lamin band was compared with emerlin using UViband software (UVitech Ltd). When lamin C was present in immunoprecipitation reactions, it was recovered at approximately 1:1 ratio with emerlin (Table 1). The ratio of lamin B to lamin C was ~1:3 (Fig. 1D; Table 1) when these two lamins were co-immunoprecipitated with emerlin. The ratio of lamin A to lamin C was ~1:2 (Fig. 1D; Table 1) when these two lamins were mixed with emerlin. Finally, the ratio of lamin A to

Table 1. Densitometric analysis of fluorographs from lamin and emerlin co-immunoprecipitations*

Experiment	Protein mixture	% area	Ratio lamin:emerlin	Ratio lamin:lamin
1	Emerlin	0.419		
	Lamin C	0.393	1:1.1	1:2.1 (lamin A:C)
	Lamin A	0.188	1:2.3	
2	Emerlin	0.433		
	Lamin C	0.416	1:1.04	1:2.85 (lamin B:C)
3	Emerlin	0.388		
	Lamin B	0.354	1:1.1	1:1.37 (lamin A:B)
	Lamin A	0.258	1:1.5	

*Fluorographs from lamin and emerlin co-immunoprecipitation reactions (Fig. 1) were scanned with a Kodak scanning densitometer using UViband software. The proportion of each band, as a percentage of the area of bands within each single lane was determined. The ratio of % area of each lamin to emerlin and each lamin pair within a single experiment was calculated.

lamin B was ~2:3 (Fig. 1D; Table 1) when these two lamins were mixed with emerlin. These data suggest that, although emerlin is able to interact with all three lamins in vitro, its preferred interaction is with lamin C.

Abnormal targeting of emerlin in a cell line SW13 with altered expression and organisation of lamins A and C

To investigate lamin-emerlin interactions in vivo we compared the distribution of emerlin and the lamin B binding protein LAP2β in two cell lines with very different lamin complements. The expression of the different lamins in HeLa cells was investigated by immunoblotting with specific antibodies against lamins A/C, A, C, B₁ and B₂. All four lamins were expressed at high levels (Fig. 2A). A similar investigation was performed on the adrenal cortex carcinoma cell line SW13 (Paulin-Levasseur et al., 1989). In this cell line lamin A was expressed at greatly reduced levels (compared with HeLa) and was undetectable with some antibodies (JoL4). By contrast, lamins B₁ and B₂ were expressed at similar levels in SW13 and HeLa, whereas lamin C was expressed at reduced but readily detectable levels (Fig. 2B). Next, the distribution of lamins A and C were compared in HeLa and SW13 cells by immunofluorescence. Using anti-lamin-C-specific anti-sera, lamin C was detected predominantly in the nuclear rim in HeLa cells (Fig. 2C). In SW13 cells lamin C was detected at low levels in the nuclear rim but at high levels in the nucleolus (Fig. 2D). (Note that the nucleolar distribution of lamin C was confirmed by co-staining with Ki67 antibody and in all subsequent experiments co-staining with Ki67 was used to confirm nucleolar localisation of lamin C in the absence of lamin A; data not shown.) Using the lamin-A-specific mAb JoL4, lamin A was detected in nuclear speckles and at the nuclear rim in HeLa cells but was undetectable in SW13 cells (Fig. 2C,D). Lamins B₁ and B₂ were both localised exclusively at the nuclear rim in HeLa and SW13 cells (data not shown). These data suggested that, in SW13 cells, lamin A was expressed at very low levels (compared with HeLa), whereas a significant fraction of lamin C was mis-localised to the nucleolus.

Next we compared the distribution of LAP2β and emerlin in HeLa and SW13 cells. In each cell line, LAP2β was located at the NE (Fig. 2E,F). In HeLa, emerlin was detected as a distinct nuclear rim stain indicating its location at the NE (Fig. 2E). By

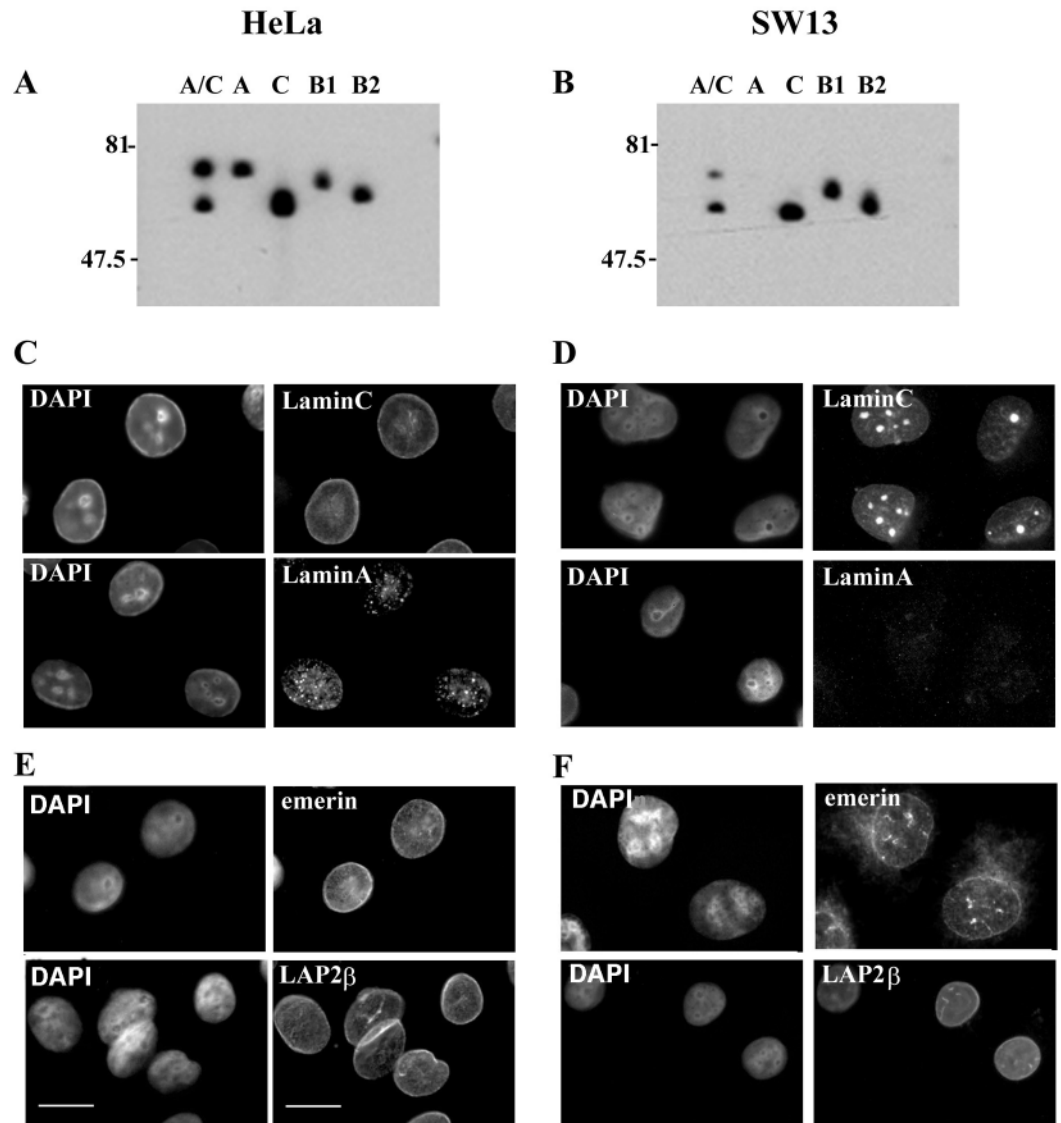


Fig. 2. Comparison of emerin and lamin C distributions in SW13 and HeLa cells. The level of expression of different lamins was compared in HeLa (A) and SW13 cells (B) by immunoblotting. Nuclei isolated from 10^6 cells were resolved on 10% SDS-PAGE, transferred to nitrocellulose and immunoblotted with mAbs JoL2 (lamin A/C), JoL4 (lamin A), LN43 (lamin B₂), rabbit polyclonal anti-lamin C or goat anti-lamin B₁ using a multi-blot apparatus. The distribution of lamin C (blocks C and D), lamin A (blocks C and D), emerin (blocks E and F) and LAP2 β (blocks E and F) in HeLa cells (blocks C and E) and SW13 cells (blocks D and F) were investigated by indirect immunofluorescence using polyclonal anti-lamin C, JoL4, MANEM5 and LAP17, respectively. In all samples the distribution of DNA was detected with DAPI. Bars, 10 μ m.

contrast, in SW13 cells, emerin was located both at the NE and within the cytoplasm. To investigate the site of emerin localisation within the cytoplasm, SW13 cells were co-stained with MANEM3 and antibodies against calreticulin. As a control, SW13 cells were co-stained with MANEM3 and the mitochondrial marker p32 (Matthews and Russell, 1998). Confocal microscopy revealed that the cytoplasmic fraction of emerin co-localised exclusively with calreticulin (Fig. 3) but did

not co-localise with p32 (data not shown). These data suggest that in SW13 cells a significant fraction of emerin resides in the ER.

To further investigate the behaviour of emerin in HeLa and SW13 cells, cDNA encoding full-length human emerin was sub-cloned into pEGFP (pEGFP-emerin) and expressed in each cell line following transient transfection. As expected, in HeLa cells GFP-emerin was localised exclusively in the NE (Fig. 4E). When the same construct was transfected into SW13 cells

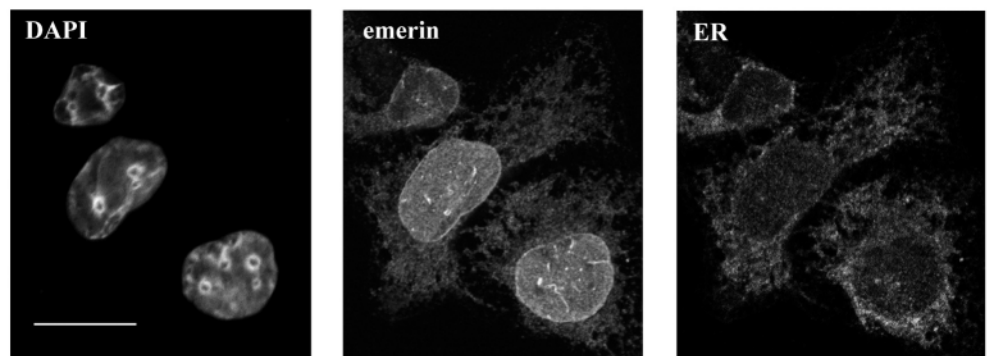


Fig. 3. Co-localisation of emerin and calreticulin in SW13 cells. The distribution of emerin (MANEM3) was compared with the distribution of calreticulin (ER) in SW13 cells by indirect immunofluorescence and confocal microscopy. The distribution of DNA was detected using DAPI. Bar, 10 μ m.

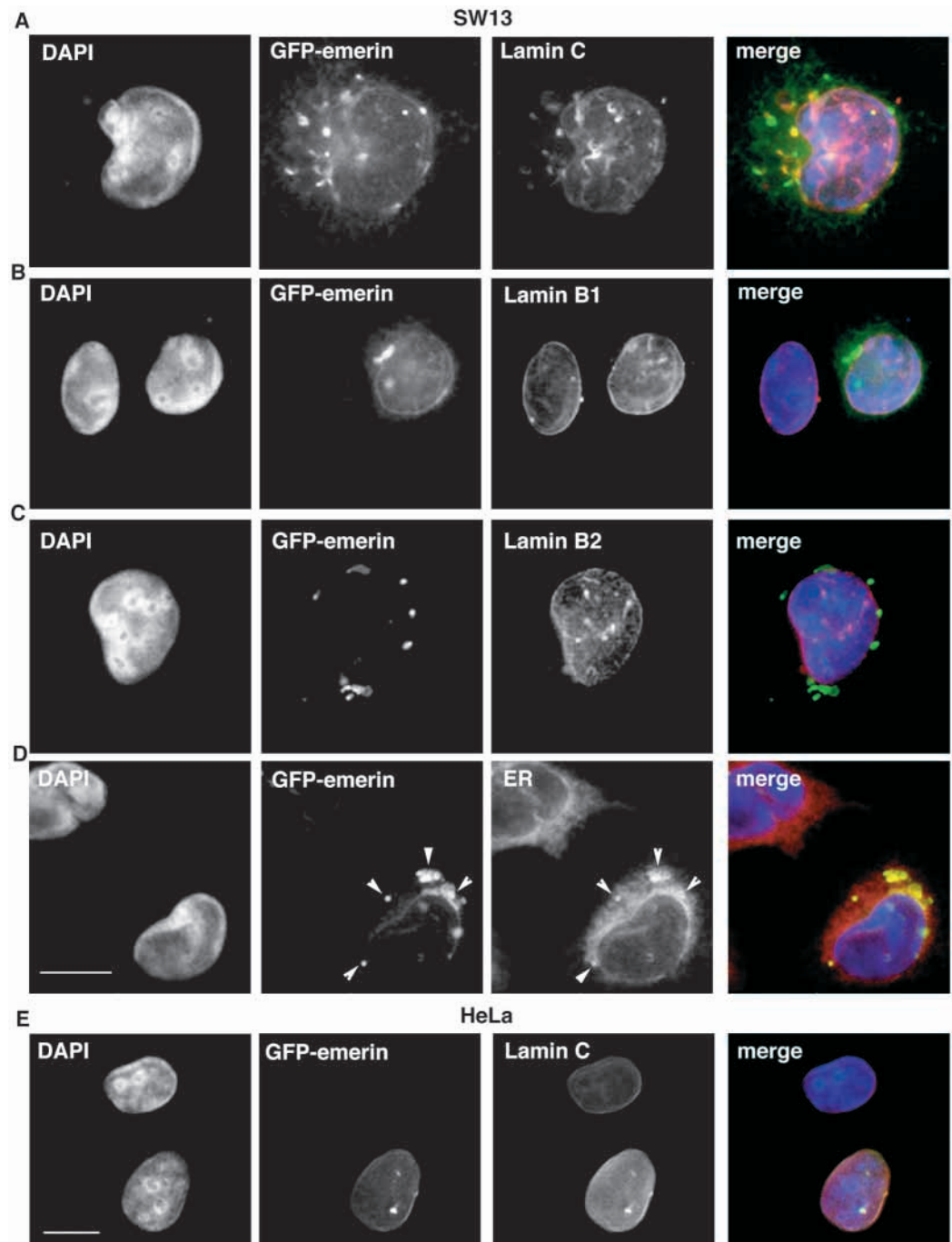


Fig. 4. GFP-emerin forms aggregates within a sub-domain of the ER in SW13 cells. GFP-emerin was transiently expressed in SW13 cells (rows A-D) or HeLa cells (row E) and its distribution was compared with lamin C (row A), lamin B₁ (row B), lamin B₂ (row C) or calreticulin (row D, ER) after fixation. In each sample the distribution of DNA was revealed with DAPI. Images are displayed as individual black and white panels and as three-colour merged images. In merged images 'yellow' indicates spectral overlap between red and green signals. Arrowheads (row D) indicate the position of emerlin aggregates within the ER. Bars, 10 μ m.

the majority of GFP-emerin was localised in the cytoplasm, where it accumulated in large granular structures (Fig. 4A-D). Co-staining with calreticulin revealed that although the granular structures were distinct from the majority of the ER they did contain ER proteins (Fig. 4D). Transfected cells were also co-stained with specific antibodies against lamins C, B₁ or B₂. Surprisingly, a readily detectable fraction of lamin C (Fig. 4A), but no lamin B₁ (Fig. 4B) or B₂ (Fig. 4C), relocated from the nucleus to co-distribute with GFP-emerin in some of the cytoplasmic granules.

A number of conclusions can be drawn from these data. First, the absence of lamin A from the NE correlates with a significant fraction of lamin C being mis-localised to the nucleolus and a

fraction of emerlin residing in the ER. Second, when GFP-emerin is overexpressed in SW13, it forms cytoplasmic aggregates, probably within an ER sub-domain that traps some lamin C but no lamins B₁ or B₂. Taken together, these data suggest that emerlin interacts with lamins A and/or C *in vivo*.

Emerlin is localised in the ER in a range of cell lines that display abnormal levels of expression and distributions of lamins A and C

Birkitt's lymphoma cell lines

A comparison of emerlin and lamin C localisation in HeLa and SW13 cell lines indicated that lamin A might organise both proteins at the NE. To investigate the generality of this

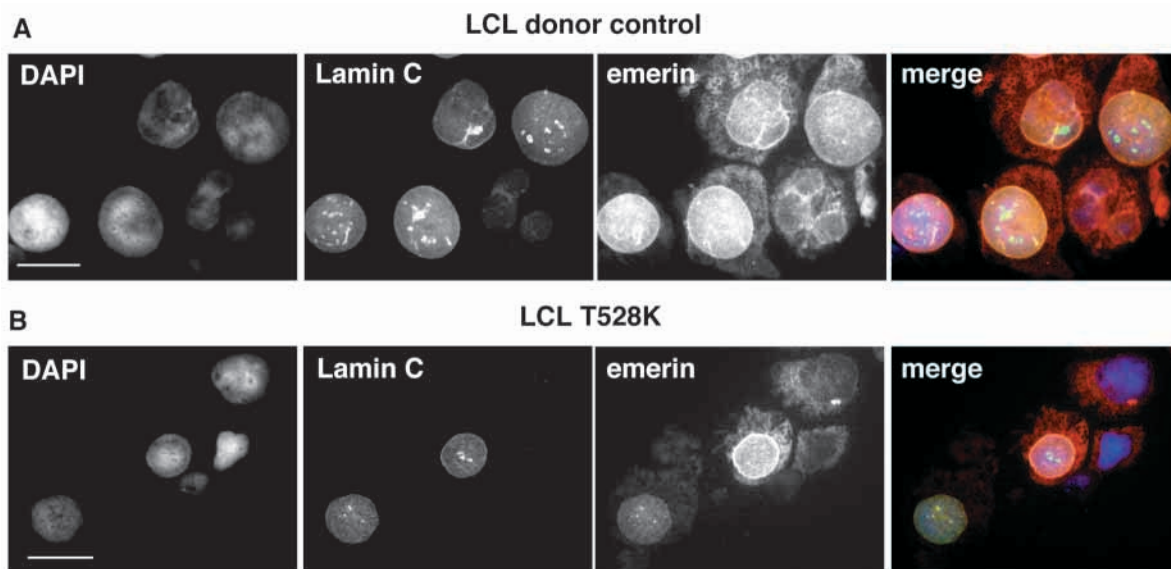


Fig. 5. The distribution of lamin C and emerin in lymphoblastoid cell lines. EBV-transformed lymphoblastoid cell lines were obtained from a donor control (row A) and from an AD EDMD patient with a single point mutation giving rise to an amino acid substitution at position T528K (row B). Each cell line was co-stained with rabbit anti-lamin C (Lamin C) and MANEM5 (emerin). The distribution of DNA in each sample was detected with DAPI. Panels show individual black and white micrographs or three-colour merged images (merge) in which the distribution of DAPI is shown in blue, lamin C in green and emerin in red/orange. In merged images, 'yellow' indicates spectral overlap between red and green signals. Bar, 10 μ m.

Table 2. Expression and distribution of lamin, LAP2 β and emerin*

Cell line	LamA		LamA/C		LamC		LamB ₁		LamB ₂		LAP ₂		Emerin	
	Exp	Dist	Exp	Dist	Exp	Dist	Exp	Dist	Exp	Dist	Exp	Dist	Exp	Dist
HeLa	++	rim	++	rim	++	rim	++	rim	++	rim	++	rim	++	rim
SW13	-	NA	+	rim/no	+	rim/no	++	rim	++	rim	++	rim	++	ER
Ramos (Birkitt's lymphoma)	-	NA	+	no	+	no	++	rim	++	rim	++	rim	++	ER
NL-SCLC2	+/-	abs	+/-	abs	ND		++	rim	++	rim	++	rim	++	ER
NCI-H125	+ to ++	rim	+ to ++	rim	ND		++	rim	++	rim	++	rim	++	rim
LCL-control	+	rim	+	rim	+	rim	++	rim	++	rim	++	rim	++	rim+ER
LCL-AD EDMD	+	rim/abs	+	rim/abs	+	rim/abs	++	rim	++	rim	++	rim	++	rim/ER

*JoL4 was used to detect lamin A (LamA); JoL2 was used to detect lamins A/C (LamA/C); rabbit anti-lamin C was used to detect lamin C (LamC); goat anti-lamin B1 was used to detect lamin B₁ (LamB₁); LN43 was used to detect lamin B₂ (LamB₂); LAP17 was used to detect LAP2 β (LAP₂); MANEM5 was used to detect emerin. Dist, distribution detected by immunofluorescence; Exp, level of expression detected by western blotting; -, undetectable; +/-, very low levels of expression; +, medium levels of expression; ++, high levels of expression; abs, absent; ER, staining in endoplasmic reticulum (where an ER distribution of emerin was detected, this was confirmed by co-localisation with anti-calreticulin); NA, not applicable; ND, not done; no, nucleolar staining (where nucleolar staining of lamin C was detected, this was confirmed by co-localisation with Ki67); rim, nuclear rim staining.

phenomenon we compared the distribution of emerin and LAP2 β in other human cell lines that are deficient for lamin A/C expression or which display altered distributions of lamin C. A Burkitt's lymphoma cell line (Ramos) did not express lamin A and expressed reduced levels of lamin C. Moreover, in immunofluorescence experiments lamin C was localised exclusively in the nucleolus. The levels of expression and distribution of lamins B₁ and B₂ appeared normal (Table 2). LAP2 β was localised exclusively at the NE in Ramos cells. By contrast, emerin was distributed exclusively in the ER (Table 2).

Small cell lung carcinomas

Previous investigations have shown that small cell lung carcinomas express greatly reduced levels of lamins A and C compared with non-small-cell lung carcinomas (Kaufman et

al., 1991). Therefore, we compared the distribution of LAP2 β and emerin in non-small-cell and small cell lung carcinoma cell lines. The non-small-cell lung carcinoma line used in this investigation (NCI-H125) expressed high levels of lamins A, C, B₁ and B₂ that were distributed mainly at the NE (Table 2). In this cell line, LAP2 β and emerin were both located exclusively at the NE. In common with most examples (Broers et al., 1993), the small cell lung carcinoma cell line used in the study (NL-SCSC2) did not express lamin A or lamin C but expressed high levels of lamins B₁ and B₂. LAP2 β was localised at the NE in the small cell lung carcinoma, whereas emerin was localised in the ER (Table 2).

EDMD cell lines

Finally, we compared emerin localisation in EBV-transformed

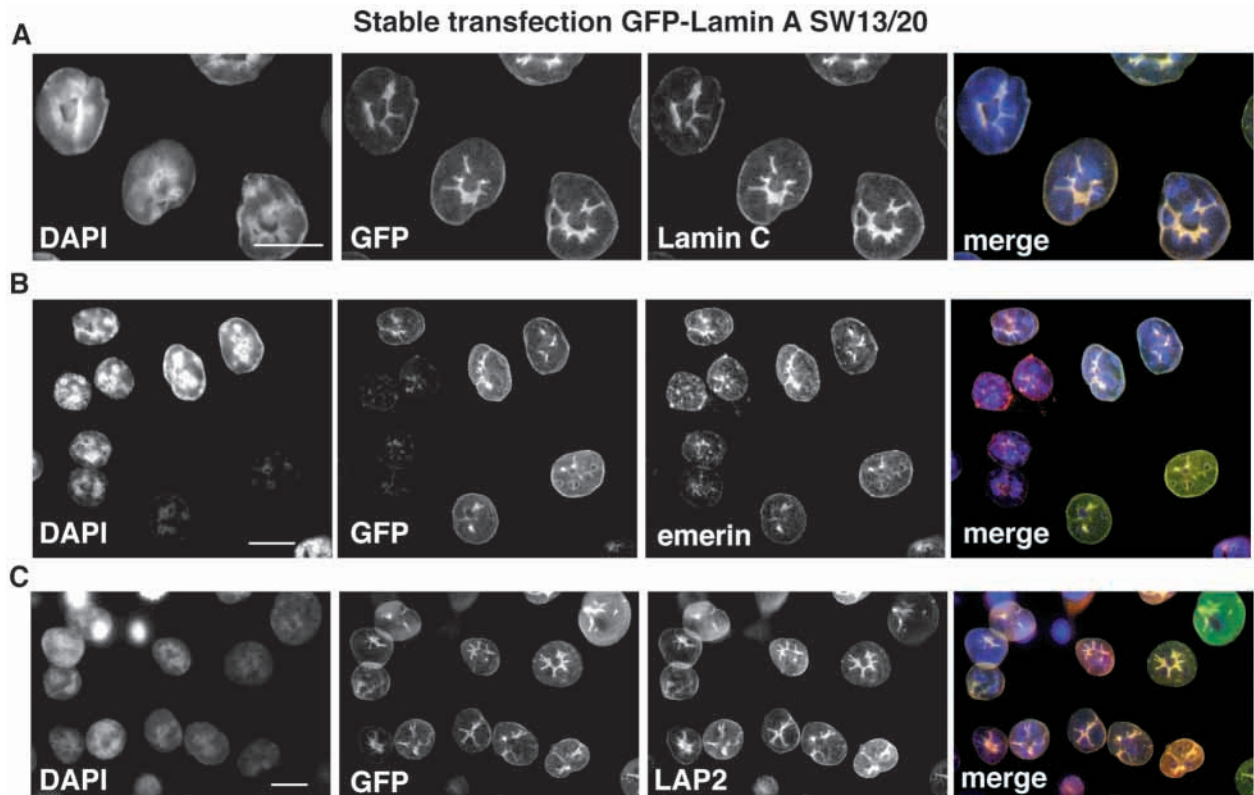


Fig. 6. The distribution of emerin and lamin C in SW13 cells stably transfected with GFP-lamin A (SW13/20). The distribution of lamin C, emerin and LAP2 β in SW13 cells was investigated by antibody staining using rabbit anti-lamin C (Lamin C, row A), MANEM5 (emerin, row B) and LAP17 (LAP2, row C), respectively. In each case, antibody distribution was compared with the distribution of GFP-lamin A (GFP). The distribution of DNA in each sample was detected with DAPI. Each panel displays individual black and white images and three-colour merged images (merge) in which DAPI is displayed in blue, GFP-lamin A in green and antibody staining in red. In merged images 'yellow' indicates spectral overlap between red and green signals. Bars, 10 μ m.

lymphoblastoid cell lines (LCL) obtained from a control donor and from a patient with autosomal dominant EDMD (AD EDMD). The control LCL expressed relatively low levels of lamins A and C but both were located in the nuclear rim (Table 2; Fig. 5A). Lamin B₁ and B₂ expression and distribution (Table 2) in this cell line appeared normal. Although some emerin was located in the ER (presumably as a consequence of the relatively low-level expression of lamins A/C), the majority was located at the NE (Fig. 5A). The AD EDMD cell line was obtained from a patient having a missense mutation in the lamin A/C tail (T528K). In this patient expression of lamins A and C was variable, with some cells expressing apparently normal levels of the proteins at the NE, whereas in other cells both proteins were absent (Table 2; Fig. 5B). Again, lamins B₁ and B₂ appeared normal both in terms of level of expression and distribution (Table 2). The variable level of A-type lamin expression in the AD EDMD patient permitted a side-by-side comparison of emerin distribution in those cells expressing lamins A/C and in those that do not. Cells in which lamins A and C were expressed and localised at the NE (lamin C is shown in Fig. 5B) also contained significant quantities of emerin at the NE. By contrast, in adjacent cells that did not express lamins A and C, emerin was located exclusively in the ER/NE (Fig. 5B; the ER localisation was confirmed by co-staining with anti-calreticulin (not shown)). Therefore, in a range of cell lines, localisation of emerin at the NE correlates with expression and localisation of lamins A and C at the NE.

In addition, lamin C localisation at the NE may also depend upon lamin A.

Stable and transient expression of GFP-lamin A in SW13 cells causes the relocalisation of lamin C and emerin to the NE

Our immunofluorescence data have revealed a strong correlation between abnormal expression and distribution of lamins A and C and localisation of emerin in the ER. To investigate whether this represented a causal relationship we carried out transient and stable transfection experiments on SW13 with GFP-lamins. Initially, we selected a number of cell lines that had been stably transfected with GFP-lamin A. One such cell line (SW13/20) is shown here, since it is representative. Stable transfection with GFP-lamin A in SW13 resulted in levels of expression of the fusion protein that were approximately fourfold higher than levels of expression of lamin C (not shown). Importantly, all A-type lamins (including endogenous lamin C) were located predominantly in the NE rather than in the nucleolus (Fig. 6A). In SW13/20 cells both LAP2 β (Fig. 6C) and emerin (Fig. 6B) were localised exclusively at the NE. Next we performed transient transfection experiments with GFP-lamins A, B₁ and C. Typically in these experiments 15% of cells expressed the GFP-fusion protein. When SW13 was transfected with GFP-lamin A, the GFP-fusion protein was localised at the NE (Fig. 7A,B). Importantly, the endogenous lamin C became

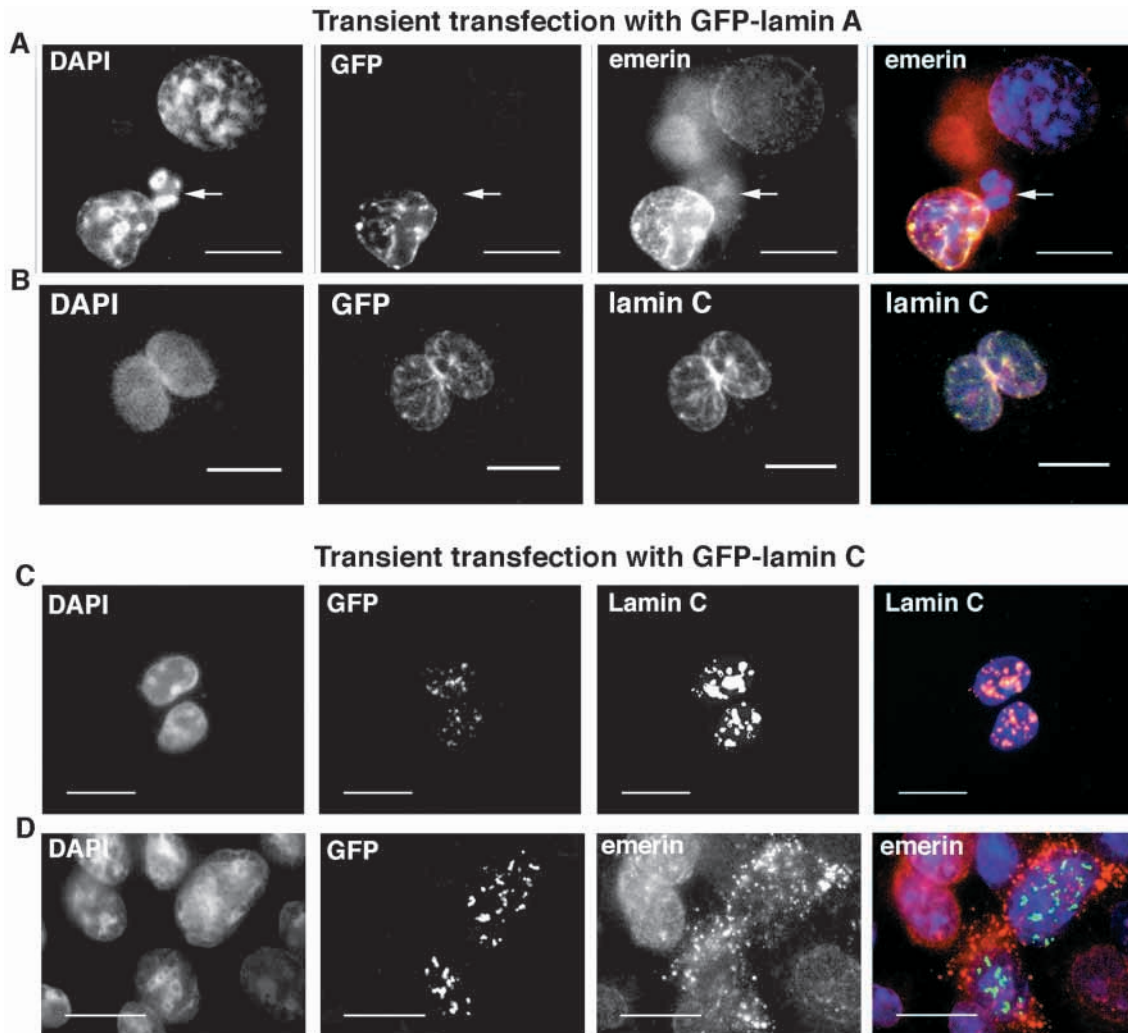


Fig. 7. Transient transfection of SW13 with GFP-lamin A, but not GFP-lamin C, rescues endogenous emerin and lamin C distributions. The distribution of lamin C and emerin was investigated in SW13 cells following transient transfection with GFP-lamin A (rows A,B) or GFP-lamin C (rows C,D). The distribution of endogenous lamin C and emerin was detected by immunofluorescence as described in Materials and Methods. The distribution of DNA was detected with DAPI. In each panel the distributions of DNA, GFP or antibody staining are presented as individual black and white images or as three-colour merged images in which antibody staining is shown in red, GFP-lamin A is shown in green and DAPI is shown in blue. In merged images 'yellow' indicates spectral overlap between red and green signals. The arrows in A indicate the position of a mitotic cell. Bars, 10 μ m.

predominantly co-localised with the GFP rather than in the nucleolus (Fig. 7B). Emerin was also localised at the NE in cells expressing GFP-lamin A but not in surrounding untransfected cells (Fig. 7A, arrowhead; the arrow identifies a mitotic cell lying adjacent to the cell expressing GFP-lamin A; the cytoplasmic emerin observed next to the transfected cell is within the mitotic cell). GFP-lamin B₁ was also localised to the NE in transfected SW13 cells but failed to cause the re-localisation of endogenous lamin C or emerin to the NE (data not shown). Finally, when SW13 was transfected with GFP-lamin C, the fusion protein accumulated in nucleoplasmic granules (Fig. 7C,D; note that when GFP-lamin C is transiently expressed in HeLa cells it localises exclusively to the NE (data not shown)). These granules did not influence the distribution of endogenous lamins (endogenous lamin C remained in the nucleolus; Fig. 7C) and emerin remained in the cytoplasm (Fig. 7D). As with previous experiments the nucleolar distribution of lamin C and the ER localisation of emerin was confirmed

by co-localisation with Ki67 and calreticulin, respectively (data not shown). These data strongly support the view that localisation of emerin to the NE depends upon the presence of lamins A and C within the lamina. Furthermore, the data also suggest that the presence of lamin A within the lamina is necessary (but possibly not sufficient) for lamin C localisation to the NE.

Dominant negative mutants of lamin B₁ selectively eliminate lamins A and C from the lamina and cause emerin to accumulate in cytoplasmic granules

To further investigate the relationship between emerin localisation at the NE and the presence of lamins A and C in the lamina, we used dominant negative mutants of lamin B₁ to specifically disrupt A-type lamins. We have previously described the creation of a dominant negative mutant of lamin B₁ that is capable of disrupting the lamina of sperm pronuclei assembled in vitro (Ellis et al., 1997). This mutant protein

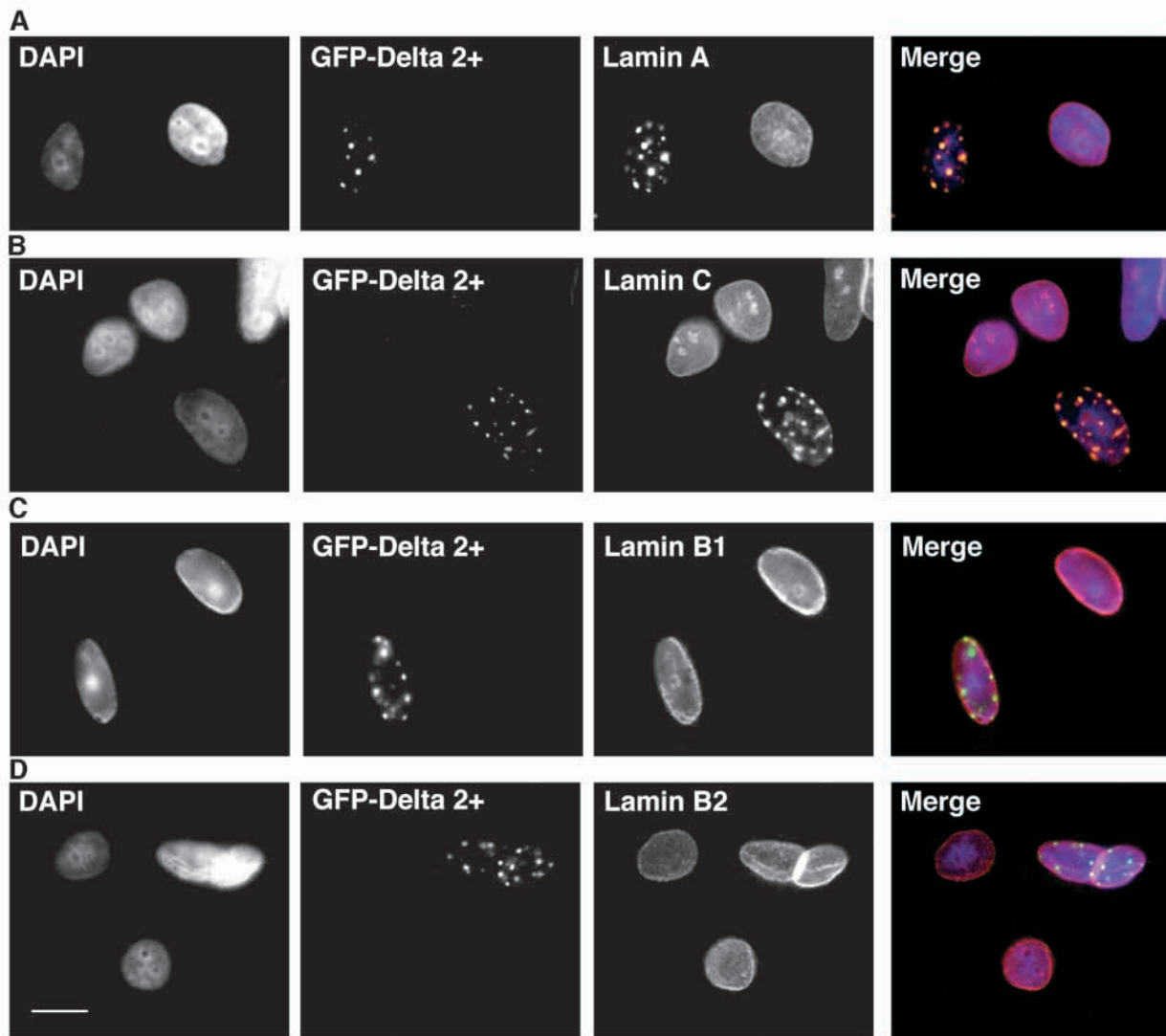


Fig. 8. The effects of a dominant negative mutant of lamin B₁ on lamin distribution in HeLa cells. A GFP-fusion of a dominant negative lamin B₁ mutant (GFP-Delta 2+) was transiently transfected into HeLa cells. The distribution of lamin A (row A), lamin C (row B), lamin B₁ (row C) and lamin B₂ (row D) in transfected cells was compared with untransfected cells by antibody staining with JoL2, rabbit anti-lamin C, goat anti-lamin B₁ and LN43, respectively. The distribution of DNA was detected with DAPI. Each panel displays either individual black and white images or three-colour merged images in which DAPI is shown in blue, GFP-Delta 2+ in green and antibody staining in red. Bar, 10 μ m.

(delta 2+) was fused to GFP and expressed in transient transfection assays in HeLa cells. Following transfection, GFP-delta 2+ accumulated as small nucleoplasmic granules that formed over 48 hours (Fig. 8A-D). Both lamin A (Fig. 8A) and lamin C (Fig. 8B) relocated from the nuclear lamina to the nucleoplasmic granules over the same period of time. By contrast, lamins B₁ (Fig. 8C) and B₂ (Fig. 8D) remained in the nuclear lamina. To confirm that lamins B₁ and B₂ remained in the nuclear lamina, transfected cells were extracted *in situ* with detergents, nucleases and ammonium sulphate. Following this procedure both B-type lamins were retained in the insoluble lamina, demonstrating that their solubility properties were unaffected by the presence of the mutant protein (data not shown). Thus in these transfection experiments, GFP-delta 2+ exerted a dominant effect over A-type lamins, causing their redistribution from the lamina to nucleoplasmic granules, but had seemingly no effect on B-type lamins. Next we

investigated the effects of the dominant negative mutants on emerin and LAP2 β distribution. When HeLa cells were transfected with GFP-delta 2+ and then stained with anti-emerin antibodies, the majority of emerin was located in cytoplasmic granules rather than the NE (Fig. 9A). By contrast, LAP2 β remained in the NE (Fig. 9B).

The cytoplasmic granules observed in transfected HeLa were unlike the ER distribution of emerin observed in lamin A/C-deficient cell lines. Therefore, to investigate the location of the cytoplasmic emerin, transfected cells were stained with anti-calreticulin antibodies (TRITC) and anti-emerin antibodies (Cy5). A typical result is shown (Fig. 10) and reveals that emerin did not co-localise with the majority of calreticulin in transfected cells. Instead, emerin was mainly located in granules lying close to the NE. However, calreticulin did accumulate within these granules (Fig. 10, arrowheads) suggesting that the granules were within the ER.

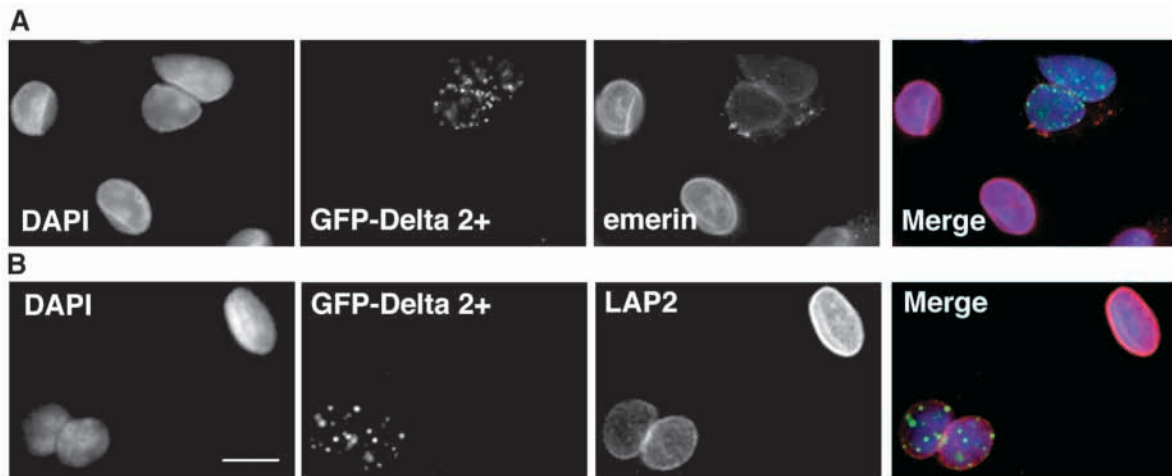


Fig. 9. The influence of GFP-Delta 2+ on the distribution of emerin and LAP2 β in HeLa cells. HeLa cells were transiently transfected with GFP-delta 2+ and the distributions of emerin (row A) and LAP2 β (row B) were compared in transfected and untransfected cells by immunofluorescence using MANEM5 (emerin) and LAP17 (LAP2). The distribution of DNA was detected with DAPI. Panel displays individual black and white micrographs or three-colour merged images in which DAPI is shown in blue, GFP-Delta 2+ in green and antibody staining in red. In merged images 'yellow' indicates spectral overlap between red and green images. Bar, 10 μ m.

DISCUSSION

Lamin C is the preferred in vitro binding partner of emerin

The data presented here demonstrate that lamins A, C and B₁ all interact with emerin in vitro. However, in competition reactions the preferred interaction of in vitro translated emerin is with lamin C. This finding, although novel, is consistent with the results of previous investigations. In a recent study, Östlund et al. (1999) reported that the nucleoplasmic domain of emerin is both necessary and sufficient for targeting integral membrane proteins of the ER to the INM. We have shown that this domain of emerin binds to lamins following translation of both proteins in rabbit reticulocyte lysates. Emerin also co-immunoprecipitates from C2C12 myoblast lysates in a complex containing lamins A, C and B₁, as well as actin (Fairley et al., 1999). It is not clear which protein (or proteins) within this complex emerin was associating with, since A-type lamins form hetero-oligomeric complexes with B-type lamins in cell lysates (Dyer et al., 1999) and actin also binds to lamin A in vitro (Sasseville et al., 1998). Therefore, it is possible that, in cell lysates, the complex containing emerin, lamin B₁, lamin C and actin is formed through association of all three proteins with lamin A. Direct interactions between the nucleoplasmic domain of emerin and lamin A have been detected using the BIAcore biosensor (Clements et al., 2000). Based on the data presented here and previously published evidence we conclude that emerin probably associates with residues conserved between A-type and B-type lamins, possibly as a multimer (Clements et al., 2000), but that its preferred association is with lamin C.

Evidence for a hierarchy of lamina associations mediated by lamin A

We investigated emerin localisation in two different human cell lines that were deficient for synthesis of lamins A and C and

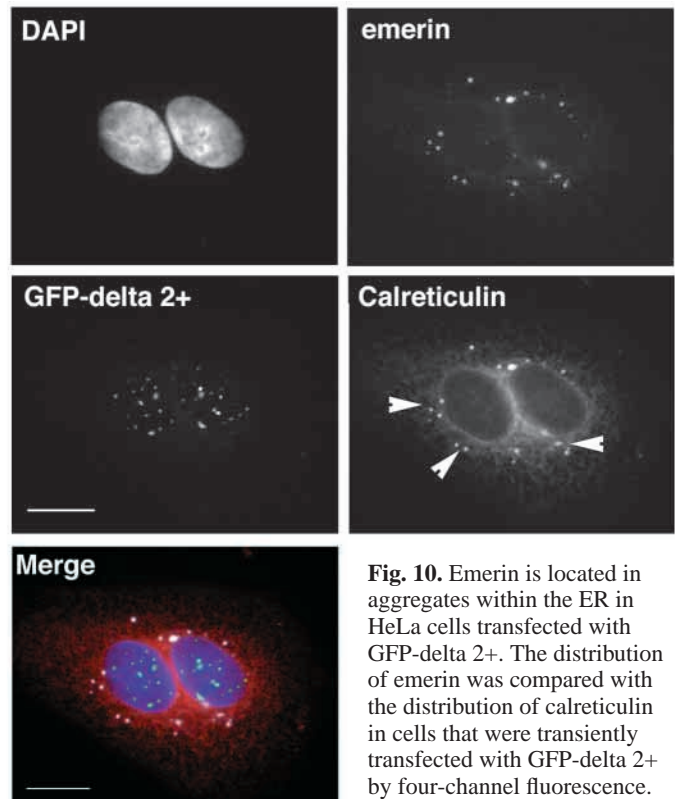


Fig. 10. Emerin is located in aggregates within the ER in HeLa cells transfected with GFP-delta 2+. The distribution of emerin was compared with the distribution of calreticulin in cells that were transiently transfected with GFP-delta 2+ by four-channel fluorescence. Transfected cells were stained

with DAPI to reveal the distribution of DNA; with MANEM5 (Cy5) to reveal the distribution of emerin; and with rabbit anti-calreticulin (TRITC) to reveal the distribution of calreticulin within the ER. Images are displayed either as individual black and white panels or a four-colour merged image in which DAPI is displayed in blue, GFP-delta 2+ in green, calreticulin in red, and emerin in white. It should be noted that when calreticulin staining was omitted no bleed through between the Cy5 and the TRITC filters was observed. Bars, 10 μ m.

two further cell lines that were deficient for lamin A synthesis and in which lamin C was mis-localised to the nucleolus. In each cell line either all or a majority of emerin was mis-localised to the ER. In addition, we used a dominant negative mutant of lamin B₁ that selectively eliminates lamins A and C but not lamins B₁ and B₂ from the NE of HeLa cells. A consequence of eliminating lamins A and C from the NE was that emerin relocated from the NE to the ER, where it formed insoluble inclusions.

Our data suggest that lamin A has a central role in tethering both emerin and lamin C to the NE. The following evidence supports this model. Association of lamin C with the NE in two of the cell lines reported here is dependent upon the presence of lamin A, and in its absence lamin C organisation is disrupted such that most (SW13) or all (Ramos) is mis-localised to the nucleolus. In SW13 cells the failure of lamin C to be incorporated into the lamina is a direct consequence of the absence of lamin A since transient or stable expression GFP-lamin A causes significant re-localisation of endogenous lamin C to the NE. The failure of lamin C to be incorporated into the lamina in the absence of lamin A may result from weak associations between lamin C and lamin B filaments or because lamin C is not isoprenylated and carboxy methylated and cannot accumulate at the NE on its own. Thus lamin A may be required to carry lamin C to the NE or to mediate its association with lamina filaments or both. It is unclear why, in the absence of lamin A, lamin C becomes mis-localised to the nucleolus. However, it is possible that the nucleolus is a default location for some proteins that might otherwise form damaging structures (e.g. aggregates) when they are unable to accumulate at their normal sites of assembly.

Evidence that lamin C incorporation into the lamina is dependent on the presence of lamin A has been reported previously. When fluorescently labelled lamin C was microinjected into Swiss 3T3 cells it forms small aggregates in the nucleoplasm which persist for several hours (an analogous situation was observed in this study when GFP-lamin C was transiently expressed in SW13 cells). When lamin A and lamin C were injected together into Swiss 3T3 cells they are both incorporated into the NE rapidly (Pugh et al., 1997). In agreement with these findings, when lamin C was transfected into cells arrested in S-phase it remained in the nucleoplasm, whereas lamin A was incorporated into the NE under similar conditions. However, if the transfected cells were released from S-phase and permitted to divide, presumably allowing transfected lamin C to interact with soluble lamin A, the lamin C became incorporated into the NE during the following G₁ phase (Horton et al., 1992).

We observed that emerin is mis-localised to the ER in four different human cell lines that display abnormal expression or localisation of lamins A and C. Moreover, expression of GFP-lamin A in one of these lines resulted in relocation of emerin from the ER to the NE. In all cell lines employed in the study, lamins B₁ and B₂ were expressed and localised normally and LAP2β was localised at the NE. Thus, even though emerin can bind to lamin B₁ *in vitro*, the presence of this protein in the lamina is not sufficient to anchor emerin at the INM. Instead these data suggest that the presence of lamins A and C at the NE is necessary for emerin localisation at the INM. The data reported here is entirely consistent with the recent description of the lamin A/C-knockout mouse. In lamin A/C^{-/-} mice,

emerin is located mainly in the ER in most tissues (Sullivan et al., 1999). Consistent with this data, when we used dominant negative mutants of lamin B₁ to selectively eliminate lamins A and C from the NE in HeLa cells, emerin relocated from the INM to inclusions within the ER.

Our data can be explained by a hierarchical series of associations between lamin A, lamin C and emerin. In this hierarchy, lamin A may mediate the association of lamin C with the lamina and, once present, lamin C may stabilise the association of emerin with the INM. In previous studies associations between lamins A/C and emerin have been reported but the possibility that lamin A and lamin C perform different functions at the INM were not considered. For example, a clear temporal correlation between emerin and lamin A/C association with the reforming NE at telophase has been reported (Manilal et al., 1999). Similar temporal correlations were reported in live GFP-imaging studies (Haraguchi et al., 2000). In addition, one study reported a striking spatial correlation in which lamins A/C and emerin co-associate with the reforming NE in discrete foci at telophase, whereas lamin B₁ and LAP2β re-associates throughout the NE (Dabauvalle et al., 1999). This spatial correlation between lamin A/C and emerin incorporation at the reforming NE is all the more striking because A-type lamins do not return to the nucleus until after the formation of a transport-competent envelope, whereas emerin-containing membranes associate with chromatin much earlier. Although all three studies indicated a structural complex involving emerin, lamin A and lamin C at the INM, none of the studies was able to distinguish between different roles for lamin A and lamin C in the complex. Five observations led us to present a new hypothesis to explain lamin-emerin associations at the INM. (1) emerin's preferred *in vitro* binding partner is lamin C. (2) Emerin is never present in the NE in the absence of lamin C. (3) Lamin C is not incorporated into the lamina in the absence of lamin A. (4) Lamin C can be sequestered into emerin aggregates in the ER when GFP-emerin is overexpressed in lamin A-deficient cells. (5) We have demonstrated previously that lamin A is incorporated into the lamina through association with lamin B filaments (Dyer et al., 1999). Therefore, we propose that the hierarchy of lamina associations involving emerin is as follows: lamin A associates with lamin C as either dimers or tetramers in the nucleoplasm. Lamin A/C then associates with B-type lamina filaments, this association being mediated by lamin A. The association of lamin A with the lamina allows incorporation of lamin C but in the absence of lamin A, lamin C accumulates at default sites in the nucleus. Once incorporated into the lamina, lamin C associates with emerin at the INM. The association between emerin and lamin C may stabilise and tether both proteins at the lamina. Thus in the absence of lamin C, emerin is not stably associated with the lamina and we speculate that, in the absence of emerin, lamin C may not be stably associated with the INM. This model provides an explanation for the accumulation of lamin C in cytoplasmic aggregates when GFP-emerin was overexpressed in SW13 cells. Presumably, in this instance, emerin aggregates were a preferred location compared with the default location in nucleoli.

Our data suggest that emerin can bind to lamin B₁ *in vitro*. However, we found no evidence to suggest that B-type lamins influences emerin behaviour *in vivo*. B-type lamins have a

number of specific binding partners at the NE, including LAP2 β and LBR (reviewed by Vaughan et al., 2000). It is possible that potential emerin binding sites on B-type lamins, organised as lamina filaments, are unavailable because they are occupied by other INM proteins with higher binding affinities. Thus, although emerin can associate with B-type lamins, it does not do so in living cells.

Disease causing mutations in the lamin A/C gene and their relationship to lamina structure

Since the report early last year that mutations in the gene encoding lamins A and C cause AD-EDMD, it has become clear that a large range of lamin A/C mutations give rise to a number of diseases (Bonne et al., 1999; Brodsky et al., 2000; Cao et al., 2000; Fatkin et al., 1999; Raffaele et al., 2000). The mutations so far identified map to different regions of the lamin A/C protein depending upon the disease. Missense mutations causing EDMD map either to highly conserved residues in the lamin A/C tail (Bonne et al., 1999; Raffaele et al., 2000), or to equally highly conserved residues throughout the coiled-coil domain (Raffaele et al., 2000). The spread of mutations causing AD-EDMD suggest that lamin A/C makes a number of molecular interactions at the INM and that different mutations disrupt different interactions. Reported interactions are with emerin (present study; Fairley et al., 1999; Clements et al., 2000), lamin B (present study; Dyer et al., 1999), lamin A to lamin C (present study; Pugh et al., 1997), the chromatin binding protein LAP2 α (Dechat et al., 2000), LAP1C (Powell and Burke, 1990), the tumour suppressor protein p110^{RB} (Ozaki et al., 1994) and chromatin (Höger et al., 1991; Glass et al., 1993). If the model presented above is correct, mutations in lamin A/C and perhaps in emerin might give rise to loss or weakened association of lamin C with the lamina in EDMD. This in turn would lead to the possibility of abnormal lamin C complexes forming elsewhere in the nucleus (e.g. the nucleolus). Indeed, in a preliminary investigation of LCLs from 20 EDMD patients, abnormal nucleoplasmic distributions of lamin C were observed in a majority of cases (E. Wang, M.W. and C.J.H., unpublished). Given the range of associations reported for A-type lamins, abnormal nuclear localisation of lamin C may result in significant and deleterious gain of function. Therefore, abnormal nuclear distributions of lamin C rather than an absence of emerin from the INM may promote EDMD and other lamin diseases.

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