J. Biochem. 129, 321–327 (2001)

Interaction between Emerin and Nuclear Lamins[1]

Masayo Sakaki,* Hisashi Koike,† Nobuhiro Takahashi,* Noboru Sasagawa,* Shigeo Tomioka,‡ Kiichi Arahata,§ and Shoichi Ishiura*,2

*Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, 3-8-1 Komaba, Meguro-ku, Tokyo 153-8902; †Department of Biochemistry, Meiji Pharmaceutical University, 2-522-1 Noshio, Kiyose, Tokyo 204-8588; ‡Department of Molecular Biology, Institute of Molecular and Cellular Bioscience, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-0032; and §Department of Neuromuscular Research, National Institute of Neuroscience, NCNP, Tokyo 187-8502

Received September 5, 2000; accepted December 5, 2000

Emerin is an inner nuclear membrane protein that is involved in X-linked recessive Emery-Dreifuss muscular dystrophy (X-EDMD). Although the function of this protein is still unknown, we revealed that C-terminus transmembrane domain–truncated emerin (amino acid 1–225) binds to lamin A with higher affinity than lamin C. Screening for the emerin binding protein and immunoprecipitation analysis showed that lamin A binds to emerin specifically. We also used the yeast two-hybrid system to clarify that this interaction requires the top half of the tail domain (amino acid 384–566) of lamin A. Lamin A and lamin C are alternative splicing products of the lamin A/C gene that is responsible for autosomal dominant Emery-Dreifuss muscular dystrophy (AD-EDMD). These results indicate that the emerin-lamin interaction requires the tail domains of lamin A and lamin C. The data also suggest that the lamin A–specific region (amino acids 567–664) plays some indirect role in the difference in emerin-binding capacity between lamin A and lamin C. This is the first report that refers the difference between lamin A and lamin C in the interaction with emerin. These data also suggest that lamin A is important for nuclear membrane integrity.

Key words: emerin, Emery-Dreifuss muscular dystrophy, lamin, nuclear envelope protein, nuclear matrix.

Emery-Dreifuss muscular dystrophy (EDMD) is caused by mutations in one of two genes. The X-linked recessive form of EDMD is caused by the loss of the nuclear membrane protein emerin (*1*, *2*). The autosomal dominant form of EDMD is caused by mutations in the lamin A/C gene (*3*); lamin is an intermediate filament protein that constitutes the nuclear lamina. Humans have at least three lamin genes (lamin A/C, B1, and B2) that are expressed independently during development and differentiation (*4*). Lamin A and lamin C are alternatively spliced products of the lamin A/C gene (Fig. 1, A and B), and are expressed primarily, but not exclusively, in differentiated non-proliferating cells.

It is known that emerin is localized at the inner nuclear membrane in various tissues by its transmembrane domain at the C-terminus. This hydrophobic C-terminus is not only essential for nuclear membrane targeting but also is involved in the stabilization of the protein. The presence of this region is necessary but not sufficient for protein targeting to the nuclear rim. So, emerin might have interactions distinct from those of lamin with some other components of the inner nuclear membrane, and this selective interaction might be required to achieve stability and function of rigorously moving nuclei in tissues such as those in skeletal muscle, heart, and joints. The physiological function of emerin, however, has not yet been determined (*2*, *5*).

To reveal the function of emerin and the mechanism of this muscle degenerative disease (X-EDMD), several trials have been done to identify the emerin binding proteins. Up to now, the interactions of emerin with lamins and nuclear actin have been studied by immunoprecipitation analysis (*6*), and the direct interaction of emerin with lamin A has been investigated by biomolecular interaction analysis (*7*).

In our study, we constructed an emerin affinity column using recombinant emerin expressed in *Escherichia coli*. With this affinity column, we attempted to identify candidate emerin-binding proteins. This approach for screening emerin-binding proteins is useful and practical, because large numbers of soluble proteins can be handled without limitation. After narrowing the number of candidates for emerin-binding proteins, we performed immunoprecipitation analysis using four antibodies against nuclear membrane proteins. In these experiments, the C-terminus transmembrane-truncated emerin binds specifically to lamin A, but no specific interaction of lamin C with emerin could be detected. Therefore, we concluded that emerin binds to lamin A more strongly than to lamin C.

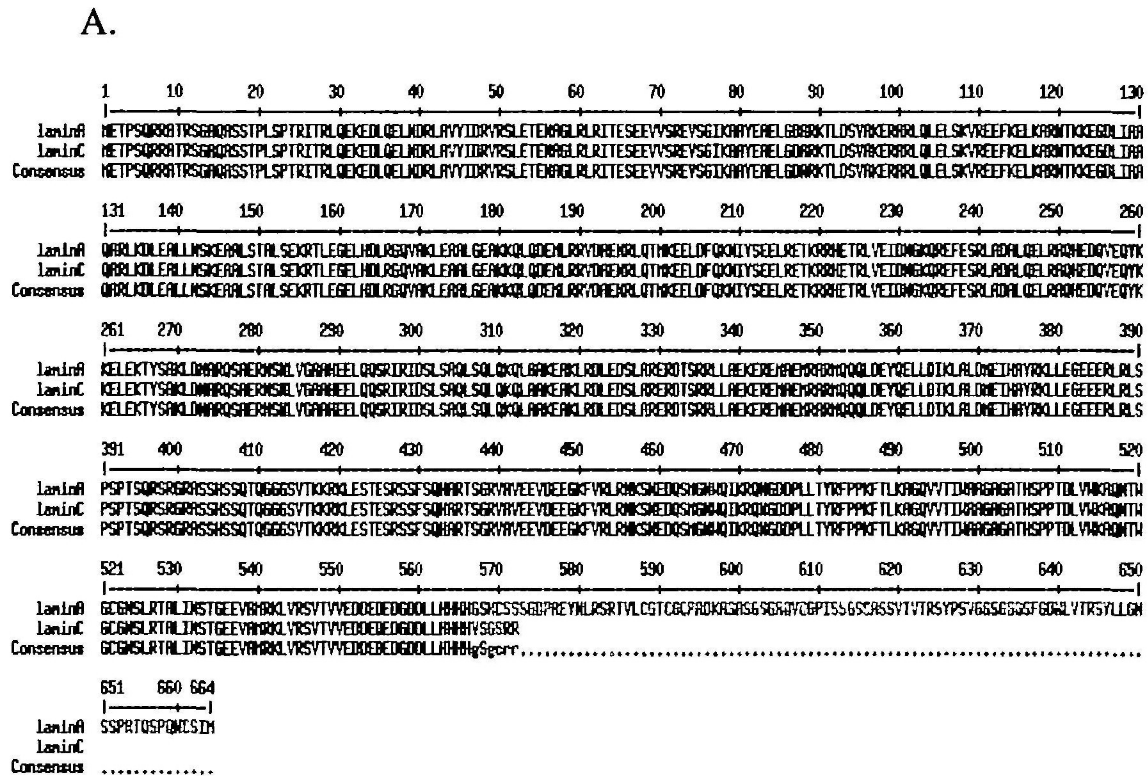
Next we also clarified the region of lamin A that is critical for the interaction with emerin using the yeast two-hybrid system. We used C-terminus truncated emerin as the bait, and transformed it with prey plasmids containing various truncated lamin A genes into yeast strain Y187. The resulting constructs were then analyzed by the β-galactosidase assay. A comparison of β-galactosidase units between the several lamin A deletion constructs showed that

[1] This work was supported in part by a Grant-in-Aid from the Ministry of Health and Welfare of Japan, and Japan Space Forum.

[2] To whom correspondence should be addressed. Phone: +81-3-5454-6739, Fax: +81-3-5454-6739, cishiura@mail.ecc.u-tokyo.ac.jp

© 2001 by The Japanese Biochemical Society.

Downloaded from http://jb.oxfordjournals.org/ at Peking University on October 1, 2012



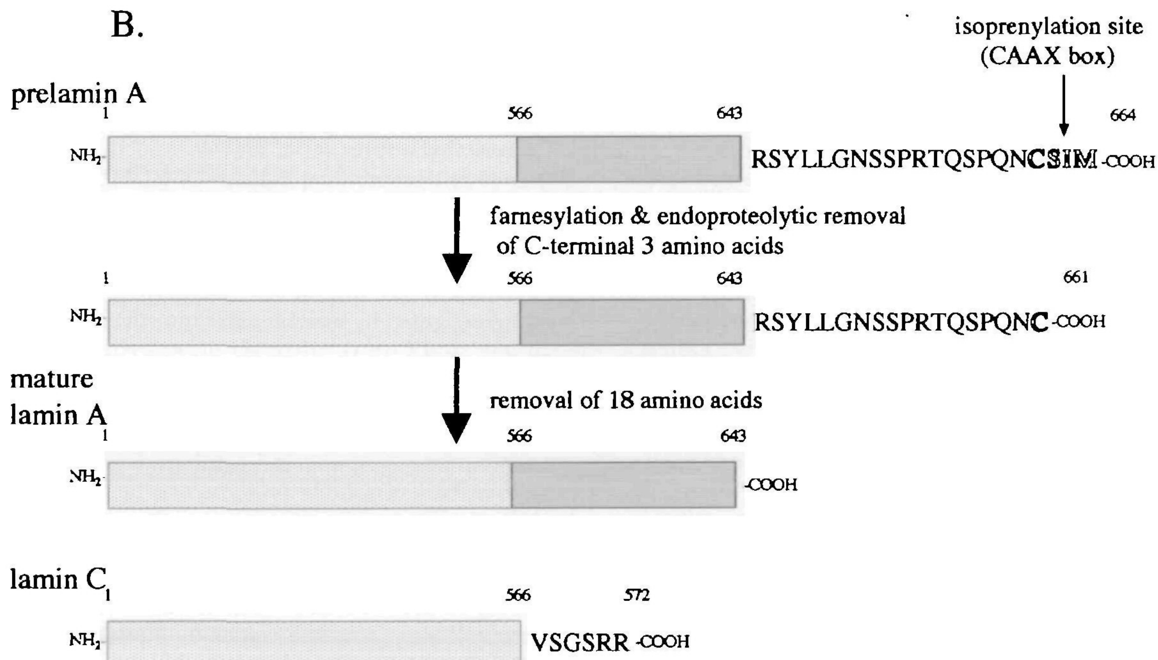


Fig. 1. **Differences in the peptide sequences of lamin A and lamin C.** A: Multiple alignments of human lamin A and lamin C. Lamin A and lamin C are encoded by the same gene. The differences in these two proteins are generated by alternative splicing. B: Lamin A is synthesized as prelamin A (664 polypeptides), and undergoes posttranslational modification. The initial step in this processing is the isoprenylation of a CAAX box. Furthermore, the C-terminus 18 amino acids of prelamin A are removed to yield mature lamin A. The difference between mature lamin A and lamin C is 77 amino acids (from residue 567 to 643) in the C-terminus of lamin A.

the tail domain of lamin A is important for binding to emerin. This is the first report to mention the difference between lamin A and lamin C in physiological activity, and the results suggest that lamin A plays some role in the etiology of the muscle degeneration or cardiac defects in EDMD patients.

Downloaded from http://jb.oxfordjournals.org/ at Peking University on October 1, 2012

There have been many reports referring to the difference between lamin A and lamin C in nuclear assembly pathways or expression patterns. For example, when lamin A or C is microinjected into Swiss 3T3 cells, the incorporation of recombinant lamin C into the nuclear lamina is slower than that of lamin A, and proceeds *via* intranuclear foci. Hence, it was concluded that the incorpoation of lamin A and lamin C into the nuclear lamina proceeds through different pathways (*8*). The relationship between the expression of muscle-specific proteins and that of lamin A has also been reported (*9*). An experiment using chicken embryonic muscle tissue has shown that the expression of lamin A increases during myoblast differentiation, and precedes the expression of a number of muscle-specific genes.

With regard to protein interactions, there is a report that lamin A also binds to chromatin and, by this interaction, lamin A is involved in the control of gene expression at the transcription or replication level (*10*). It has also been reported that lamin A colocalizes with the RNP (*11*) and the RNA splicing factor in the nucleoplasm (*12*). Possible explanations for these interactions have not been provided. However, the data indicate that lamin A plays some key role in the control of gene expression. Therefore it is possible that emerin, the intracellular partner of lamin A, is also involved in the control of gene expression.

MATERIALS AND METHODS

Purification of Recombinant Emerin—Emerin was expressed as a recombinant His-tagged fusion protein in *E. coli*. Cells were suspended in denaturing buffer [8 M urea, 0.5 M NaCl, 20 mM Tris/HCl (pH 7.5), 5 mM imidazole] and disrupted by sonication. The cell lysate was incubated overnight at 4°C, then centrifuged at 1,000 ×*g* for 30 min, and the supernatant was applied to a Ni-column prepared by stuffing 4 ml Ni-NTA agarose gel (QIAGEN, Germany) into a 1.5 cm × 2.6 cm column. The column was first washed with 20 ml low concentration imidazole buffer [8 M urea, 0.5 M NaCl, 20 mM Tris/HCl (pH 7.5), 20 mM imidazole] to remove non-specific binding proteins, and then emerin was eluted in 10 ml elution buffer [8 M urea, 0.5 M NaCl, 20 mM Tris/HCl (pH 7.5), 100 mM imidazole].

The urea was removed by dialysis, with the urea concentration of urea in the dialysate halved every 24 h until 1 M urea. Finally, in the last two steps, the concentration of urea was reduced to 0.2 M and to 0 M. The dialyzed sample solution was centrifuged at 1,000 ×*g* for 10 min yielding purified soluble emerin in the supernatant.

Preparation of Nuclear Membrane Proteins—Rat skeletal muscle (60 g) or liver (40 g) was homogenized at 4°C in 10 volumes of homogenizing buffer [0.25 M sucrose, 50 mM Tris/HCl (pH 7.5), 25 mM KCl, 5 mM $MgCl_2$] with a Teflon-glass homogenizer. The homogenate was centrifuged at 200 ×*g* for 10 min to remove tissue fragments. The supernatant was further centrifuged at 600 ×*g* for 10 min to yield nuclei. The pellet was suspended in 2 volumes of homogenizing buffer, and resuspended in 6 volumes of high-density buffer [2.3 M sucrose, 50 mM Tris/HCl (pH 7.5), 25 mM KCl, 5 mM $MgCl_2$]. This solution was loaded into a centrifugation tube with a 1/5 volume of high density buffer at the bottom, and centrifuged at 12,400 ×*g* for 30 min. The purified nuclear pellet was resuspended in 1 volume of homogenizing buffer, and sonicated to separate the nuclear matrix and genomic DNA from the nuclear membranes. This sonicated solution was then centrifuged at 54,000 ×*g* for 30 min to yield nuclear membranes. The pellet was solubilized in denaturing buffer containing 8 M urea by incubation overnight at 4°C. The denaturing buffer was replaced with bind buffer [0.5 M NaCl, 5 mM Tris/HCl (pH 7.5), 5 mM imidazole] by the same dialysis steps as in "Purification of recombinant emerin."

Emerin Affinity Column Chromatography—Purified His-tag emerin was re-applied onto a Ni-column. The nuclear extracts from rat skeletal muscle and liver were separately applied over the emerin loaded Ni-column. The column was first washed with low imidazole buffer without urea [0.5 M NaCl, 20 mM Tris/HCl (pH 7.5), 20 mM imidazole], and then the emerin-binding proteins were eluted along with emerin with the same elution buffer used in the purification of emerin.

Antibodies—The antibody against emerin recognized the sequence SRSSLDLSYYPTSSST, which corresponds to residues 173–188 of emerin, and that for lamin A recognized the sequence CSSSGDPAEYN, which corresponds to residues 570–580 of lamin A. The antibody against lamin C recognized the sequence LHHHHVSGSRR, which corresponds to residues 562–572 of lamin C (*2*, *5*). The anti-emerin and anti-LAP1 antibodies (MA1-074, Affinity Bioreagents) are mouse antibodies, while the anti–lamin A and anti–lamin C antibodies are rabbit antibodies. As a second antibody, biotinylated mouse IgG or rabbit IgG was used.

Immunoprecipitation—Four antibodies against nuclear membrane proteins, emerin, lamin A, lamin C, and LAP1 (lamina associated polypeptide 1), were used. The mixture of purified emerin and nuclear membrane extracts was incubated for at least 1 h at 4°Cwith 50 µl of 50% (w/v) protein A-Sepharose (Amersham Pharmacia Biotech AB, Uppsala, Sweden) in TNE buffer [150 mM NaCl, 20 mM Tris/HCl (pH 7.5), 10 mM EDTA] without antibodies. After incubation, proteins binding non-specifically to protein A-Sepharose were removed with the beads. The supernatant was transferred into a new microtube, 50 µl of 50% (w/v) protein A-Sepharose was added to the solution. The mixture was incubated for 1 h at 4°C, after which the antibodies were added and the mixture was incubated for 1 more hour. The beads were washed five times with TNE buffer. The immunoprecipitates were analyzed by SDS-PAGE and Western blotting.

SDS-PAGE and Western Blot Analysis—The sample eluted from the emerin affinity column or the immunoprecipitates was diluted in SDS-PAGE sample buffer, and analyzed by SDS–polyacrylamide gel electrophoresis. Proteins were stained with CBB (Coomassie Brilliant Blue) or transferred onto PVDF membranes (Finetrap NT32; Nihon Eido, Tokyo) and stained with each antibody. Results were visualized with a Vectastain ABC kit (Vector Lab. Burlingame, CA, USA) and POD immunostain set (WAKO, Osaka).

The Yeast Two-Hybrid Assay—The Matchmaker yeast two-hybrid system (CLONTECH Laboratories, USA) was used for interaction assays. To generate plasmids for use in this assay, cDNAs were cloned into pGAD424, which expresses fusion proteins with GAL4 transcription activation domains, or pAS2-1-C (provided by H. Sorimachi, University of Tokyo, Tokyo), which expresses fusion proteins with the GAL4 DNA-binding domain. The desired plasmids

Downloaded from http://jb.oxfordjournals.org/ at Peking University on October 1, 2012

were co-transformed into *Saccharomyces cerevisiae* strain Y187 and the yeast cells were plated onto Trp-Leu- synthetic medium to select for plasmids. Yeast transformation and β-galactosidase activity assays using CPRG (COMVOS GmbH, Mannheim, Germany) as the substrate were performed according to the Yeast Protocols Handbook (CLONTECH Laboratories).

Plasmid Construction—Complementary DNAs were generated by polymerase chain reaction using a GeneAmp PCR System 9600 thermocycler (Perkin Elmer Instruments, USA). To generate various DNA fragments encoding parts of emerin or lamin A, custom oligonucleotide primers (GENSET KK, Kyoto) designed with restriction endonuclease sites at their 5′-ends were used with clones for human emerin and lamin A as templates. Amplified cDNAs were purified and cloned into the plasmids of choice by standard methods. Full-length emerin and C-terminus transmembrane truncated emerin (amino acids from 1 to 225) were cloned into pAS2-1-C. Full-length emerin, lamin A, lamin C, C-terminus transmembrane truncated emerin, and various fragments of lamin A were cloned into pGAD-424. Fragments lam A (1–566), lam A (1–384), lam A (385–664), and lam A (567–664) were cut using *Eco*RI–*Bam*HI restriction enzymes and ligated into the corresponding sites in pGAD424. Sequence analysis using a Shimadzu DNA sequencer DSQ-2000L (Shimadzu, Kyoto) and a Thermo Sequenase kit (Amersham Pharmacia Biotech AB, Uppsala, Sweden) verified that these cDNAs were ligated in-frame.

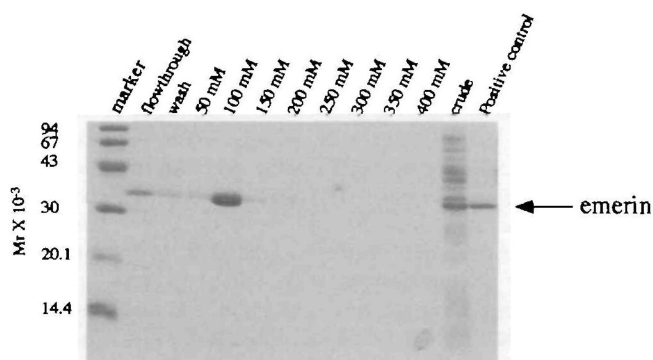


Fig. 2. **Purification of emerin by Ni-NTA agarose chromatography.** Urea-denatured emerin was loaded onto a Ni-NTA agarose column. After washing the column with a low concentration of imidazole buffer, Ni-bound His-tagged emerin was eluted. The most effective concentration of imidazole for the elution of emerin was found to be 100 mM. Protein sample were analyzed by 15% SDS-PAGE with CBB staining.

RESULTS

Expression and Purification of Emerin—C-terminus transmembrane domain-truncated emerin was expressed in *E. coli* (BL21 DE3) with 6× His at the N-terminus. SDS-PAGE and immunoblot analysis using the antibody against emerin showed the presence of emerin at the predicted molecular mass (approximately 32 kDa). Crude *E. coli* extracts were solubilized in 8 M urea and loaded onto Ni-NTA agarose. His-tag fusion emerin was purified as a single protein (Fig. 2). When the buffer containing the emerin was changed from denaturing to physiological by dialysis, the emerin remained in the soluble form.

Screening of Emerin-Binding Proteins by Emerin Affinity Column Chromatograyshy—Purified emerin was reloaded onto Ni-NTA agarose, and the nuclear membrane protein extracts were applied to this emerin affinity column. The non-specific column binding proteins were washed out, and the emerin-binding proteins were eluted with emerin (Fig.

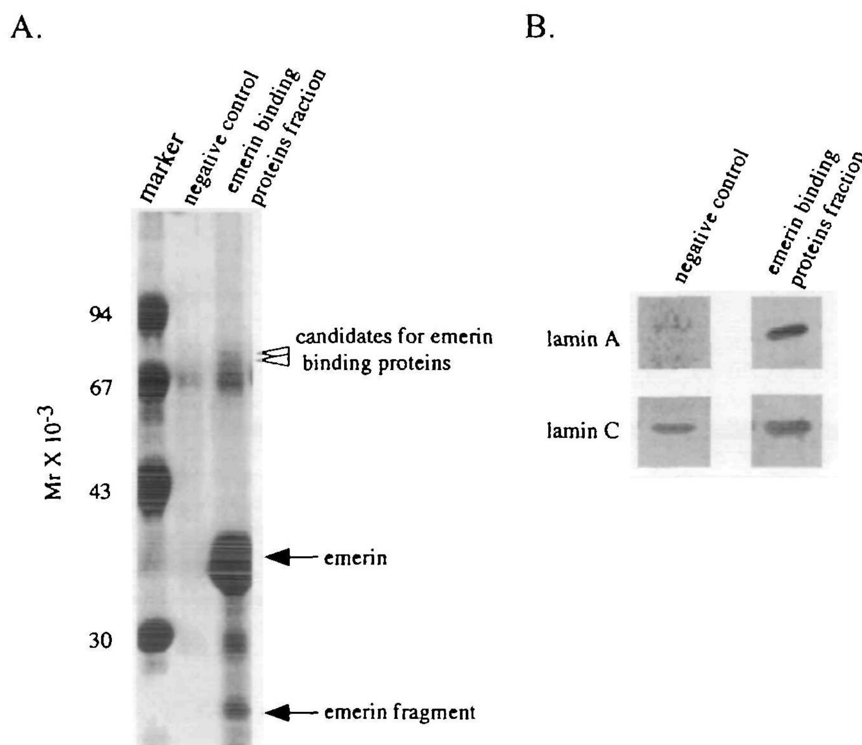


Fig. 3. **Screening of emerin-binding proteins by emerin affinity column chromatography.** Purified emerin was reloaded onto a Ni-NTA agarose column, and the nuclear membrane proteins of rat liver extracts were applied on top. Emerin binding proteins were separated by this emerin affinity column. A: CBB staining of the SDS-PAGE gel. Arrowheads indicate possible emerin binding proteins. B: Western blot analysis of lamin A and lamin C stained by anti-lamin A and lamin C antibodies.

Downloaded from http://jb.oxfordjournals.org/ at Peking University on October 1, 2012

3a). The eluted proteins were analyzed by peptide sequence analysis and Western blotting analysis. Although several proteins were eluted with emerin, some of them (67 kDa, 68 kDa) were reproducibly obtained by the same procedure without emerin. Therefore we concluded that these are nonspecific proteins. On the other hands, lamin A (70 kDa) was concentrated on the emerin affinity column, and eluted with emerin (Fig. 3a), whereas lamin C was also eluted from the negative control column without emerin (Fig. 3b). Therefore, lamin A seems to bind to emerin specifically, while lamin C does not.

Immunoprecipitation—To confirm the above results, immunoprecipitation experiments were performed using recombinant emerin and nuclear membrane extracts. The antibodies used for these experiments were anti-emerin, -lamin A, -lamin C, and -LAP1 (lamina associated polypeptides 1). SDS-PAGE and Western blot analysis showed that the anti–lamin A antibody co-immunoprecipitated emerin, but the other two antibodies, anti–lamin C and anti-LAP1, did not (Fig. 4). The anti-emerin immunoprecipitates contained large amounts of emerin, but Western blotting and immunoassay with anti–lamin A antibody did not show the presence of lamin A in this complex (see "DISCUSSION"). We also concluded from this experiment that emerin binds to lamin A specifically without the C-terminus transmembrane domain.

Binding of Nuclear Proteins to Emerin—We used the yeast two-hybrid assay to reveal slight differences in the binding capacities of several nuclear membrane proteins to emerin. Lamin A and lamin C showed slight differences in their binding strength to C-terminus transmembrane truncated emerin. The β-galactosidase activity of lamin A is higher than that of lamin C. Therefore we concluded that lamin A binds to emerin more strongly than lamin C. It was also shown that molecules of C-terminus truncated emerin bind to each other. This indicates that C-terminus truncated emerin can form homodimers or multimers.

Identification of the Lamin A Domain That Is Required for the Interaction with Emerin—Next we examined the interactions of various portions of lamin A with emerin using the yeast two-hybrid assay (Fig. 5). The tail domain of lamin A, from amino acids 384 to 664, showed higher binding activity than the negative control, while the lamin A–specific 98 amino acids from 567 to 664 showed no excess activity in comparison with the control. Therefore, the first half of the lamin A tail domain (amino acids 384 to 566) is responsible for emerin binding. This region is also present in lamin C and thought to be an emerin-binding

Downloaded from http://jb.oxfordjournals.org/ at Peking University on October 1, 2012

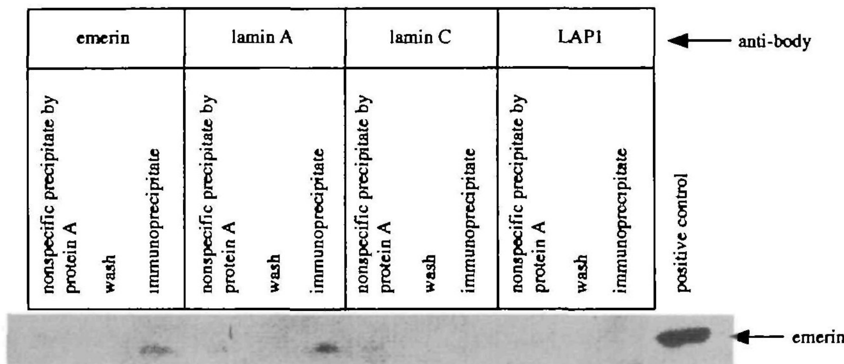


Fig. 4. **Immunoprecipitation of nuclear extracts by four antibodies against nuclear envelope proteins.** Emerin was immunoprecipitated by both anti-emerin and anti–lamin A antibodies, but the anti–lamin C antibody did not immunoprecipitate emerin. The Western blot was stained with the emerin antibody. To remove the nonspecific binding to protein A Sepharose, precipitation without antibodies was first performed. Emerin was not included in this fraction.

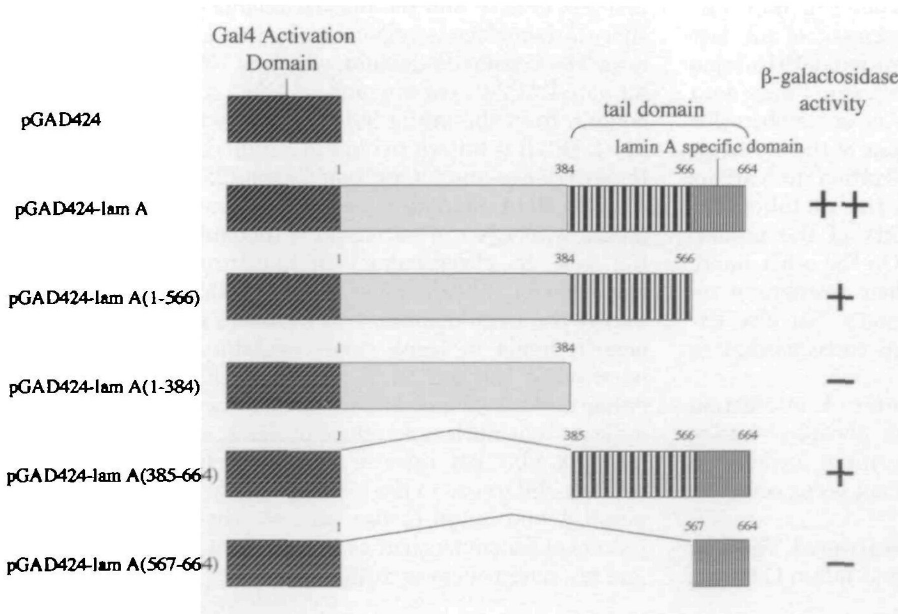


Fig. 5. **Evaluation of the emerin binding ability of nuclear proteins using the yeast two-hybrid system.** The yeast two-hybrid assay was performed to identify the region of lamin A that is responsible for the binding to emerin. C-terminus truncated emerin was cloned into pAS2-1-C as a bait plasmid and various fragments of lamin A were cloned into pGAD424 as prey plasmids. From comparison of the transformants of various lamin A deletion constructs, the top half of the tail domain was identified as an emerin-binding site. The lamin A–specific C-terminal domain plays no direct role in the binding to emerin, but is thought to be important for maintaining a functional structure that promotes binding to emerin.

site. These results suggest that the lamin A–specific C-terminal region, amino acids 567 to 664, might have some structural function that promotes binding to emerin.

DISCUSSION

Our results can be positively interpreted that a specific emerin–lamin A interaction occurs *in vivo*. Posttranslational modification is essential for lamin A to localize at nuclear lamina (*13*, *14*). The modification occurs mainly in three steps. The initial step is the isoprenylation of a CAAX (CSIM, located at the C terminus from residues 661 to 664) box cysteine. The second step is the endoproteolytic removal of the carboxyl-terminal 3 amino acids and the subsequent methylation of the carboxyl-terminal cysteine. The last step is the removal of 18 amino acids from the carboxyl terminus. All of these steps take place in the nucleus. Prelamin A is transported to the nucleus where it undergoes maturation (*15*). However the lamin A that is extracted from the nuclear membrane is thought to be a mature form. Since the difference in the peptide sequences of mature lamin A and lamin C lies in the C-terminus 77 amino acids sequence from residue 567 to 643, it is possible that this C-terminal region of lamin A may form some structures that allow or help emerin to bind to lamin A. Structural analysis of the emerin–lamin A complex will reveal whether this prediction is correct or not.

We used C-terminus truncated emerin in the affinity purification and immunoprecipitation experiments. Therefore, we can conclude that emerin binds to lamin A without its C-terminal predicted transmembrane domain. In our experiments, however, we could not co-immunoprecipitate lamin A with the anti-emerin antibody. Since the amount of emerin relative to other nuclear envelope proteins is exceedingly high, lamin A might be lost due to binding the surplus emerin in the immunoprecipitation experiment. It is reasonable to think that the amount of lamin A that was co-immunoprecipitated with emerin by anti-emerin antibody was too small to be detected by immunoblot assay.

Several reports have shown that the C-terminal transmembrane domain is necessary for the nuclear membrane targeting of emerin (*5*, *16*). Mutations in this region result in greatly reduced amounts of expressed emerin. More surprisingly, mutations involving the replacement of the last eight amino acids of emerin with an additional 101 amino acids results in the disappearance of emerin. These data indicate that insufficient hydrophobicity or some obstacles to membrane targeting result in alterations of the biochemical properties of emerin and mislocalization to various organelles or cytoplasm. Depending on the mutation, incompleteness may bring about instability of the protein resulting in digestion in the lysosomes. On the other hand, proper targeting of emerin to the nuclear membrane requires not only the transmembrane domain but also the latter half of the nucleoplasmic domain corresponded to residues 109–225 (*5*).

We also clarified that the emerin–lamin A interaction does not require such modifications as phosphorylation, myristoylation or glycosylation, because emerin expression in *E. coli* indicates that modifications do not occur correctly in prokaryotes.

In our study, two questions remain unanswered. The first is why the difference between lamin A and lamin C brings about a difference in their interaction with emerin. Lamin A and lamin C are products of the same gene, and are thought to construct nuclear lamina by forming heterodimers or homodimers under dephospholylation conditions. The phosphorylation of lamins causes the nuclear envelope to disperse in the cell cycle–dependent manner. The functions of these two lamins as components of the nuclear lamina are thought to be similar. This major function to reinforce the nuclear structure by forming nuclear lamina is essential so that all tissues contain lamins as structural proteins. However, the tissue specificity of the defects in EDMD patients (*17*, *18*) indicates that nuclear membrane proteins have other functions, especially in skeletal and cardiac muscle cells or in fat tissues in the case of lipodystrophy patients. So it is rationally considered that each prothein has its own function in addition to its role as a structural protein in various tissues. The difference in the binding capacity to emerin between lamin A and lamin C is thought to derive from the additional function in skeletal and cardiac muscle. From the results of the yeast two-hybrid assay, lamin A is superior to lamin C in terms of the interaction with emerin, although both lamin A and lamin C are able to bind to emerin (data not shown). The region that is required for binding to emerin was determined to be not the lamin A–specific region, but the top half of the tail domain, which is also contained in lamin C. These results suggest that lamin A and lamin C interact with emerin through this common region, and that the lamin A–specific region is thought to be involved in the promotion of binding to emerin. Structural analysis of this two protein complexes may lead to an understanding of the mechanism.

The second question is what are the additional functions of these nuclear membrane proteins. Nuclear membrane proteins such as lamins are believed to have functions in addition to the maintenance of nuclear structure as components of nuclear lamina. For example, lamin A interacts with chromatin, and by this interaction, lamin A controls gene expression at the level of transcription or replication (*10*). It has also been reported that lamin A colocalizes with RNP (*19*) and RNA splicing factor (*12*) in the nucleoplasm. LAP2 (lamina associated polypeptide 2)-β is an inner nuclear membrane protein that shows similarity to emerin. LAP2-β, emerin and the nuclear membrane protein MAN1 share a homologous region approximately 40 amino acids long. The conserved domain, which is 70% identical among human LAP2-β, emerin and MAN1, is termed the LEM domain from the initial letters of these three proteins (*20*, *21*). LAP2-β is known to bind to lamin, chromatin and BAF, the barrier-to-autointegration factor (*22*). BAF is a small dimeric DNA–binding protein that forms oligomeric complexes with DNA *in vitro*, and is thought to play an essential role in chromosomal architecture. The region responsible for the binding of LAP2-β to BAF is thought to be part of the LEM domain. This indicates the possibility that emerin binds to some gene regulatory proteins, and is involved in the control of muscle-specific gene expression either directly or indirectly. These strong pieces of evidence indicate that nuclear envelope proteins, such as emerin and lamin A, play key roles in the control of gene expression. And the difference in the binding ability to emerin between lamin A and lamin C also suggests the existence of some unknown functions such as involvement in gene regulation and the metabolism of mRNA.

Downloaded from http://jb.oxfordjournals.org/ at Peking University on October 1, 2012

We thank Dr. Yuichi Tsuchiya for providing us with the anti-emerin, lamin A and lamin C antibodies, and the emerin constructs with meaningful technical advice.

REFERENCES

1. Bione, S., Maestrini, E., Rivella, S., Mancini, M., Regis, S., Romeo, G., and Toniolo, D. (1994) Identification of a novel X-linked gene responsible for Emery-Dreifuss muscular dystrophy. *Nat. Genet.* **8**, 323–327
2. Yorifuji, H., Tadano, Y., Tsuchiya, Y., Ogawa, M., Goto, K., Umetani, A., Asaka, Y., and Arahata, K. (1997) Emerin, deficiency of which causes Emery-Dreifuss muscular dystrophy, is localized at the inner nuclear membrane. *Neurogenetics* **1**, 135–140
3. Bonne, G., Di Barletta, M.R., Varnous, S., Becane, H.M., Hammouda, E.H., Merlini, L., Muntoni, F., Greenberg, C.R., Gary, F., Urtizberea, J.A., Duboc, D., Fardeau, M., Toniolo, D., and Schwartz, K. (1999) Mutations in the gene encoding lamin A/C cause autosomal dominant Emery-Dreifuss muscular dystrophy. *Nat. Genet.* **21**, 285–288
4. Moir, R.D., Spann, T.P., and Goldman, R.D. (1995) The dynamic properties and possible functions of nuclear lamins. *Int. Rev. Cytol.* **162B**, 141–182
5. Tsuchiya, Y., Hase, A., Ogawa, M., Yorifuji, H., and 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
6. Fairley, E.A., Kendrick-Jones, J., and Ellis, J.A. (1999) The Emery-Dreifuss muscular dystrophy phenotype arises from aberrant targeting and binding of emerin at the inner nuclear membrane. *J. Cell Sci.* **112**, 2571–2582
7. Clements, L., Manilal, S., Love, D.R., and Morris, G.E. (2000) Direct interaction between emerin and lamin A. *Biochem. Biophys. Res. Commun.* **267**, 709–714
8. Pugh, G.E., Coates, P.J., Lane, E.B., Raymond, Y., and Quinlan, R.A. (1997) Distinct nuclear assembly pathways for lamins A and C lead to their increase during quiescence in Swiss 3T3 cells. *J. Cell Sci.* **110**, 2483–2493
9. Lourim, D. and Lin, J.J. (1989) Expression of nuclear lamin A and muscle-specific proteins in differentiating muscle cells in ovo and in vitro. *J. Cell Biol.* **109**, 495–504
10. Gotzmann, J. and Foisner, R. (1999) Lamins and lamin-binding proteins in functional chromatin organization. *Crit. Rev. Eukaryot. Gene Expr.* **9**, 257–265
11. Neri, L.M., Raymond, Y., Giordano, A., Capitani, S., and Martelli, A.M. (1999) Lamin A is part of the internal nucleoskeleton of human erythroleukemia cells. *J. Cell Physiol.* **178**, 284–295
12. Jagatheesan, G., Thanumalayan, S., Muralikrishna, B., Rangaraj, N., Karande, A.A., and Parnaik, V.K. (1999) Colocalization of intranuclear lamin foci with RNA splicing factors. *J. Cell Sci.* **112**, 4651–4661
13. Beck, L.A., Hosick, T.J., and Sinensky, M. (1990) Isoprenylation is required for the processing of the lamin A precursor. *J. Cell Biol.* **110**, 1489–1499
14. Lutz, R.J., Trujillo, M.A., Denham, K.S., Wenger, L., and Sinensky, M. (1992) Nucleoplasmic localization of prelamin A: implications for prenylation-dependent lamin A assembly into the nuclear lamina. *Proc. Natl. Acad. Sci. USA* **89**, 3000–3004
15. Sinensky, M., Fantle, K., Trujillo, M., McLain, T., Kupfer, A., and Dalton, M. (1994) The processing pathway of prelamin A. *J. Cell Sci.* **107**, 61–67
16. Ellis, J.A., Brown, C.A., Tilley, L.D., Kendrick-Jones, J., Spence, J.E., and Yates, J.R. (2000) Two distal mutations in the gene encoding emerin have profoundly different effects on emerin protein expression. *Neuromuscul. Disord.* **10**, 24–30
17. Funakoshi, M., Tsuchiya, Y., and Arahata, K. (1999) Emerin and cardiomyopathy in Emery-Dreifuss muscular dystrophy. *Neuromuscul. Disord.* **9**, 108–114
18. Buckley, A.E., Dean, J., and Mahy, I.R. (1999) Cardiac involvement in Emery Dreifuss muscular dystrophy: a case series. *Heart* **82**, 105–108
19. Wilson, K.L. (2000) The nuclear envelope, muscular dystrophy and gene expression. *Trends Cell Biol.* **10**, 125–129
20. Lin, F., Blake, D.L., Callebaut, I., Skerjanc, I.S., Holmer, L., McBurney, M.W., Paulin-Levasseur, M., and Worman, H.J. (2000) MAN1: An inner nuclear membrane protein that shares the LEM domain with lamina-associated polypeptide 2 and emerin. *J. Biol. Chem.* **275**, 4840–4847
21. Dechat, T., Vlcek, S., and Foisner, R. (2000) Review: lamina-associated polypeptide 2 isoforms and related proteins in cell cycle-dependent nuclear structure dynamics. *J. Struct Biol.* **129**, 335–345
22. Furukawa, K. (1999) LAP2 binding protein 1 (L2BP1/BAF) is a candidate mediator of LAP2-chromatin interaction. *J. Cell Sci.* **112**, 2485–2492

Downloaded from http://jb.oxfordjournals.org/ at Peking University on October 1, 2012