ACADEMIC PRESS

Available online at www.sciencedirect.com

SCIENCE DIRECT®

Experimental Cell Research 291 (2003) 122–134

Experimental Cell Research

www.elsevier.com/locate/yexcr

Failure of lamin A/C to functionally assemble in R482L mutated familial partial lipodystrophy fibroblasts: altered intermolecular interaction with emerin and implications for gene transcription

Cristina Capanni,[b] Vittoria Cenni,[b] Elisabetta Mattioli,[b] Patrizia Sabatelli,[a] Andrea Ognibene,[b] Marta Columbaro,[c] Veena K. Parnaik,[d] Manfred Wehnert,[e] Nadir M. Maraldi,[a,b] Stefano Squarzoni,[a] and Giovanna Lattanzi[a,*]

[a] *ITOI, CNR, Unit of Bologna, c/o IOR, Bologna, Italy*
[b] *Laboratory of Cell Biology, Istituti Ortopedici Rizzoli, Bologna, Italy*
[c] *Neuromuscular Unit, Istituti Ortopedici Rizzoli, Bologna, Italy*
[d] *Centre for Cellular and Molecular Biology, Hyderabad 500 007, India*
[e] *Institute of Human Genetics, University of Greifswald, Germany*

Received 10 December 2002, revised version received 9 July 2003

Abstract

Familial partial lipodystrophy is an autosomal dominant disease caused by mutations of the *LMNA* gene encoding alternatively spliced lamins A and C. Abnormal distribution of body fat and insulin resistance characterize the clinical phenotype. In this study, we analyzed primary fibroblast cultures from a patient carrying an R482L lamin A/C mutation by a morphological and biochemical approach. Abnormalities were observed consisting of nuclear lamin A/C aggregates mostly localized close to the nuclear lamina. These aggregates were not bound to either DNA-containing structures or RNA splicing intranuclear compartments. In addition, emerin did not colocalize with nuclear lamin A/C aggregates. Interestingly, emerin failed to interact with lamin A in R482L mutated fibroblasts in vivo, while the interaction with lamin C was preserved in vitro, as determined by coimmunoprecipitation experiments. The presence of lamin A/C nuclear aggregates was restricted to actively transcribing cells, and it was increased in insulin-treated fibroblasts. In fibroblasts carrying lamin A/C nuclear aggregates, a reduced incorporation of bromouridine was observed, demonstrating that mutated lamin A/C in FPLD cells interferes with RNA transcription.

Keywords: Lamin A/C; Familial partial lipodystrophy; Lamin-emerin interaction; Chromatin disorganization; Intranuclear lamin A/C speckles; Gene transcription

Introduction

Familial partial lipodystrophy (FPLD, MIM 151660) is an inherited disease caused by mutations of the *LMNA* gene coding for alternatively spliced lamins A and C [1]. The clinical phenotype of FPLD is characterized by loss of fat in the trunk and limbs and accumulation of fat tissue in neck and face occurring during the peripubertal phase [2]. Additionally, variable degrees of resistance to insulin action, together with hypertriglyceridemia, may occur [2].

In FPLD, *LMNA* missense mutations substitute a highly conserved arginine at position 482, or lysine at position 486 in the C-terminal globular domain of lamin A/C [1]. In the last few years, several diseases have been associated to lamin A/C mutations, either dominant such as FPLD, autosomal Emery-Dreifuss muscular dystrophy (EDMD2) [3], dilated cardiomyopathy and conduction system disease (CMD-CD) [4], and limb girdle muscular dystrophy 1B (LGMD 1B) [5], or recessive such as autosomal recessive Emery-Dreifuss muscular dystrophy [6], mandibuloacral

* Corresponding author. ITOI, CNR, Unit of Bologna, c/o IOR, Via di Barbiano 1/10, I-40136 Bologna, Italy.
E-mail address: lattanzi@jolly.bo.cnr.it (G. Lattanzi).

0014-4827/$ – see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/S0014-4827(03)00395-1

dysplasia [7], and Charcot-Marie Tooth neuropathy type 2 [8]. Recently, Hutchison-Gilford progeria syndrome has been ascribed to laminopathies, as lamin A/C mutations and nuclear defects have been found in patients [9–11]. The increasing number of *LMNA*-related diseases strongly suggests that lamin A/C is involved in a number of seemingly unrelated tissue-specific functions. This hypothesis is further supported by the well-known up-regulation of lamin A/C expression in differentiated cells [12–16]. In the knockout mouse model for lamin A/C, a phenotype resembling Emery-Dreifuss muscular dystrophy with Charcot-Marie Tooth neuropathy-like changes has been described, while adipose tissue and insulin sensitivity are nearly normal [17,18].

Lamin A/C interacts with the nuclear envelope protein emerin [19–23], the protein absent or mutated in X-linked Emery-Dreifuss muscular dystrophy (EDMD1). Moreover, anchorage of emerin at the nuclear rim is dependent upon lamin A/C interaction [17]. Emerin expression and localization are not affected in FPLD [24], while emerin binding to lamin A/C in FPLD cells was not studied as far as direct molecular interaction is concerned.

In this study, we analyzed lamin A/C–emerin interaction in cultured FPLD fibroblasts from a patient carrying an R482L mutation. Coimmunoprecipitation experiments showed that emerin does not interact with lamin A in FPLD. We also considered the possibility that lack of interaction between emerin and lamin might cause redistribution of one or both proteins in the nucleus. Altered lamin distribution with dysmorphic budding nuclei in FPLD fibroblasts carrying R482Q and R482W mutations has been reported, while the authors did not find altered distribution of emerin in FPLD cells [24]. In accordance with these reported data, we never found mislocalization of emerin in R482L FPLD fibroblasts. Nevertheless, lamin A/C was redistributed into nuclear aggregates in a high percentage of cells, forming a pattern not described before in FPLD.

Lamins have been implicated in many nuclear activities including nuclear growth, DNA replication, and apoptosis [25] and, more recently, in RNA transcription [26,27]. Abnormal localization of lamin A/C was previously described in cells transfected with lamin A mutants [26,28–30] and could be associated with alteration of the transcriptional machinery. We checked the transcription rate of FPLD fibroblasts and found reduced RNA polymerase II activity in cells carrying abnormal lamin A/C aggregates.

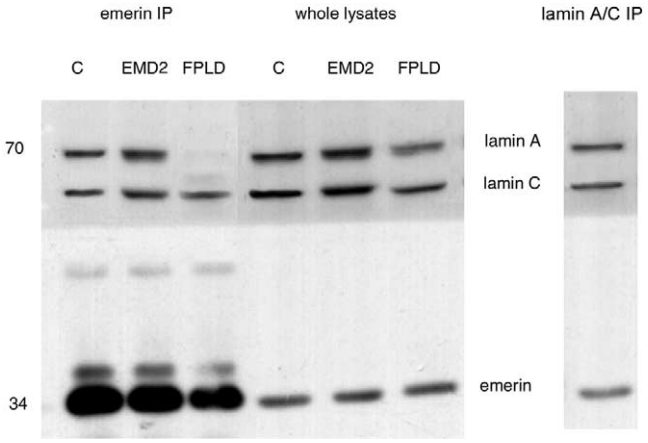


Fig. 1. Lamin A does not coprecipitate with emerin in R482L FPLD fibroblasts. Emerin was immunoprecipitated from whole cell lysates of control (C), EDMD2 (LMNA mutation R401C), and FPLD (*LMNA* mutation R482L) fibroblasts. The immuno-complexes were separated by SDS-PAGE followed by immunoblotting with anti-emerin and anti-lamin A/C antibodies. Immunoprecipitation with anti-emerin antibody is shown in the left panel; immunoblotting of whole lysastes with anti-lamin A/C and anti-emerin antibody is shown in the middle panel; immuno precipitation of a FPLD lysate with anti-lamin A/C antibody is shown in the right panel. Molecular weight markers are indicated in kDa.

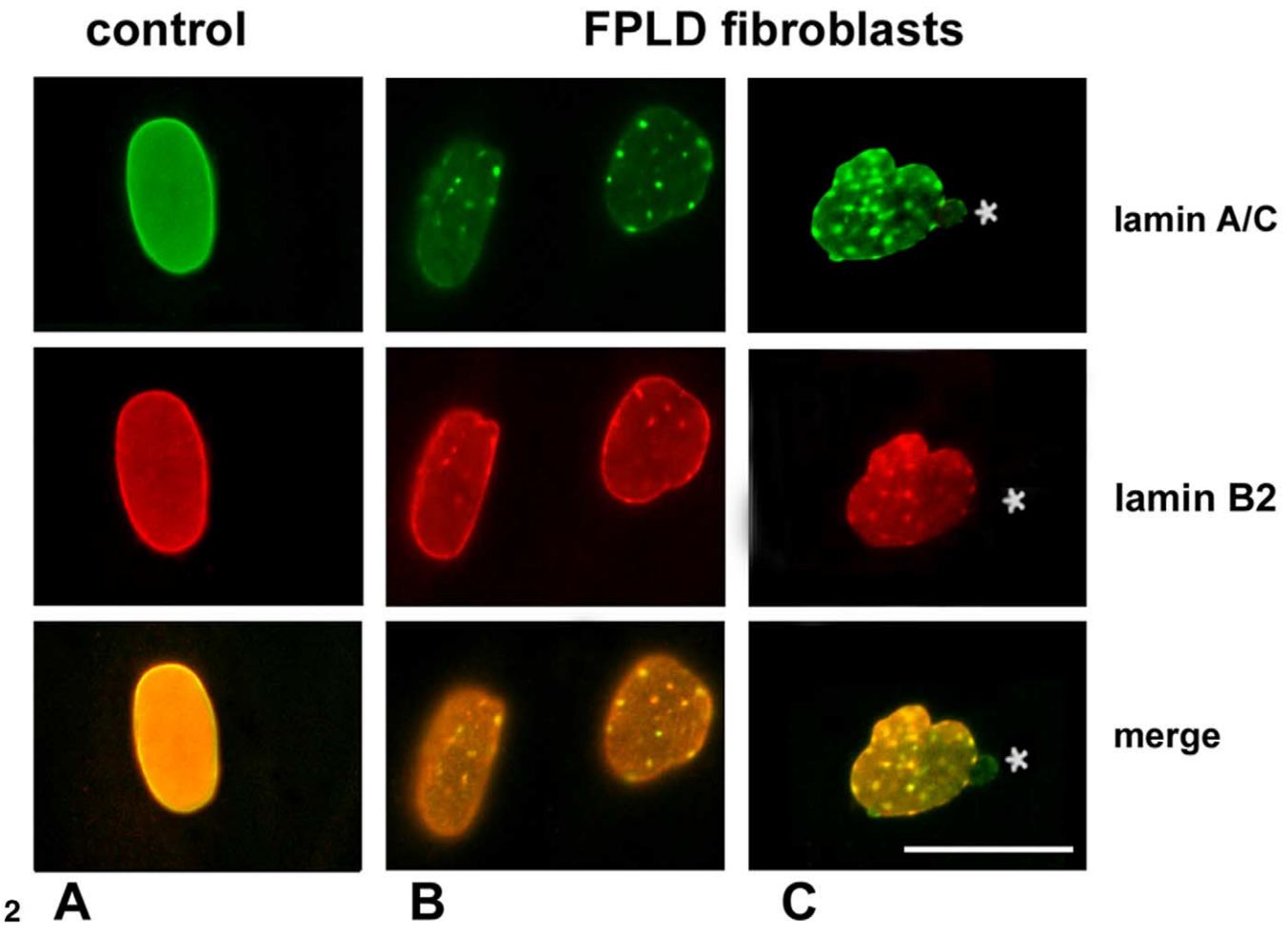
Materials and methods

Cell culture and synchronization

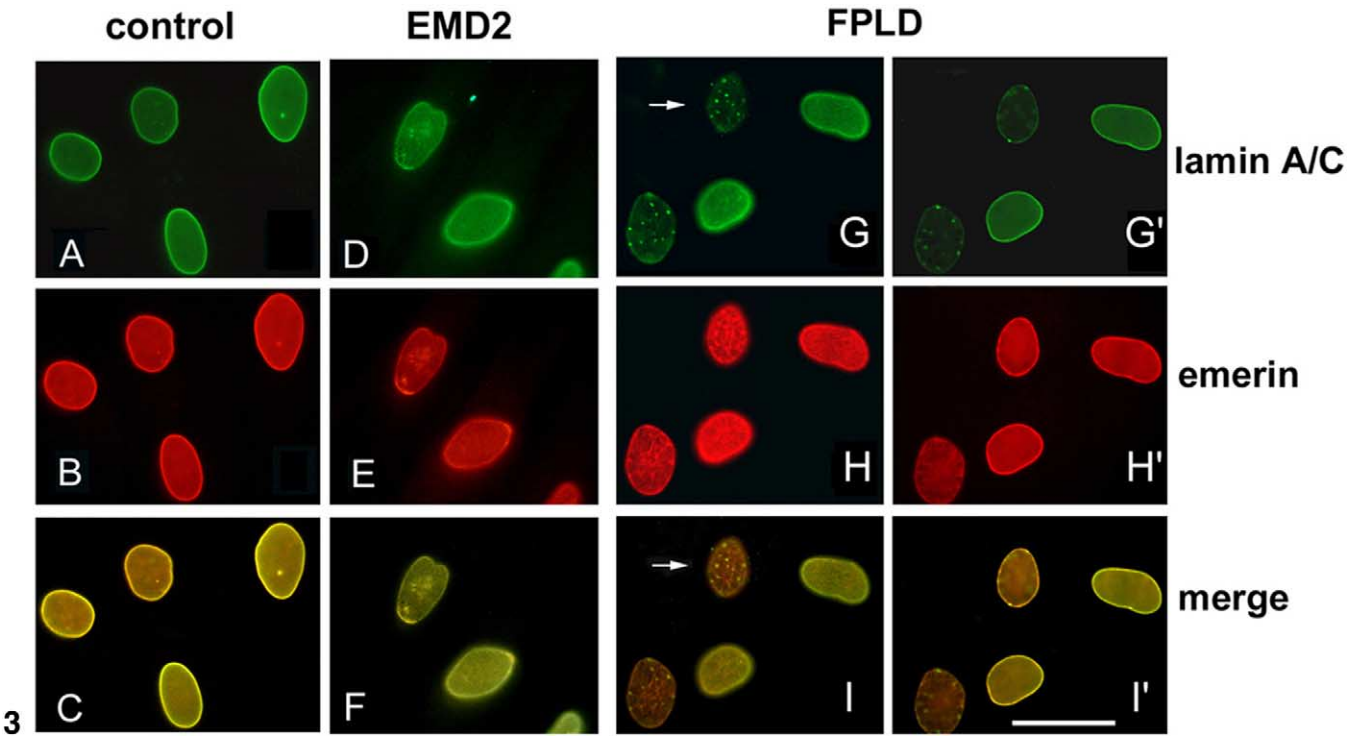
Skin biopsies were obtained from FPLD patient G-9956 carrying an R482L lamin A/C mutation and from unaffected control or EDMD2 (patient G-10877; *LMNA* mutation R401C) patients following a written consent. Fibroblast cultures were established from skin biopsy explants and grown in D-MEM containing 10% fetal calf serum and antibiotics. Cells at passages 10 to 15 were employed. Accumulation of cells in G1 phase was obtained by serum starvation of semiconfluent fibroblast cultures [31], while synchronization of fibroblasts in the G2-M phase of the cell cycle was obtained by demecolcine treatment (25 ng/ml for 24 h) and release [32]. G0 fibroblasts (resting cells) were obtained by serum deprivation of confluent fibroblast cultures for 7 days. All these cells along with not-synchronous fibroblasts (NS) were subjected to methanol fixation and double immunofluorescence staining with anti-PCNA and anti-lamin A/C. PCNA staining (not shown) allowed us to

Fig. 2. Structural abnormalities of the nuclei in R482L FPLD cells. (A) Control nuclei showed lamin A/C and lamin B staining at the nuclear rim. (B) Lamin A/C nuclear aggregates are detected in 15–20% of FPLD fibroblast nuclei. (C) In FPLD nuclei, lamin B is absent from the blebbed areas (*) in which lamin A/C organization is also altered. A nucleus carrying both nuclear lamin A/C aggregates and anomalous distribution of lamin B is shown. Bar, 10 μm.

Fig. 3. Emerin and lamin A/C do not colocalize to nuclear lamin A/C aggregates in R482L FPLD cells. Control fibroblasts are shown in A, B, and C. EDMD2 fibroblasts are shown in D, E, and F: nuclear lamin A/C aggregates are not observed in EDMD2 nuclei, while nuclear lamina abnormalities can be detected in these cells as well as in FPLD fibroblasts (Fig. 2). For FPLD fibroblasts (G–I′) different focal planes are shown: pictures obtained at the nuclear lamina plane are shown in G, H, and I; pictures obtained at the equatorial plane of the nucleus are shown in G′, H′, and I′. As shown in G and I (arrows), lamin A/C nuclear aggregates are mostly observed at the nuclear lamina level, while just a few aggregates are detected at the middle plane of the nucleus, i.e., at internal nuclear sites (G′, I′). No aggregates appear in anti-emerin staining (H, H′) Bar, 10 μm.


control
FPLD fibroblasts
lamin A/C
lamin B2
merge
A
B
C

2


control
EMD2
FPLD
lamin A/C
emerin
merge
A
D
G
G'
B
E
H
H'
C
F
I
I'

3

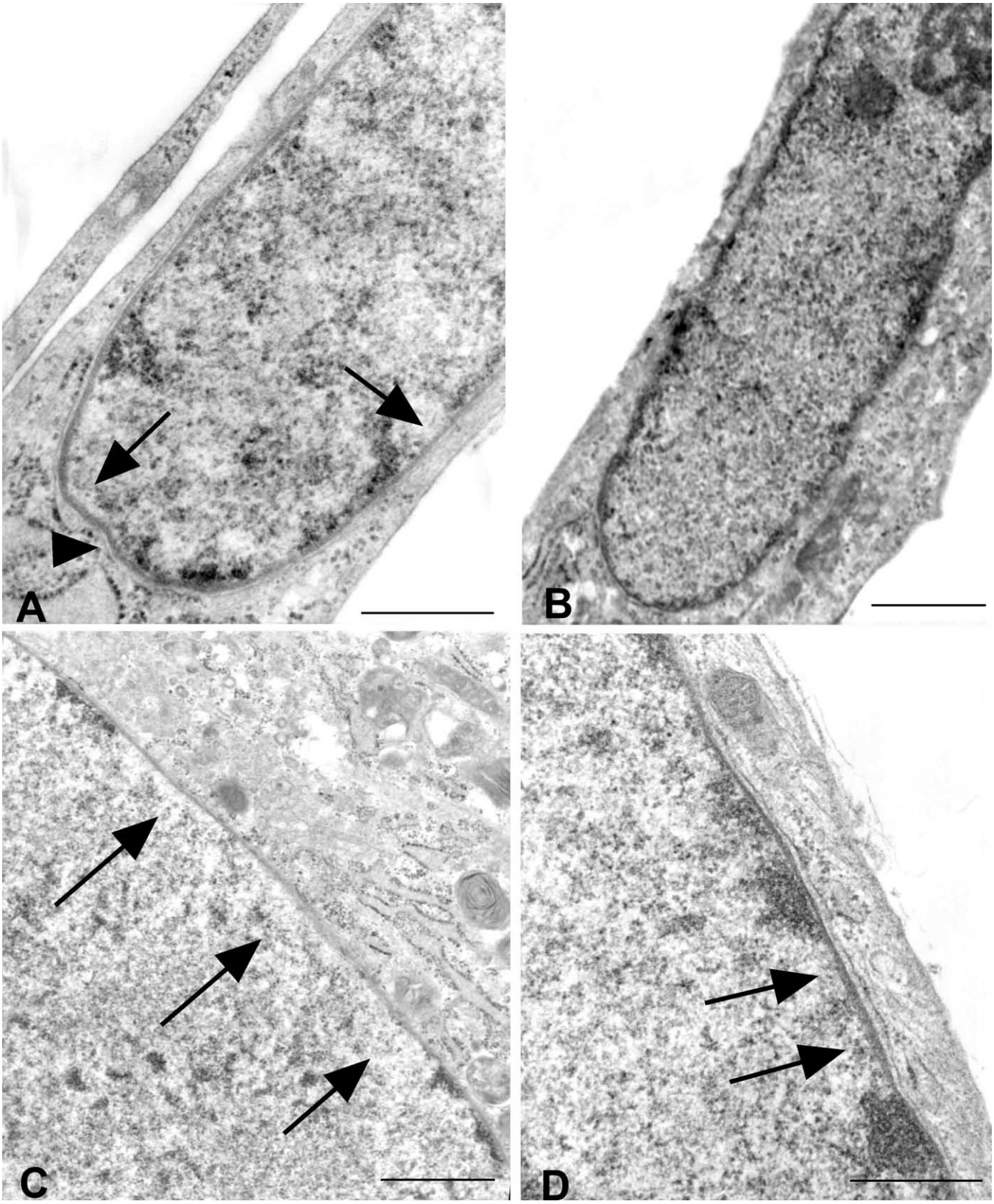


Fig. 4. Electron microscopy analysis of R482L FPLD fibroblast nuclei. Alterations of the chromatin pattern were observed in FPLD nuclei (A, C, D), compared to controls (B): they consisted mainly in lack of heterochromatin at the nuclear periphery, close to the nuclear lamina (A, C, D) (arrows). Detachment of heterochromatin from the nuclear lamina was also observed (A, arrowhead). Nuclear pores were generally absent in the areas corresponding to the peripheral heterochromatin loss (D, arrows). Bars: (A, D), 0.5 μm, (B, C), 1 μm.

distinguish between S-phase cells and G1 or G2 cells. The G2/M population of synchronized fibroblasts is referred to as G2 in the text and figure legends, as only G2 cells were considered in our analysis of lamin A/C aggregate distribution.

Insulin treatment (10 μM, Sigma) of serum-starved fibroblasts was performed for 4 h.

FACS analysis

To check the distribution of cells in the cell cycle, FPLD fibroblasts and controls at 50% of confluence were retrieved by trypsin detachment, fixed in 70% ethanol for 5 h, resuspended in PBS containing 5 μg/ml RNase for 15 min at 37°C, washed in PBS and counter-stained with propidium iodide. Samples were analyzed by a FACStar Plus flow cytometer (Becton Dickinson, Palo Alto, CA) equipped with an argon ion laser tuned at 488-nm wavelength, 50 mW light output.

Antibodies

Anti-lamin A/C goat polyclonal antibody (Santa Cruz) or anti-lamin A/C monoclonal antibody (Novocastra) were used for immunofluorescence staining and for Western blot analysis. The anti-lamin A/C monoclonal antibody was used for the immunoprecipitation experiments. Anti-lamin A 2H10 [33] was employed for immunofluorescence staining. Monoclonal anti-emerin and anti-lamin B2 were obtained from Novocastra. Anti-SC35 monoclonal antibody was purchased from Sigma, and anti-PCNA monoclonal antibody from Santa Cruz. Monoclonal anti-BrdU antibody was from BD-PharMingen. Secondary antibodies were CY3-conjugated anti-mouse IgG (Sigma), FITC-conjugated anti-goat IgG (DAKO), FITC-conjugated anti-mouse IgM (Sigma), HRP conjugated anti-mouse Ig, and anti-goat Ig (Amersham-Pharmacia-Biotech).

Immunoprecipitation and Western blotting

Subconfluent cells were extracted by addition of RIPA buffer (20 mM Tris-HCl, pH 7.0, 1% NP-40, 150 mM NaCl, 10% glycerol, 10 mM EDTA, 20 mM NaF, 5 mM sodium pyrophosphate, 1 mM Na_3VO_4, 1 mM PMSF, 10 μg/ml leupeptin, 10 μg/ml pepstatin) at 4°C. Precleared cell lysates were incubated for 3 h with anti-emerin, or anti-lamin A/C antibody (3 μg per sample), or mouse IgG to provide a negative control, plus 30 μl of 50% (v/v) of protein A/G agarose slurry (Santa Cruz) at 4°C with gentle rocking. Pellets were washed twice with PBS plus 1% NP-40, twice in TNE (10 mM Tris-Cl, pH 7.5, 100 mM NaCl, 1 mM EDTA), and boiled in Laemmli sample buffer.

Immunoprecipitated proteins and whole cell lysates obtained in RIPA buffer were subjected to Western blotting. Samples were loaded onto 12% SDS-PAGE gel, blotted onto nitrocellulose, and probed with specific anti-lamin A/C or emerin antibodies or HRP-conjugated secondary antibodies and developed with enhanced chemiluminescence (ECL) reaction (Amersham-Pharmacia-Biotech).

In situ nuclear matrix preparation

Unfixed fibroblast cultures were washed in PBS, treated with a buffer containing 10 mM Tris-HCl (pH 7.4), 150 mM NaCl, 5 mM $MgCl_2$, 1% NP-40, 1 mM PMSF, 10 μg/ml leupeptin, and aprotinin for 15 min at room temperature. Detergent-treated cells were washed twice in the absence of NP-40, subjected to DNase treatment (20 U/ml for 1 h at room temperature) and high salt extraction in 2 M NaCl containing buffer for 5 min at room temperature. This treatment led to complete removal of chromatin (as demonstrated by the absence of DAPI staining of nuclei, not shown). Samples were fixed with 2% paraformaldehyde and processed for immunofluorescence labeling.

In situ transcription

Living fibroblasts were treated according to published protocols [26]. Briefly, cells were permeabilized in a buffer containing 20 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$, 0.5 mM EGTA, 25% glycerol, 10 μg/ml digitonin, 1 mM PMSF, RNasin (20 U/ml). Transcription assay was performed for 5 min in a buffer containing 50 mM Tris-HCl, pH 7.4, 100 mM KCl, 10 mM $MgCl_2$, 0.5 mM EGTA, 25% glycerol, 2 mM ATP, 0.5 mM CTP, 0.5 mM GTP, 0.5 mM BrUTP, 1 mM PMSF and RNasin (20 U/ml) (Promega). α-Amanitin (10 μg/ml) (Sigma) was added to the transcription buffer to obtain negative controls (not shown). Cells were washed in PBS and fixed in 2% paraformaldehyde at room temperature. Incorporated nucleotides were revealed by immunofluorescence labeling with anti-BrdU antibody. Chromatin was counterstained with 4,6-diamidino-2-phenylindole (DAPI, 0.1 μg/ml) (Sigma).

Immunofluorescence

Fibroblasts grown on coverslips were fixed with methanol at −20°C or with 2% paraformaldehyde at 4°C. Paraformaldehyde-fixed fibroblasts were permeabilized with 0.15% Triton X-100 in PBS. All preparations were incubated with PBS containing 4% BSA to saturate nonspecific binding. Incubation with primary antibodies was performed overnight at 4°C, while secondary antibodies were applied for 1 h at room temperature. Slides were mounted with an antifade reagent in glycerol and observed with a Nikon E 600 fluorescence microscope equipped with a digital camera.

Electron microscopy

FPLD fibroblasts and controls were fixed with 2.5% glutaraldehyde–0.1 M phosphate buffer, postfixed with 1%

OsO_4 in veronal buffer, dehydrated in ethanol, scraped, and embedded in epoxy resin. Ultrathin sections were cut from two different embedded blocks of mutated and control cells, respectively, counterstained with uranyl acetate-lead citrate, and observed with a Philips EM 400 T electron microscope, operated at 100 KV. For each sample, about 250 nuclei were evaluated.

Results

Lamin A and emerin do not interact in cell lysates from FPLD R482L fibroblasts

Lamin A/C has been demonstrated to bind directly emerin [20]. This interaction has been shown to involve mainly lamin A as for the strength of binding [21]. It depends mostly on lamin A for retaining emerin at the nuclear envelope [23]. The sequence that binds emerin includes amino acids 384 to 566 [21] in the tail domain shared by lamins A and C. Since the mutated FPLD sequence is within this domain, we immunoprecipitated FPLD fibroblast lysates with anti-emerin antibody to check if emerin-lamin binding was affected by the R482L mutation. An immunoblot of the immunoprecipitated complex showed that lamin A failed to coprecipitate with emerin in these cells (Fig. 1), while the two proteins coprecipitated in control fibroblasts as well as in EDMD2 fibroblasts cultured under the same conditions (Fig. 1). On the contrary, lamin C was still bound to emerin in FPLD fibroblasts (Fig. 1). The amount of both lamin A and C was not affected in the whole cell lysates from FPLD fibroblasts suggesting that the proteins are expressed at normal levels (Fig. 1). Moreover, the emerin-immunoblotted band was not reduced in FPLD whole cell lysates with respect to controls (Fig. 1). As expected, immunoprecipitation of lamin A/C from FPLD fibroblasts with an anti-lamin A/C monoclonal antibody allowed us to detect coprecipitation of emerin (Fig. 1), thus demonstrating that the interaction between emerin and lamin A/C is preserved through lamin C binding.

Lamin A/C distribution is dramatically affected in FPLD R482L fibroblasts

To check lamin A/C distribution in cultured FPLD fibroblasts we immunolabeled methanol or paraformaldehyde fixed cells with anti-lamin A/C (Fig. 2). Nuclear lamina staining was observed in 80% of labeled fibroblasts, while a marked reduction of labeling was observed at the nuclear rim in 15–20% of FPLD cells (338 out of 1890 nuclei), with lamin A/C being mainly distributed into nuclear aggregates (Fig. 2A). The same pattern of distribution was observed with an anti-lamin A antibody (not shown). The number of nuclear lamin A/C aggregates varied from 10 to 30 per nucleus and the aggregates appeared to be uniformly distributed throughout the nuclei (Fig. 2). Lamin A/C aggregates were not observed in control fibroblasts (Fig. 2).

We immunolabeled the same samples with anti-lamin B2 antibody and found that lamin A and lamin B2 colocalized in some, but not all the intranuclear aggregates (Fig. 2). This finding suggests that partial redistribution of lamin B in these FPLD fibroblasts does occur. In addition, alterations of the lamin A/C lattice (Fig. 2B) were observed in about 15% of fibroblasts as compared with only 4% in controls and 10% in EDMD2 fibroblasts (Fig. 3). In FPLD cells, lamin A/C was irregularly organized at one pole of the nucleus, with a sponge-like appearance, and lamin B2 was absent from the same area (Fig. 2B). This finding is in agreement with data reported by Vigouroux et al. [24]. Interestingly, in 3.5% of fibroblasts we could observe both these lamina abnormalities and the presence of lamin A/C aggregates (Fig. 2B). It is conceivable that both defects result from impaired interaction of lamin A/C with other nuclear binding partners.

Emerin is not detected at nuclear lamin A/C aggregates

Lamin A/C aggregates were not observed in control fibroblasts from either unaffected controls or EDMD2 patient carrying R401C *LMNA* mutation (Fig. 3A). We wondered if emerin was still retained at the nuclear envelope in R482L FPLD fibroblasts or if it was relocalized due to the lack of binding to lamin A as observed by immunoprecipitation (Fig. 1A) or as a consequence of the apparent mislocalization of lamin A/C. Double immunofluorescence labeling using antibodies against lamin A/C and emerin (Fig. 3) showed that emerin localization was not affected in FPLD fibroblasts compared to control fibroblasts (Fig. 3), while the intensity of emerin staining appeared slightly reduced in cells bearing the lamin A/C aggregates (Fig. 3). In EDMD2 fibroblasts lamin A/C aggregates were not found, while nuclear lamina holes and thickening of the nuclear rim were observed (Fig. 3). In FPLD cells, emerin was never detected at the lamin A/C aggregates (Fig. 3). Double labeling of emerin and lamin A/C also allowed us to define the exact localization of lamin A/C aggregates observed in FPLD fibroblasts. As also shown in Fig. 3, the aggregates are not visible in the equatorial plane of the nucleus, i.e., they are mostly localized close to the nuclear lamina.

Peripheral heterochromatin distribution is altered in FPLD nuclei

Electron microscopy of FPLD fibroblast nuclei showed an altered distribution of peripheral chromatin in a high percentage of nuclei (about 20%, 53 nuclei out of 250 observed) compared to controls (no alterations detected)

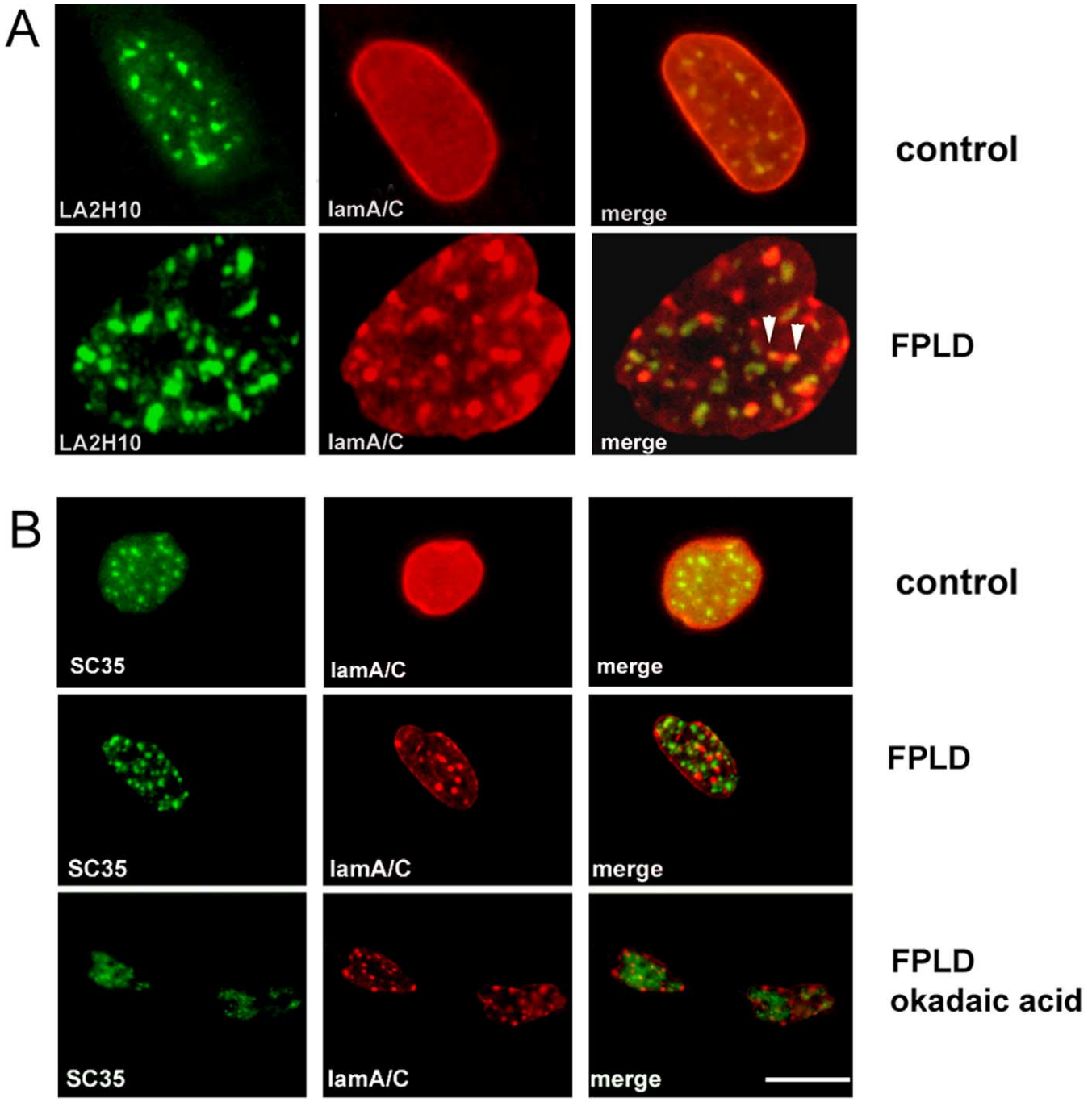


Fig. 5. Lamin A/C aggregates partially colocalize with intranuclear lamin A/C sites and splicing factor components. (A) Anti-lamin A (2H10) antibody was used to label intranuclear lamin A foci in control and FPLD fibroblasts. 2H10-labeled sites, mostly, did not colocalize with lamin A/C aggregates of R482L FPLD cells. However, in a minor percentage the aggregates colocalized with internal lamin A foci (arrowhead). (B) Antisplicing factor SC35 antibody staining allowed us to demonstrate that redistribution of splicing sites by okadaic acid treatment does not affect lamin A/C aggregates. SC35 and lamin A/C were double-labeled in control fibroblasts and in R482L FPLD fibroblasts before and after okadaic acid treatment. Bar, 2.4 μm for (A) and 10 μm for (B).

(Fig. 4). Lack of heterochromatin was observed at the nuclear periphery, close to the nuclear lamina (arrows); in these areas the nucleoplasm appeared less electron dense than normal (Figs. 4A and C); nuclear pores were generally absent in the areas corresponding to the peripheral heterochromatin loss (Fig. 4D). Detachment of chromatin from the nuclear lamina was also observed (arrowhead Fig. 4A). Neither thickening of the nuclear lamina nor nuclear envelope breaches were detected. These observations indicate that altered organization of the nuclear lamina can affect the chromatin arrangement. This is consistent with previously published data demonstrating altered chromatin organization in EDMD cells and muscle tissue bearing lamin A/C [34] or emerin [35] mutations.

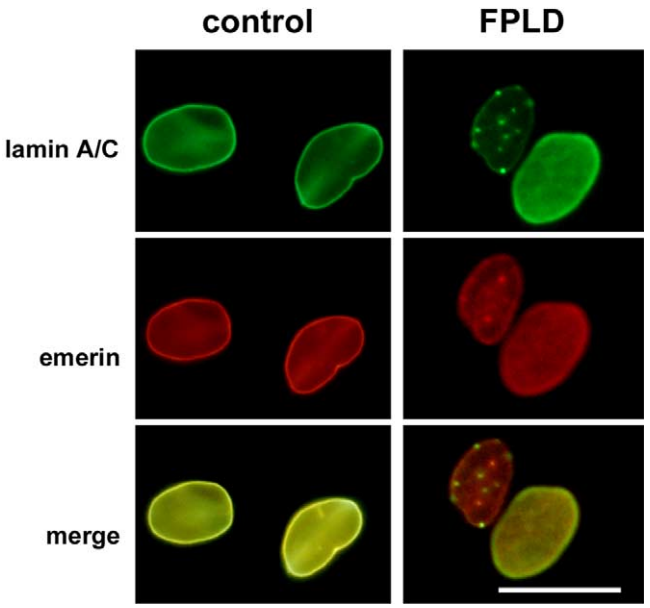


Fig. 6. Double staining of emerin and lamin A/C was performed in in situ nuclear matrix preparations. FPLD fibroblasts carrying lamin A/C aggregates show a slightly reduced emerin staining at the nuclear rim. In FPLD nuclei, lamin A/C aggregates are bound to the nuclear matrix after DNase treatment and high salt extraction. Bar, 10 μm.

Lamin A/C aggregates do not correspond to intranuclear lamin A/C sites

Intranuclear lamin A foci have been previously described in G1 cells [31] and also in interphase cells by labeling with a specific antibody [33]. We wondered if lamin A/C aggregates observed in FPLD fibroblast nuclei could correspond to intranuclear structures observed in normal cells and could be detected in FPLD cells better than in control fibroblasts due to epitope unmasking.

Immunolabeling of FPLD fibroblasts was performed with the 2H10 antibody, which selectively binds to lamin A at intranuclear sites [33]. In most nuclei, the lamin A/C aggregates detected by anti-lamin A/C antibody did not colocalize with 2H10 labeled sites (Fig. 5). It should be noted that intranuclear lamin A foci are found throughout the nucleus (except the nucleolus) whereas the abnormal aggregates are mostly localized toward the nuclear periphery. However, in a lower percentage of cells, the two fluorescence signals partially coincided (Fig. 5). This finding suggests that the abnormal distribution of lamin A/C in FPLD fibroblasts could represent an alteration of a dynamic situation, at least in part involving intranuclear lamin A foci.

To check if lamin A/C aggregates were structurally or functionally related to intranuclear lamin A/C foci known to be associated with mRNA splicing compartments, we treated FPLD fibroblasts with okadaic acid. Okadaic acid is a phosphatase inhibitor known to cause redistribution of splicing factor SC35 and of several components of the splicing factor complexes [36]. As shown in Fig. 5, SC35 was redistributed after okadaic acid incubation, but lamin A/C aggregates were not affected by the treatment. Thus, we can conclude that the nuclear aggregates do not correspond to lamin A/C-containing structures present inside the nucleus in normal cells [33], but represent abnormal structures that form close to the nuclear lamina as a consequence of R482L lamin A/C mutation.

The lamin A/C aggregates observed in FPLD nuclei were not extracted by detergent treatment and were still detectable after DNase digestion and high salt extraction (Fig. 6), thus suggesting that they were bound to the nuclear matrix.

The presence of lamin A/C aggregates is increased in G1 and G2 cells

To rule out the possibility that mutated lamin A/C could interfere with cell cycle progression, fluorescence-activated cell-sorting analysis (FACS analysis) of cultured FPLD fibroblasts was performed. A distribution of the FPLD fibroblast population in the cell cycle phases comparable to that observed in control fibroblasts was detected (not shown).

Immunofluorescence labeling with anti-lamin A/C antibody was then performed on synchronized FPLD fibroblasts, to evaluate the rate of lamin A/C aggregate formation in the different cell cycle phases. Fibroblasts synchronized in the G1 phase by serum starvation and release or in the G2 phase by demecolcine treatment showed increased lamin A/C aggregates with respect to nonsynchronous FPLD cells (Fig. 7). In addition, starved cells obtained after 7 days of serum deprivation, completely lost lamin A/C aggregates. We did not observe any lamin A/C aggregates in S phase cells (as determined by proliferating cell nuclear antigen (PCNA) and lamin A/C double staining of asynchronous

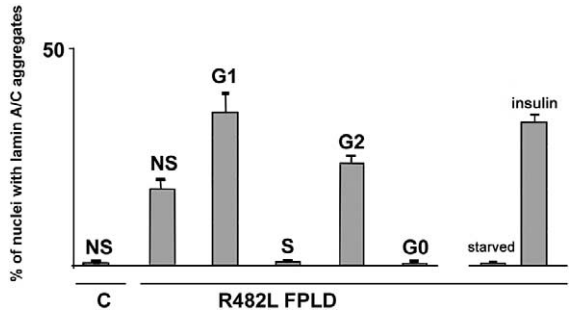


Fig. 7. Increased nuclear lamin A/C aggregates in G_1 cells. The nuclear aggregates are not detected in G0 fibroblasts and in S-phase cells. Control (C) and mutant R482L (FPLD) fibroblasts were synchronized as described in Materials and methods and double-stained with lamin A/C and PCNA to detect cells in different phases of the cell cycle. In G1 cells the percentage of nuclei with lamin aggregates was 35% (out of 1500 examined). Starved cells (starved) were obtained after 7 days of serum depletion and treated with 10 μM insulin for 4 h (insulin). In insulin-treated fibroblasts 32% of nuclei (out of 1320 examined) showed lamin aggregates. The mean of the results of three different counts performed in different samples +/− standard deviation are reported per each point. (NS, not synchronous; G1, G1 phase; S, S phase; G2, G2 phase; G0, G0 phase.)

cells, not shown). Thus, abnormal lamin A/C structures did not occur in G0 cells or, alternatively, in cells with low transcriptional activity. This finding suggests that formation of lamin A/C aggregates is a failure of correct lamin A/C assembly into a functionally active structure. This hypothesis is supported by the observation that insulin treatment of serum-depleted FPLD cells raised to 32% the percentage of nuclei bearing lamin A/C aggregates (Fig. 7).

Transcriptional activity is altered in FPLD R482L fibroblasts

To check whether abnormal lamin A/C organization could interfere with RNA polymerase II activity, we performed an in situ transcription assay that allowed us to determine the relative amount of incorporated bromouridine (BrU) into transcribed RNA. A bright punctate staining was observed in the nuclei of control fibroblasts (Fig. 8A) and in FPLD nuclei with normal lamin A/C organization at the nuclear lamina. In all FPLD fibroblasts bearing abnormal lamin A/C aggregates (15–20% of the total population) we found strongly reduced incorporation of BrU as determined by immunofluorescence (Fig. 8B). These findings demonstrate that the R482L mutation of *LMNA,* which causes FPLD, interferes with RNA polymerase II-mediated transcription. In fact, in cells showing complete absence of BrU fluorescence in the nucleoplasm, nucleolar staining was often observed (not shown), suggesting that RNA polymerase I-mediated transcription is not affected. It should be noted that DAPI staining of nuclei (Fig. 8A) allowed us to rule out the possibility that BrU-negative nuclei were apoptotic: in fact, neither hypercondensed chromatin nor micronuclei were found in fibroblasts showing reduced transcriptional activity.

Discussion

The overall evaluation of our results shows that:(1) mutation of lamin A/C at R482 affects interaction of the nuclear lamina protein with both nuclear envelope (emerin) and nuclear lamina (lamin B2) components; (2) in R482L FPLD fibroblasts, lamin A/C is redistributed in a significant percentage of nuclei and an altered nuclear lamina assembly is observed mostly in G1 and G2 cells, but not in G0 fibroblasts; (3) in FPLD fibroblast nuclei, altered heterochromatin distribution close to the nuclear lamina occurs and heterochromatic areas are frequently lacking; (4) the lamin A/C aggregates observed in FPLD fibroblasts are not bound to DNA or splicing factor compartments, but a low percentage of aggregates colocalize with intranuclear lamin A sites; (5) a reduced RNA transcription rate is observed in FPLD nuclei carrying lamin A/C aggregates, as determined by in situ transcription assay.

R482L lamin A/C mutation causes disruption of lamin organization

In FPLD fibroblast nuclei carrying an R482L mutation of lamin A/C, we observed the formation of lamin A/C aggregates localized close to the nuclear lamina. This abnormal distribution of lamin A/C has never been observed before in FPLD nuclei and can be strictly related to the amino acid substitution present in these cells. An altered organization of lamin A/C, similar to the one observed in this study, has been described by Lloyd et al.[28] in HeLa cells transiently transfected with an E203G lamin A/C mutation, which is associated with dilated cardiomyopathy (CMD1A). These authors suggest that the E203G *LMNA* mutation can affect the self-assembly of the molecule, thus causing formation of intranuclear aggregates [28]. In R482L FPLD fibroblasts, it is more probable that the amino acid substitution may affect an interaction with other nuclear partner(s) [28,37] necessary for proper nuclear lamina organization. The nuclear lamin A/C aggregates present in R482L FPLD fibroblasts represent anomalous lamin A/C structures not anchored to nuclear components involved in either DNA replication or RNA splicing. Thus, it appears likely that the lamin A/C aggregates observed in FPLD R482L fibroblasts are formed as a consequence of lacking functional interaction usually occurring in normal fibroblasts. The variable impact on the lamina structure of R482L lamin A/C mutation can be explained by hypothesizing that a functional interaction occurring in a percentage of an asynchronous cell population may be impaired in FPLD fibroblasts. This hypothesis is supported by the fact that accumulation of cells in G1, as well as insulin treatment, increase the rate of altered nuclei. The latter is an intriguing result, suggesting that some post-translational modification of lamin A/C, such as phosphorylation, may occur in the nuclei with altered lamin A/C distribution. On the other hand, insulin signaling plays a major role in adipocyte differentiation through the SREBP1-PPARγ pathway [38]: thus, lamin A/C distribution in response to insulin stimulus should be investigated in an adipocyte cellular model, and intermolecular interactions between lamin A/C and its possible partner protein SREBP1 [28] or chromatin [39] should be evaluated.

R482L mutated lamin A fails to interact with emerin

Moreover, we demonstrate that lamin A fails to bind emerin in FPLD R482L fibroblasts, while lamin C, whose interaction with emerin is not disrupted, is sufficient to retain the nuclear envelope protein at its proper localization. The finding that lamin A and emerin do not coprecipitate in R482L FPLD fibroblasts was an unexpected finding. Other authors reported that the interaction between emerin and lamin is not affected in FPLD fibroblasts carrying different mutations [24,40] or in in vitro pull-down assays performed with lamin A/C R482W mutant [41]. We suggest that a different amino acid substitution (R482L instead of

R482Q/W) [24,37,41] elicits different effects on the intermolecular interactions involving the mutated C-terminal domain. In this respect, it is noteworthy that an Ig-like domain has been recently identified in the carboxy-terminal globular domain of lamin A, which has been suggested to be involved in intermolecular interactions [37,42]. Moreover, loss of a basic side chain at the carboxy-terminus of lamin A/C has been demonstrated in FPLD cells [37]. Thus, we hypothesize that the presence of a nonpolar amino acid such as leucine within this domain impairs lamin A–emerin binding. Another explanation for the failure of emerin to bind lamin A could be the absence of an interaction with a third molecule necessary to stabilize the emerin-lamin A complex. In fact, other interactions with lamin A/C could also be altered in nuclei carrying the R482L mutation and thus elicit a pathogenic effect. As far as the site of interaction between LAP2α and lamin A/C is within the mutated C-terminal domain of lamin A/C [29], the interaction with LAP2α is possibly also interrupted in R482L FPLD. Reduced in vitro binding of lamin A/C mutants found in FPLD to the sterol response element binding protein 1 (SREBP1) has been recently demonstrated [28]. The evidence of impaired emerin–lamin A binding as reported in this study represents the first evidence of altered in vivo protein–protein interaction in FPLD cells. This supports the hypothesis of a major role of abnormal lamin A interference with its partner protein(s) in the pathogenesis of FPLD [37].

Chromatin disorganization is a common feature of laminopathies

We previously described altered lamin distribution in EDMD1 fibroblasts [35] and altered chromatin organization in both EDMD1[35] and EDMD2 fibroblasts [34]. A markedly altered heterochromatin arrangement and areas devoid of heterochromatin have been observed also in FPLD fibroblasts (Fig. 4). This observation was not unexpected, since not only lamin A/C is mutated in FPLD, but also emerin-lamin A binding is impaired in R482L FPLD cells: thus, a nuclear envelope defect is again found to be associated with altered chromatin organization. This finding suggests that a common mechanism leading to chromatin disorganization may underlie the pathology caused by nuclear envelope–nuclear lamina defects [43]. This hypothesis is strongly supported by a recent report by Stierlé et al. [39] demonstrating that the lamin A/C sequence around the amino acid R482 contains a DNA-binding domain that allows a stable DNA interaction in cooperation with the NLS-containing peptide [39]. Interestingly, these authors further show that no DNA sequence specificity is required for lamin A/C binding, again suggesting that lamin A/C could be involved in a more general mechanism regulating chromatin organization.

Blebbed areas lacking lamin B were recently described in FPLD fibroblasts carrying different amino acid substitutions by Vigouroux et al. [24]. Also, these authors showed that transfection of normal fibroblasts with a vector encoding an R482W mutated lamin A/C causes in a percentage of nuclei an altered lamin A/C organization with holes and lack of lamin B in the same areas [24]. This finding is consistent with our results obtained with R482L FPLD cells.

Mutated R482L lamin A/C can affect RNA transcription

Further evidence to the hypothesis that nuclear lamin A/C aggregates are formed due to the lack of a normal functional interaction is given by the finding that the nuclear aggregates are not found in serum-starved cells, while their frequency increases in G1 and G2 fibroblasts. Thus, the aggregates must correspond to anomalous lamin A/C structures forming in actively transcribing cells. It is noteworthy that lamin A/C aggregates were not observed in S-phase cells, so that we can rule out that they represent a failure of lamin A/C to correctly assemble into structures involved in DNA replication. On the basis of these results, we further evaluated the transcriptional activity of R482L FPLD cells. Involvement of nuclear envelope proteins in the regulation of gene expression has been demonstrated for LAP2 beta and its associated germ-cell-less protein [44]. Moreover, a report by Spann et al. [26] shows that a mutated lamin A impairs RNA polymerase II-mediated transcription in transfected cells. Our results agree with those of Spann et al. [26], as far as we demonstrated a reduced BrU incorporation in R482L-mutated nuclei bearing lamin A/C aggregates. This finding provides the first evidence that RNA transcription is affected in a laminopathy, concomitantly with failure of lamin A/C to correctly assemble at the nuclear lamina. It is noteworthy that the presence of lamin A/C aggregates in FPLD nuclei appears to be dependent on the functional status of the cell (cells accumulated in G1 showed aggregates to a higher percentage). Thus, virtually all FPLD fibroblasts could undergo an alteration of lamin A/C arrangement, and as a consequence an altered RNA transcription rate, under a determined functional condition. Lamin A/C has been shown to interact with SREBP1 [28] as well as with MOK2 transcription factor and to affect the nuclear localization of the latter [45]. Concerning the diseases associated with nuclear envelope defects, a recent expression profiling in EDMD1 cells using cDNA microarray analysis showed altered expression of several genes, including *LMNA* [46]. Thus, altered gene expression in laminopathies, as previously speculated by several authors [16,43,47], is now gaining experimental evidence. Nevertheless, more than one mechanism is likely to be involved in the pathogenesis of laminopathies [48], and proved and potential nuclear binding partners of lamins may be linked to the pathogenetic mechanism. In this respect, it is worthy to note that a transactivation-deficient mutant of *PPARG* encoding peroxisome proliferator activated receptor (PPAR)-γ has been recently identified in a FPLD not linked to *LMNA* mutation [49]. The analysis of the in vivo interaction be-

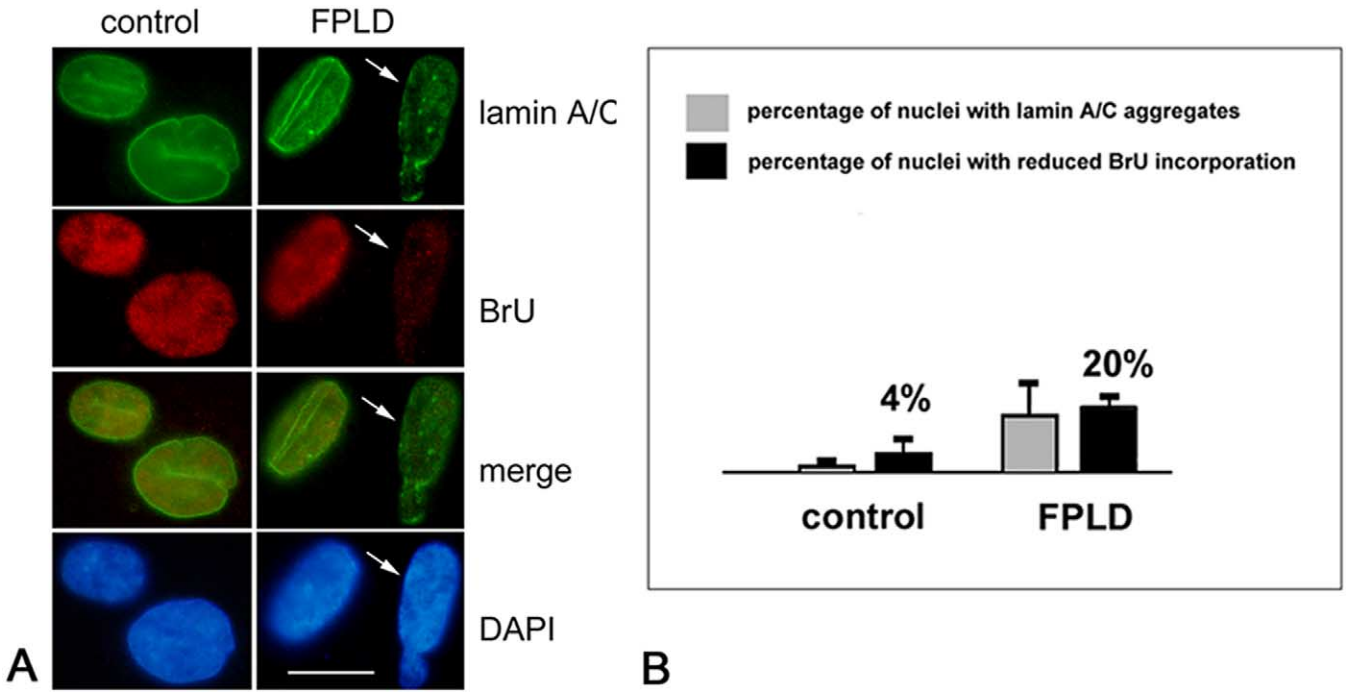


Fig. 8. (A) Reduced BrU incorporation in FPLD nuclei with abnormal lamin A/C distribution. BrU incorporation in control nuclei was detected as bright fluorescence staining (CY3 labeling, red). The marked FPLD nucleus (arrows) bearing lamin A/C aggregates shows reduced BrU staining. Fluorescence staining of incorporated BrU is not affected in the FPLD nucleus with normal lamin A/C at the nuclear lamina. DAPI staining of chromatin is also shown. Bar, 5 μm. (B) This figure is representative of the results obtained in three different experiments. A total of 450 nuclei were counted and the mean values for each cell population are reported as percentage of observed nuclei. To perform a quantitative analysis of reduced transcriptional activity, in situ transcription assay was performed for a shorter period (2 min), in order to obtain a BrU fluorescence signal of altered FPLD nuclei under the detection level. Under these experimental conditions, only negative nuclei were considered as showing reduced BrU incorporation.

tween lamin A/C and PPAR-γ or SREBP1 [28] could give an insight into the altered mechanism leading to FPLD.

Acknowledgments

The study was supported by an EU grant MYO-Cluster QLG1-1999-00870, by Italian Ministero della Salute, P.F. No. 83/2001 and FIRB Project No. RBNE01JJ45. M.W. was supported by a grant of the Deutsche Forschungsgemeinschaft We 1470/12-3. V.K.P. was supported by the Council for Scientific and Industrial Research, India. The authors are indebted to Dr. K. Mohnike for referring FPLD patient G-9956 to L. Cattini for the FACS analysis and to I. Mura for the technical support.

References

[1] S. Shackleton, D.J. Lloyd, S.N.J. Jackson, R. Evans, M.F. Niermeijer, B.M. Singh, H. Schmidt, G. Brabant, S. Kumar, P.N. Durrington, S. Gregory, S. O'Rahilly, R.C. Trembath, *LMNA*, encoding lamin A/C, is mutated in partial lipodystrophy, Nature Genet. 24 (2000) 153–156.

[2] R.A. Hegele, Molecular basis of partial lipodystrophy and prospects for therapy, Trends Mol. Med. 7 (2001) 121–126.

[3] G. Bonne, M. Raffaele di Barletta, S. Varnous, H.M. Becane, E.H. Hammouda, L. Merlini, F. Muntoni, C.R. Greenberg, F. Gary, J.A. Urtizberea, D. Duboc, M. Fardeau, D. Toniolo, K. Schwartz, Mutations in the gene encoding lamin A/C cause autosomal dominant Emery-Dreifuss muscular dystrophy, Nature Genet. 21 (1999) 285–288.

[4] D. Fatkin, C. MacRae, T. Sasaki, M.R. Wolff, M. Porcu, M. Frenneaux, J. Atherton, H.J. Vidaillet Jr, S. Spudich, U. De Girolami, J.G. Seidman, C. Seidman, F. Muntoni, G. Muehle, W. Johnson, B. McDonough, Missense mutations in the rod domain of the lamin A/C gene as causes of dilated cardiomyopathy and conduction-system disease, N. Engl. J. Med. 341 (1999) 1715–1724.

[5] A. Muchir, G. Bonne, A.J. van der Kooi, M. van Meegen, F. Baas, P.A. Bolhuis, M. deVisser, K. Schwartz, Identification of mutations in the gene encoding lamins A/C in autosomal dominant limb girdle muscular dystrophy with atrioventricular conduction disturbances (LGMD1B), Hum. Mol. Genet. 9 (2000) 1453–1459.

[6] M. Raffaele di Barletta, E. Ricci, G. Galluzzi, P. Tonali, M. Mora, L. Morandi, A. Romorini, T. Voit, K. Helene Orstavik, L. Merlini, C. Trevisan, V. Biancalana, I. Housmanowa-Petrusewicz, S. Bione, R. Ricotti, K. Schwartz, G. Bonne, D. Toniolo, Different mutations in the LMNA gene cause autosomal dominant and autosomal recessive Emery-Dreifuss muscular dystrophy, Am. J. Hum. Genet. 66 (2000) 1407–1412.

[7] G. Novelli, A. Muchir, F. Sangiuolo, A. Helbling-Leclerc, M.R. D'Apice, C. Massart, F. Capon, P. Sbraccia, M. Federici, R. Lauro, C. Tudisco, R. Pallotta, G. Scarano, B. Dallapiccola, L. Merlini, G. Bonne, Mandibuloacral dysplasia is caused by a mutation in LMNA-encoding lamin A/C, Am. J. Hum. Genet. 71 (2002) 426–431.

[8] A. De Sandre-Giovannoli, M. Chaouch, S. Kozlov, J.M. Vallat, M. Tazir, N. Kassouri, P. Szepetowski, T. Hammadouche, A. Vandenberghe, C.L. Stewart, D. Grid, N. Levy, Homozygous defects in LMNA, encoding lamin A/C nuclear-envelope proteins, cause autosomal recessive axonal neuropathy in human (Charcot-Marie-Tooth disorder type 2) and mouse, Am. J. Hum. Genet. 70 (2002) 726–736.

[9] Eriksson, M., Brown, W.T., Gordon, L.B., Glynn, M.W., Singer, J., Scott, L., Erdos, M.R., Robbins, C.M., Moses, T.Y., Berglund, P., Dutra, A., Pak, E., Durkin, S., Csoka, A.B., Boehnke, M., Glover, T.W., Collins, F.S., Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. Nature 423 (2003) 293–298.

[10] De Sandre-Giovannoli, A., Bernard, R., Cau, P., Navarro, C., Amiel, J., Boccaccio, I., Lyonnet, S., Stewart, C.L., Munnich, A., Le Merrer, M. Levy, N. Lamin A truncation in Hutchinson-Gilford progeria. Science 300 (2003) 2055.

[11] Cao, H. Hegele, R.A. LMNA is mutated in Hutchinson-Gilford progeria (MIM 176670) but not in Wiedemann-Rautenstrauch progeroid syndrome (MIM 264090). J. Hum. Genet. 48 (2003) 271–274.

[12] D. Lourim, J.J. Lin, Expression of nuclear lamin A and muscle-specific proteins in differentiating muscle cells in ovo and in vitro, J Cell Biol. 109 (1989) 495–504.

[13] J.L. Broers, B.M. Machiels, G.J. van Eys, H.J. Kuijpers, E.M. Manders, R. van Driel, F.C. Ramaekers, Dynamics of the nuclear lamina as monitored by GFP-tagged A-type lamins, J. Cell Sci. 112 (1999) 3463–3475.

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

[15] A.L. Olins, H. Herrmann, P. Lichter, M. Kratzmeier, D. Doenecke, D.E. Olins, Nuclear envelope and chromatin compositional differences comparing undifferentiated and retinoic acid- and phorbol ester-treated HL-60 cells, Exp. Cell Res. 268 (2001) 115–127.

[16] B. Burke, C.L. Stewart, Life at the edge: the nuclear envelope and human disease, Nature Rev. Mol. Cell Biol. 3 (2002) 575–585.

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

[18] D.A. Cutler, T. Sullivan, B. Marcus-Samuels, C.L. Stewart, M.L. Reitman, Characterization of adiposity and metabolism in *Lmna*-deficient mice, Biochem. Biophys. Res. Commun. 291 (2002) 522–527.

[19] E.A. Fairley, J. Kendrick-Jones, J.A. Ellis, The Emery-Dreifuss muscular dystrophy phenotype arises from aberrant targeting and binding of emerin at the inner nuclear membrane, J. Cell Sci. 112 (1999) 2571–2582.

[20] L. Clements, S. Manilal, D.R. Love, G.E. Morris, Direct interaction between emerin and lamin A, Biochem. Biophys. Res. Commun. 267 (2000) 709–714.

[21] M. Sakaki, H. Koike, N. Takahashi, N. Sasagawa, S. Tomioka, K. Arahata, S. Ishiura, Interaction between emerin and nuclear lamins, J. Biochem. 129 (2001) 321–327.

[22] K.K. Lee, T. Haraguchi, R.S. Lee, T. Koujin, Y. Hiraoka, K.L. Wilson, Distinct functional domains in emerin bind lamin A and DNA-bridging protein BAF, J. Cell Sci. 114 (2001) 4567–4573.

[23] A. Vaughan, M. Alvarez-Reyes, J.M. Bridger, J.L.V. Broers, F.C.S. Ramaekers, M. Wehnert, G.E. Morris, W.G.F. Whitfield, C.J. Hutchison, Both emerin and lamin C depend on lamin A for localization at the nuclear envelope, J. Cell Sci. 114 (2001) 2577–2590.

[24] C. Vigouroux, M. Auclair, E. Dubosclard, M. Pouchelet, J. Capeau, J.C Courvalin, B. Buendia, Nuclear envelope disorganization in fibroblasts from lipodystrophic patients with heterozygous R482Q/W mutations in the lamin A/C gene, J. Cell Sci. 114 (2001) 4459–4468.

[25] Y. Gruenbaum, K.L. Wilson, A. Harel, M. Goldberg, M. Cohen, Nuclear lamins—structural proteins with fundamental functions, J. Struct. Biol. 129 (2000) 313–323.

[26] T.S. Spann, A.E. Goldman, C. Wang, S. Huang, R.D. Goldman, Alteration of nuclear lamin organization inhibits RNA polymerase II-dependent transcription, J. Cell Biol. 156 (2002) 603–608.

[27] R.I. Kumaran, B. Muralikrishna, V.K. Parnaik, Lamin A/C speckles mediate spatial organization of splicing factor compartments and RNA polymerase II transcription, J. Cell Biol. 159 (2002) 783–793.

[28] D.J. Lloyd, R.C. Trembath, S. Shackleton, A novel interaction between lamin A and SREBP1: implications for partial lipodystrophy and other laminopathies, Hum. Mol. Genet. 11 (2002) 769–777.

[29] T. Dechat, B. Korbei, O.A. Vaughan, S. Vlcek, C.J. Hutchison, R. Foisner, Lamina-associated polypeptide 2alpha binds intranuclear A-type lamins, J. Cell Sci. 113 (2000) 3473–3484.

[30] K. Bechert, M. Lagos-Quintana, J. Harborth, K. Weber, M. Osborn, Effects of expressing lamin A mutant protein causing Emery-Dreifuss muscular dystrophy and familial partial lipodystrophy in Hela cells, Exp. Cell Res. 286 (2003) 75–86.

[31] J.M. Bridger, I.R. Kill, M. O'Farrell, C.J. Hutchison, Internal lamin structures within G1 nuclei of human dermal fibroblasts, J. Cell Sci. 104 (1993) 297–306.

[32] N. Adachi, M. Kobayashi, H. Koyama, Cell cycle-dependent regulation of the mouse DNA topoisomerase IIalpha gene promoter, Biochem. Biophys. Res. Commun. 230 (1997) 105–109.

[33] G. Jagatheesan, S. Thanumalayan, B. Muralikrishna, N. Rangaraj, A.A. Karande, V.K. Parnaik, Colocalization of intranuclear lamin foci with RNA splicing factors, J. Cell Sci. 112 (1999) 4651–4661.

[34] P. Sabatelli, G. Lattanzi, A. Ognibene, M. Columbaro, C. Capanni, L. Merlini, N.M. Maraldi, S. Squarzoni, Nuclear alterations in autosomal-dominant Emery-Dreifuss muscular dystrophy, Muscle Nerve. 24 (2001) 826–829.

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

[36] T. Misteli, J.F. Caceres, D.L. Spector, The dynamics of a pre-mRNA splicing f factor in living cells, Nature 387 (1997) 523–527.

[37] S. Dhe-Paganon, E.D. Werner, Y.I. Chi, S.E. Shoelson, Structure of the globular tail of nuclear lamin, J. Biol. Chem. 277 (2002) 17381–17384.

[38] M. Caron, M. Auclair, C. Vigouroux, M. Glorian, C. Forest, J. Capeau, The HIV protease inhibitor indinavir impairs sterol regulatory element-binding protein-1 intranuclear localization, inhibits preadipocyte differentiation, and induces insulin resistance, Diabetes 50 (2001) 1378–1388.

[39] V.V. Stierlé, J. Couprie, C. Ostlund, I. Krimm, S. Zinn-Justin, P. Hossenlopp, H.J. Worman, J.C. Courvalin, I. Duband-Goulet, The carboxyl-terminal region common to lamins A and C contains a DNA binding domain, Biochemistry 42 (2003) 4819–4828.

[40] I. Holt, L. Clements, S. Manilal, S.C. Brown, G.E. Morris, The R482Q lamin A/C mutation that causes lipodystrophy does not prevent nuclear targeting of lamin A in adipocytes or its interaction with emerin, Eur. J. Hum. Genet. 9 (2001) 204–208.

[41] W., Raharjo, R., Enarson, P., Sullivan, T., Stewart, C.L., Burke, B. Nuclear envelope defects associated with LMNA mutations cause dilated cardiomyopathy and Emery-Dreifuss muscular dystrophy. J. Cell Sci. 1142001 4447–4457.

[42] I. Krimm, C. Ostlund, B. Gilquin, J. Couprie, P. Hossenlopp, J.P. Mornon, G. Bonne, J.C. Courvalin, H.J. Worman, S. Zinn-Justin, The Ig-like structure of the C-terminal domain of lamin A/C, mutated in muscular dystrophies, cardiomyopathy, and partial lipodystrophy, Structure (Cambr.). 10 (2002) 811–823.

[43] N.M. Maraldi, G. Lattanzi, P. Sabatelli, A. Ognibene, S.Squarzoni1, Functional domains of the nucleus: implications for Emery-Dreifuss muscular dystrophy, Neuromusc. Disorders 12 (2002) 815–823.
[44] E. Nili, G.S. Cojocaru, Y. Kalma, D. Ginsberg, N.G. Copeland, D.J. Gilbert, N.A. Jenkins, R. Berger, S. Shaklai, N. Amariglio, F. Brok-Simoni, A.J. Simon, G. Rechavi, Nuclear membrane protein LAP2β mediates transcriptional repression alone and together with its binding partner GCL (germ-cell-less), J. Cell Sci. 114 (2001) 3297–3307.
[45] C. Dreuillet, J. Tillit, M. Kress, M. Ernoult-Lange, In vivo and in vitro interaction between human transcription factor MOK2 and nuclear lamin A/C, Nucleic Acids Res. 30 (2002) 4634–4642.
[46] T. Tsukahara, S. Tsujino, K. Arahata, cDNA microarray analysis of gene expression in fibroblasts of patients with X-linked Emery-Dreifuss muscular dystrophy, Muscle Nerve 25 (2002) 898–901.
[47] K.L. Wilson, The nuclear envelope, muscular dystrophy and gene expression, Trends Cell Biol. 10 (2000) 125–129.
[48] C.J. Hutchison, Lamins: building blocks or regulators of gene expression?, Nature Rev. Mol. Cell Biol. 3 (2002) 848–858.
[49] R.A. Hegele, H. Cao, C. Frankowski, S.T. Mathews, T. Leff, PPARG F388L, a transactivation-deficient mutant, in familial partial lipodystrophy, Diabetes 51 (2002) 3586–3590.