Nuclear envelope influences on genome organization

Nuclear Envelope Influences On Genome Organization

Poonam Malik, Nikolaj Zuleger, and Eric C. Schirmer[1]

Wellcome Trust Centre for Cell Biology, University of Edinburgh, Edinburgh, EH9 3JR, UK

[1]To whom correspondence should be addressed:

Eric Schirmer (e.schirmer@ed.ac.uk)

Wellcome Trust Centre for Cell Biology

University of Edinburgh, Kings Buildings

Michael Swann Building, Room 5.22

Edinburgh, EH9 3JR, UK

Tel.: +441316507075

fax: +441316507360

Key words: Nuclear envelope (NE), nuclear periphery, inner nuclear membrane (INM), chromatin organization, chromosome positioning, gene regulation.

Abbreviations used: nuclear envelope (NE), nuclear envelope transmembrane protein (NET), inner nuclear membrane (INM), lamin B-receptor (LBR), lamina-associated polypeptide 2 (LAP2).

Abstract

The nuclear periphery is a specialized environment in the nucleus that contributes to genome organization and correspondingly to gene regulation. Mammalian chromosomes and certain genes occupy defined positions within the nucleus that are heritable and tissue-specific. Genes located at the nuclear periphery tend to be inactive and this negative regulation can be reversed when they are released from the periphery in certain differentiation systems. Recent work using specially designed systems has shown that genes can be artificially tethered to the nuclear periphery by an affinity mechanism. The next important step will be to identify the endogenous nuclear envelope and chromatin proteins that participate in affinity-driven nuclear envelope tethering and determine how they are regulated.

Introduction

Chromosomes in mammalian cells tend to occupy defined positions within the 3D-framework of the nucleus. The Bickmore laboratory first demonstrated this by showing that in fibroblasts chromosome 18 tends to be located at the nuclear periphery while chromosome 19 tends to be internal [1]. Subsequently the Misteli laboratory found that the chromosomes involved in tissue-specific tumor translocations were positioned adjacent to one another during interphase in those particular tissues [2]; thus different cell types favor certain chromosome groupings. The Bridger laboratory has further shown that chromosome positioning with respect to the nuclear periphery is altered in aging cells [3]. Peripheral localization of DNA is generally thought to correlate with silencing because i) gene poor chromosomes tend to be at the periphery [1], ii) most dense chromatin tends to be at the periphery as assessed by electron microscopy, and iii) much late-replicating DNA is at the periphery [4].

Only a portion of a chromosome located at the periphery actually interacts with the nuclear envelope (NE), the double membrane system that defines the nuclear compartment. In lymphocytes, gene poor regions and inactive genes of chromosome 7 are proximal to the NE compared to active genes that are more internal [5]. Additionally, internal chromosomes can extend loops outward that reach to the NE [6]. Thus chromosomes adopt a wide range of conformations and interactions. The logical purpose of combining chromosome-positioning patterns with such chromosome plasticity would be that it plays a role in gene regulation.

Specific Gene Regulation from the Nuclear Periphery

Several individual genes move to and from the nuclear periphery correlating with their activation state. The immunoglobulin heavy chain *IgH* locus remains inactive at the NE in early lymphocyte lineages but moves to the nuclear interior concomitant with the initiation of V(D)J recombination [7]. Similarly the *Mash1* (*Ascl1*) gene moves away from the NE when it needs to be activated for neural development [8]. *Mash1* contains heterochromatic epigenetic marks and replicates late when at the periphery and these switch to active chromatin marks and early replication when the locus is in the interior, but it is unclear whether the change in chromatin marks drives the movement or vice-versa. The cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, however, could be induced to move away from the NE upon treatment with trichostatin A, which promotes histone hyperacetylation [9]. Although this suggests that alterations in epigenetic marks can drive the movement of genes to and from the NE, *CFTR* is internal and active in some cell types and treatment of these cells with a transcriptional repressor that inhibits RNA polymerase promoted movement of *CFTR* to the periphery [9]. Thus the question of whether transcription factors or local epigenetic marks dominate in gene positioning remains unresolved. In either case, to position a gene at the NE these transcription factors or epigenetic marks must interact with proteins of the NE.

The protein complement of the NE includes an intermediate filament lamin polymer that directly underlies the membrane [10] and hundreds of transmembrane proteins, many of which are unique to the inner nuclear membrane (INM) [11]. Many different aspects of genome regulation have been linked to the NE: lamins and several INM proteins can influence replication [12,13], transcription [14,15], and signaling cascades [16,17]. Specific interactions have been shown between several INM proteins and both negative and positive transcriptional regulators [15,18,19]. Thus,

though the predominant effects of NE tethering discussed above were in gene silencing, NE localization can also direct gene activation: the *PLP*, *ERBB-2*, *COL1A1* and *IFN-γ* genes all become activated at the nuclear periphery when respectively glial, breast, bone and immune cells differentiate [20-23].

An Affinity Mechanism for NE Tethering of Genes

Three elegant recent studies used different artificial systems to inducibly tether genes to the NE [24-26]. In all systems, bacterial lac operator (lacO) sequences were inserted into the mammalian genome in different loci that were not typically close to the NE. The lac repressor (lacI) binds lacO sequences with high specificity and affinity; so these cells were transfected with lacI fused to a reporter alone or fused to the reporter plus the NE proteins lamin B1 [25], LAP2ß [24], or emerin [26]. Lamin B1 is part of the intermediate filament polymer under the NE while LAP2ß and emerin are NE transmembrane proteins predominantly at the INM. Expression of the simple lacI-reporter fusion had no effect on the position of the lacO locus within the 3-dimensional organization of the nucleus; however when lacI was also fused to the NE proteins, the locus moved to the nuclear periphery [24-26] (Figure 1).

Movement to the periphery was not observed during interphase: instead repositioning of the locus required the cells to go through mitosis (Figure 1). While chromosomes undergo moderate shape changes during interphase, they generally do not move significantly in bulk. In contrast, chromosomes undergo dramatic movements in mitosis. At the end of mitosis, many INM proteins bind to mitotic chromosomes and this helps drive NE reassembly as these proteins are embedded in mitotic vesicles [27,28]. Thus, before chromosome decondensation, affinity binding of the lacO array with lacI-NE proteins would bring the lacO-integrated chromosome

in contact with the reforming nuclear membrane. Indeed, though the lacO array is only a small part of the chromosome, tethering just this region to the NE was able to pull the entire chromosome to the periphery [24]. This is a very important observation as it shows that minimal directed high affinity interactions have the potential to drastically change the nuclear landscape. It is also noteworthy that with the advent of the high affinity interaction, other presumably weaker interactions were lost as chromosome 4 moved away from the periphery when chromosome 11 containing lacO repeats moved to the periphery [24]. Thus genes/ chromosomes compete for place based on the strength of affinity interactions. The lacO-lacI binding is disrupted by IPTG (isopropyl ß-D-1-thiogalactopyranoside), allowing the heritability of lacO repositioning to be assessed. Apparently the affinity interaction must be maintained because treatment of cells with IPTG resulted in loss of peripheral localization.

Transcription from a marker gene inserted in the lacO array was reduced when the array was at the periphery [24,26]. Some adjacent genes were also inhibited while others were unaffected, indicating that additional factors direct the mechanism of inhibition [24]. One such factor is the ability of the INM proteins that were fused to lacI to recruit specific transcriptional repressors germ cell-less, Btf and Lmo7, that each has target gene specificity and cell type specificity [15,18,19]. Thus gene repression may be an indirect effect from groupings of transcriptional repressors at the periphery. Disrupting peripheral tethering with IPTG restored the lost transcriptional activity [24,26].

Identification of Endogenous Proteins Involved in Affinity Tethering of Chromatin

An affinity mechanism likely also functions in the tethering of endogenous genes and chromosomes to the periphery. Knockdown of lamin B1 results in release of chromosome 18 from the periphery in human cells [29]. Though disruption of lamins may yield pleiotropic effects, this result makes some sense because the volume of a whole chromosome would predispose that its anchor was stable (e.g. an intermediate filament polymer), a predominant protein (~3,000,000 copies of lamins per average mammalian nucleus; [30]), and that the chromosome partner was abundant (lamins bind histones H2A/H2B [31]). Nonetheless, something must be missing from this model because lamin B1 is distributed uniformly throughout the nuclear periphery in nearly all cell types and histones H2A/H2B are distributed throughout all chromosomes. Thus this fails to explain why specifically chromosome 18 is at the periphery while 19 tends to the interior. The postulate of a missing partner is supported by observations using a set of three different antibodies to different regions of lamin B1. In various tissues different combinations of two antibodies recognized lamin B1, but the epitope recognized by the third antibody was masked. Thus, distinct regions of lamin B1 were bound to different partners in the various tissues [32]. This suggests that either chromosome 18 has histone modifications that increase its affinity for lamin B1 in the cell types where it is at the periphery or other as yet unidentified proteins are involved. The only other NE proteins shown to tether chromatin are the SUN proteins that recruit telomeres [33,34].

An affinity mechanism directing the tethering of specific chromatin to the NE in certain cell types would predispose that a unique combination of a NE protein and a specific chromatin protein occur in that cell type. For example, a type of chromatin such as epigenetically modified heterochromatin might have a higher affinity for a NE protein than euchromatin and this NE protein would be expressed highest in cell types

that have heterochromatin at the periphery. Individual genes associated with a particular differentiation pathway could also have specific affinity for the NE. For example the Msx1 protein interacts with the histone H1 variant H1b, which is in several muscle-specific genes and this interaction keeps these genes repressed until the commencement of myogenesis [35]. If either Msx1 or the H1b histone variant or the complex they form has significantly higher affinity for a specific NE protein, which is expressed differentially, this could direct specific tethering of that gene to the periphery.

The fundamentals of this hypothesis are supported by several examples of chromatin interactions with NE proteins. In addition to the lamin interaction with core histones mentioned above, several INM proteins have been found to interact with specific chromatin proteins and/ or post-translational modifications on chromatin proteins. The lamin B receptor (LBR) interacts specifically with heterochromatin protein 1 (HP1) α and γ [36] and histones H3/H4 [37]. Moreover LBR was found to pull down chromatin with a strong preference for silencing modifications [38]. Conversely, HP1 appears to have a higher affinity for NE proteins compared to other parts of the nucleus because microinjected HP1α accumulated at the periphery before eventually being distributed to other nuclear locations [39]. Barrier-to-autointegration factor (BAF) is a protein that modifies the condensed state of chromatin by crosslinking DNA/histones [40]. BAF binds the mammalian INM proteins LAP2ß, emerin and MAN1 [41-43].

Thus far, no endogenous NE proteins or chromatin proteins have been found that are involved in tethering of individual genes to the periphery. To identify additional NE proteins involved in tethering chromatin to the NE we have engaged a visual screen with lacO insertions and lacI reporters, but without fusing the reporter to

a NE protein. Instead roughly 40 INM proteins identified in a proteomic study of the NE [44] were overexpressed in cells that carried lacO insertions in different areas of the genome and the position of the locus was assayed for proximity to the NE. Several proteins were identified that when upregulated in the NE resulted in preferential accumulation of the marked chromatin region at the NE (NZ, W. Bickmore and ECS, unpublished observations). Future studies will focus on determining if these proteins have higher affinities for specific genes and/ or chromatin proteins.

Summary

Twelve different NE proteins have now been linked to human disease including lamins, INM proteins and associated soluble proteins [45,46]. NE diseases affect many different tissues including muscle, brain, fat cells, heart, skin, bone, immune cells, and also include the aging disease progeria. A favored hypothesis for how NE proteins can cause disease is alteration of gene expression due to physical disruption of regulatory contacts at the NE. This hypothesis is supported by observations that the myoD and Rb pathways are misregulated in NE-related muscular dystrophies [47,48], and that cells from patients with different NE diseases have altered distribution of dense peripheral chromatin [49-51]. Thus, determining the proteins involved in affinity tethering of specific chromatin to the NE has significant implications for the understanding of human disease.

Much work also still needs to be done to clarify whether the nuclear envelope silences genes (i) by sterically reducing accessibility to factors, (ii) by inactivating transcriptional regulators through sequestration, (iii) by bringing genes into an already silenced environment rich with enzymes that propagate inactive chromatin, or (iv) by recruiting already silenced chromatin. To fully understand genome regulation and

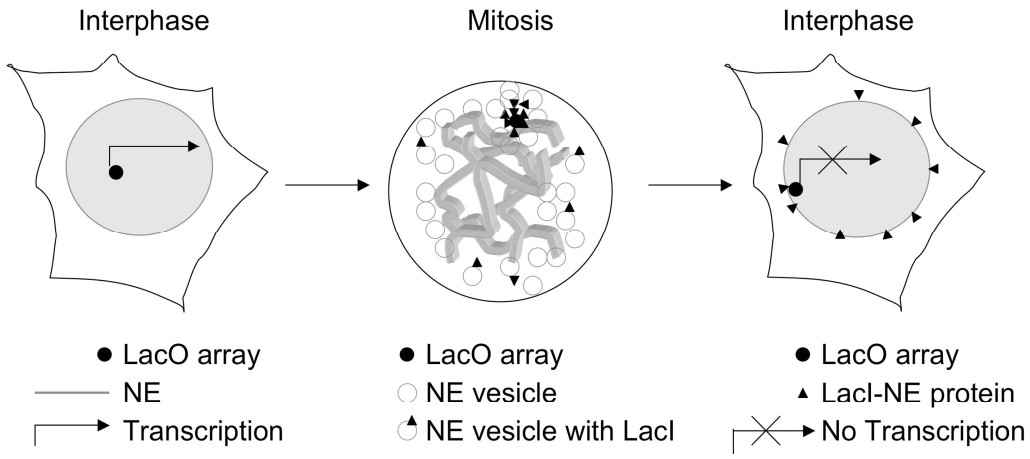
how it changes in differentiation will require further addressing the 3-dimensional constraints on the genome that, in part, appear to result from interactions at the NE.

Acknowledgements

The authors would like to acknowledge the large body of work done on this topic in non-mammalian systems that could not be cited due to space constraints. The authors would also like to thank The Royal Society and The Wellcome Trust for support, Christopher J Hutchison for organizing this meeting, and the Biochemical Society for supporting the Nuclear Envelope Diseases and Chromatin Organisation UK 2009 meeting.

Legends to Figures and Tables

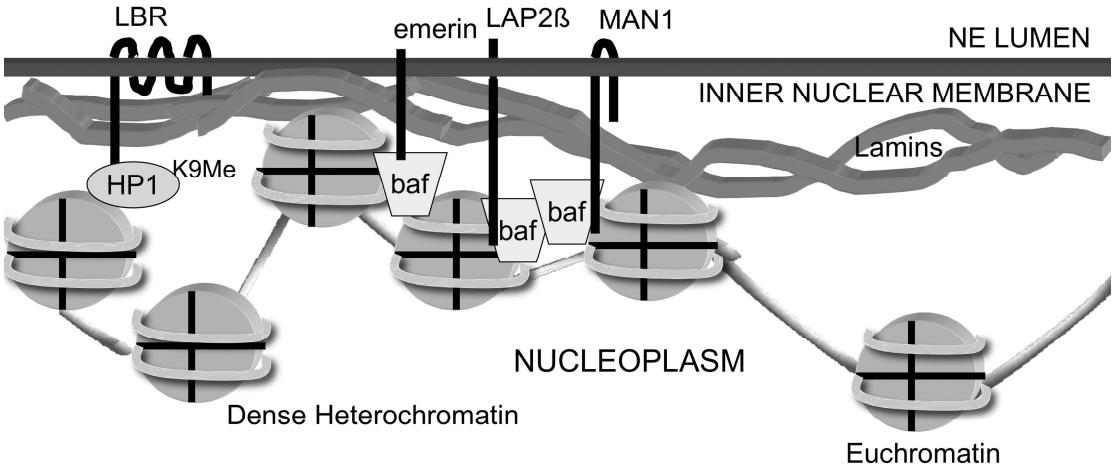
Fig. 1.



Affinity mechanism for tethering of specific chromatin at the NE.

In the lac operator system a gene inserted with the lacO locus is active when in the nuclear interior (left cell), but becomes repressed when recruited to the periphery by binding to lacI fused to a NE protein (right cell). Movement to the periphery requires going through mitosis (middle cell) where membrane vesicles carrying the NE protein-lacI fusion bring the lacO locus in association with the reforming nuclear membrane through an affinity mechanism.

Fig. 2.



Endogenous inner nuclear membrane proteins could use their affinity for certain chromatin proteins to recruit and tether chromosomes to the periphery. For example, lamins bind core histones, LBR binds to epigenetically marked histones and HP1, and emerin, LAP2ß, and MAN1 bind the BAF DNA crosslinker. Differences in the interactions between INM proteins and their chromatin partners could result in different types of chromatin (silenced/active) accumulating at the periphery.

References

1 Croft, J.A., Bridger, J.M., Boyle, S., Perry, P., Teague, P., and Bickmore, W.A. (1999) Differences in the localization and morphology of chromosomes in the human nucleus. J. Cell Biol. **145**, 1119-1131

2 Parada, L.A., McQueen, P.G., Munson, P.J., and Misteli, T. (2002) Conservation of relative chromosome positioning in normal and cancer cells. Curr. Biol. **12**, 1692-1697

3 Meaburn, K.J., Newbold, R.F., and Bridger, J.M. (2008) Positioning of human chromosomes in murine cell hybrids according to synteny. Chromosoma **117**, 579-591

4 Zink, D., Bornfleth, H., Visser, A., Cremer, C., and Cremer, T. (1999) Organization of early and late replicating DNA in human chromosome territories. Exp. Cell Res. **247**, 176-188

5 Federico, C., Cantarella, C.D., Di Mare, P., Tosi, S., and Saccone, S. (2008) The radial arrangement of the human chromosome 7 in the lymphocyte cell nucleus is associated with chromosomal band gene density. Chromosoma **117**, 399-410

6 Albiez, H., Cremer, M., Tiberi, C., Vecchio, L., Schermelleh, L., Dittrich, S., Kupper, K., Joffe, B., Thormeyer, T., von Hase, J., Yang, S., Rohr, K., Leonhardt, H., Solovei, I., Cremer, C., Fakan, S., and Cremer, T. (2006) Chromatin domains and the interchromatin compartment form structurally defined and functionally interacting nuclear networks. Chromosome Res. **14**, 707-733

7 Kosak, S.T., Skok, J.A., Medina, K.L., Riblet, R., Le Beau, M.M., Fisher, A.G., and Singh, H. (2002) Subnuclear compartmentalization of immunoglobulin loci during lymphocyte development. Science **296**, 158-162

8 Williams, R.R., Azuara, V., Perry, P., Sauer, S., Dvorkina, M., Jorgensen, H., Roix, J., McQueen, P., Misteli, T., Merkenschlager, M., and Fisher, A.G. (2006) Neural induction promotes large-scale chromatin reorganisation of the Mash1 locus. J. Cell Sci. **119**, 132-140

9 Zink, D., Amaral, M.D., Englmann, A., Lang, S., Clarke, L.A., Rudolph, C., Alt, F., Luther, K., Braz, C., Sadoni, N., Rosenecker, J., and Schindelhauer, D. (2004) Transcription-dependent spatial arrangements of CFTR and adjacent genes in human cell nuclei. J. Cell Biol. **166**, 815-825

10 Dechat, T., Pfleghaar, K., Sengupta, K., Shimi, T., Shumaker, D.K., Solimando, L., and Goldman, R.D. (2008) Nuclear lamins: major factors in the structural organization and function of the nucleus and chromatin. Genes Dev. **22**, 832-853

11 Schirmer, E.C., and Foisner, R. (2007) Proteins that associate with lamins: many faces, many functions. Exp. Cell Res. **313**, 2167-2179

12 Ellis, D.J., Jenkins, H., Whitfield, W.G., and Hutchison, C.J. (1997) GST-lamin fusion proteins act as dominant negative mutants in Xenopus egg extract and reveal the function of the lamina in DNA replication. J. Cell Sci. **110**, 2507-2518

13 Spann, T.P., Moir, R.D., Goldman, A.E., Stick, R., and Goldman, R.D. (1997) Disruption of nuclear lamin organization alters the distribution of replication factors and inhibits DNA synthesis. J. Cell Biol. **136**, 1201-1212

14 Kumaran, R.I., Muralikrishna, B., and Parnaik, V.K. (2002) Lamin A/C speckles mediate spatial organization of splicing factor compartments and RNA polymerase II transcription. J. Cell Biol. **159**, 783-793
15 Nili, E., Cojocaru, G.S., Kalma, Y., Ginsberg, D., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., Berger, R., Shaklai, S., Amariglio, N., Brok-Simoni, F., Simon, A.J., and Rechavi, G. (2001) Nuclear membrane protein LAP2beta mediates transcriptional repression alone and together with its binding partner GCL (germ-cell-less). J. Cell Sci. **114**, 3297-3307
16 Markiewicz, E., Tilgner, K., Barker, N., van de Wetering, M., Clevers, H., Dorobek, M., Hausmanowa-Petrusewicz, I., Ramaekers, F.C., Broers, J.L., Blankesteijn, W.M., Salpingidou, G., Wilson, R.G., Ellis, J.A., and Hutchison, C.J. (2006) The inner nuclear membrane protein emerin regulates beta-catenin activity by restricting its accumulation in the nucleus. EMBO J. **25**, 3275-3285
17 Osada, S., Ohmori, S.Y., and Taira, M. (2003) XMAN1, an inner nuclear membrane protein, antagonizes BMP signaling by interacting with Smad1 in Xenopus embryos. Development **130**, 1783-1794
18 Haraguchi, T., Holaska, J.M., Yamane, M., Koujin, T., Hashiguchi, N., Mori, C., Wilson, K.L., and Hiraoka, Y. (2004) Emerin binding to Btf, a death-promoting transcriptional repressor, is disrupted by a missense mutation that causes Emery-Dreifuss muscular dystrophy. Eur. J. Biochem. **271**, 1035-1045
19 Holaska, J.M., Rais-Bahrami, S., and Wilson, K.L. (2006) Lmo7 is an emerin-binding protein that regulates the transcription of emerin and many other muscle-relevant genes. Hum. Mol. Genet. **15**, 3459-3472
20 Nielsen, J.A., Hudson, L.D., and Armstrong, R.C. (2002) Nuclear organization in differentiating oligodendrocytes. J. Cell Sci. **115**, 4071-4079
21 Johnson, C., Primorac, D., McKinstry, M., McNeil, J., Rowe, D., and Lawrence, J.B. (2000) Tracking COL1A1 RNA in osteogenesis imperfecta. splice-defective transcripts initiate transport from the gene but are retained within the SC35 domain. J. Cell Biol. **150**, 417-432
22 Park, P.C., and De Boni, U. (1998) A specific conformation of the territory of chromosome 17 locates ERBB-2 sequences to a DNase-hypersensitive domain at the nuclear periphery. Chromosoma **107**, 87-95
23 Hewitt, S.L., High, F.A., Reiner, S.L., Fisher, A.G., and Merkenschlager, M. (2004) Nuclear repositioning marks the selective exclusion of lineage-inappropriate transcription factor loci during T helper cell differentiation. Eur. J. Immunol. **34**, 3604-3613
24 Finlan, L.E., Sproul, D., Thomson, I., Boyle, S., Kerr, E., Perry, P., Ylstra, B., Chubb, J.R., and Bickmore, W.A. (2008) Recruitment to the nuclear periphery can alter expression of genes in human cells. PLoS Genet. **4**, e1000039
25 Kumaran, R.I., and Spector, D.L. (2008) A genetic locus targeted to the nuclear periphery in living cells maintains its transcriptional competence. J. Cell Biol. **180**, 51-65
26 Reddy, K.L., Zullo, J.M., Bertolino, E., and Singh, H. (2008) Transcriptional repression mediated by repositioning of genes to the nuclear lamina. Nature **452**, 243-247
27 Bodoor, K., Shaikh, S., Salina, D., Raharjo, W.H., Bastos, R., Lohka, M., and Burke, B. (1999) Sequential recruitment of NPC proteins to the nuclear periphery at the end of mitosis. J. Cell Sci. **112**, 2253-2264
28 Haraguchi, T., Koujin, T., Hayakawa, T., Kaneda, T., Tsutsumi, C., Imamoto, N., Akazawa, C., Sukegawa, J., Yoneda, Y., and Hiraoka, Y. (2000) Live

fluorescence imaging reveals early recruitment of emerin, LBR, RanBP2, and Nup153 to reforming functional nuclear envelopes. J. Cell Sci. **113**, 779-794

29 Malhas, A., Lee, C.F., Sanders, R., Saunders, N.J., and Vaux, D.J. (2007) Defects in lamin B1 expression or processing affect interphase chromosome position and gene expression. J. Cell Biol. **176**, 593-603

30 Gerace, L., and Burke, B. (1988) Functional organization of the nuclear envelope. Annu. Rev. Cell Biol. **4**, 335-374

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

32 Tunnah, D., Sewry, C.A., Vaux, D., Schirmer, E.C., and Morris, G.E. (2005) The apparent absence of lamin B1 and emerin in many tissue nuclei is due to epitope masking. J. Mol. Histol. **36**, 337-344

33 Ding, X., Xu, R., Yu, J., Xu, T., Zhuang, Y., and Han, M. (2007) SUN1 is required for telomere attachment to nuclear envelope and gametogenesis in mice. Dev. Cell **12**, 863-872

34 Schmitt, J., Benavente, R., Hodzic, D., Hoog, C., Stewart, C.L., and Alsheimer, M. (2007) Transmembrane protein Sun2 is involved in tethering mammalian meiotic telomeres to the nuclear envelope. Proc. Natl. Acad. Sci. USA **104**, 7426-7431

35 Lee, H., Habas, R., and Abate-Shen, C. (2004) MSX1 cooperates with histone H1b for inhibition of transcription and myogenesis. Science **304**, 1675-1678

36 Ye, Q., and Worman, H.J. (1996) Interaction between an integral protein of the nuclear envelope inner membrane and human chromodomain proteins homologous to Drosophila HP1. J. Biol. Chem. **271**, 14653-14656

37 Polioudaki, H., Kourmouli, N., Drosou, V., Bakou, A., Theodoropoulos, P.A., Singh, P.B., Giannakouros, T., and Georgatos, S.D. (2001) Histones H3/H4 form a tight complex with the inner nuclear membrane protein LBR and heterochromatin protein 1. EMBO Rep. **2**, 920-925

38 Makatsori, D., Kourmouli, N., Polioudaki, H., Shultz, L.D., McLean, K., Theodoropoulos, P.A., Singh, P.B., and Georgatos, S.D. (2004) The inner nuclear membrane protein lamin B receptor forms distinct microdomains and links epigenetically marked chromatin to the nuclear envelope. J. Biol. Chem. **279**, 25567-25573

39 Kourmouli, N., Theodoropoulos, P.A., Dialynas, G., Bakou, A., Politou, A.S., Cowell, I.G., Singh, P.B., and Georgatos, S.D. (2000) Dynamic associations of heterochromatin protein 1 with the nuclear envelope. EMBO J. **19**, 6558-6568

40 Zheng, R., Ghirlando, R., Lee, M.S., Mizuuchi, K., Krause, M., and Craigie, R. (2000) Barrier-to-autointegration factor (BAF) bridges DNA in a discrete, higher-order nucleoprotein complex. Proc. Natl. Acad. Sci. USA **97**, 8997-9002

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

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

43 Mansharamani, M., and Wilson, K.L. (2005) Direct binding of nuclear membrane protein MAN1 to emerin in vitro and two modes of binding to barrier-to-autointegration factor. J. Biol. Chem. **280**, 13863-13870

44 Schirmer, E.C., Florens, L., Guan, T., Yates, J.R.r., and Gerace, L. (2003) Nuclear membrane proteins with potential disease links found by subtractive proteomics. Science **301**, 1380-1382

45 Foisner, R., Aebi, U., Bonne, G., Gruenbaum, Y., and Novelli, G. (2007) 141st ENMC International Workshop inaugural meeting of the EURO-Laminopathies project "Nuclear Envelope-linked Rare Human Diseases: From Molecular Pathophysiology towards Clinical Applications", 10-12 March 2006, Naarden, The Netherlands. Neuromuscul. Disord. **17**, 655-660

46 Worman, H.J., and Bonne, G. (2007) "Laminopathies": a wide spectrum of human diseases. Exp. Cell Res. **313**, 2121-2133

47 Bakay, M., Wang, Z., Melcon, G., Schiltz, L., Xuan, J., Zhao, P., Sartorelli, V., Seo, J., Pegoraro, E., Angelini, C., Shneiderman, B., Escolar, D., Chen, Y.W., Winokur, S.T., Pachman, L.M., Fan, C., Mandler, R., Nevo, Y., Gordon, E., Zhu, Y., Dong, Y., Wang, Y., and Hoffman, E.P. (2006) Nuclear envelope dystrophies show a transcriptional fingerprint suggesting disruption of Rb-MyoD pathways in muscle regeneration. Brain **129**, 996-1013

48 Melcon, G., Kozlov, S., Cutler, D.A., Sullivan, T., Hernandez, L., Zhao, P., Mitchell, S., Nader, G., Bakay, M., Rottman, J.N., Hoffman, E.P., and Stewart, C.L. (2006) Loss of emerin at the nuclear envelope disrupts the Rb1/E2F and MyoD pathways during muscle regeneration. Hum. Mol. Genet. **15**, 637-651

49 Fidzianska, A., Toniolo, D., and Hausmanowa-Petrusewicz, I. (1998) Ultrastructural abnormality of sarcolemmal nuclei in Emery-Dreifuss muscular dystrophy (EDMD). J. Neurol. Sci. **159**, 88-93

50 Goldman, R.D., Shumaker, D.K., Erdos, M.R., Eriksson, M., Goldman, A.E., Gordon, L.B., Gruenbaum, Y., Khuon, S., Mendez, M., Varga, R., and Collins, F.S. (2004) Accumulation of mutant lamin A causes progressive changes in nuclear architecture in Hutchinson-Gilford progeria syndrome. Proc. Natl. Acad. Sci. USA **101**, 8963-8968

51 Verga, L., Concardi, M., Pilotto, A., Bellini, O., Pasotti, M., Repetto, A., Tavazzi, L., and Arbustini, E. (2003) Loss of lamin A/C expression revealed by immuno-electron microscopy in dilated cardiomyopathy with atrioventricular block caused by *LMNA* gene defects. Virchows Arch. **443**, 664-671