The inner nuclear envelope as a transcription factor resting place

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Just as people head to the beaches for a well-deserved rest, accumulating evidence suggests that transcription factors take similar ‘vacations’ at the nuclear envelope. Recent studies indicate that the periphery of the nucleus provides a platform for sequestering transcription factors away from chromatin. Several transcriptional regulators, operating in different signal-transduction pathways, have been found to interact physically with components of the inner nuclear membrane. In general, this association seems to restrict access to their target genes and limit their transactivation or transrepression abilities. The mechanisms of inner nuclear membrane association are diverse, and include regulated associations with the nuclear lamina and integral membrane proteins. Together, these findings indicate that the inside of the nuclear envelope functions as a resting place for transcription factors and suggest a more direct role for the nuclear envelope in gene regulation than previously anticipated.

Keywords: gene expression; inner nuclear membrane; lamin; nuclear lamina; signal transduction

EMBO reports (2007) 8, 914–919. doi:10.1038/sj.embor.7401075

Introduction

The nuclear envelope (Franke *et al*, 1981) is arguably the most important border within the eukaryotic cell: it isolates chromatin from the cytoplasm, which allows additional levels of regulation and, perhaps, defence. Transport across the nuclear envelope occurs through nuclear pore complexes (NPCs), which span both the inner and outer nuclear membranes (INM and ONM, respectively). These membranes contain different sets of proteins and, considering the continuity of the nuclear envelope to the endoplasmic reticulum, most ONM proteins are likely to perform endoplasmic reticulum-related functions. The roles of INM proteins are less intuitive, although several functions have been attributed to them. Here, we briefly summarize the functions before discussing the newly emerging role for these proteins, which is the subject of this review.

Known and putative functions of the INM

In higher eukaryotes, the INM is associated with a network of intermediate filament proteins called lamins (Stuurman *et al*, 1998), of

which there are two types: lamin A/C and lamin B, where lamin A and C are variants of the same gene. Together, these form the nuclear lamina—a stable structure beneath the INM. Many different INM proteins bind to lamins, presumably to tightly anchor the nuclear lamina to the INM (Fig 1A).

Two nuclear envelope protein pairs, vertebrate nesprin–SUN (Sad1 and uncoordinated 84 (UNC84) homology), and *Caenorhabditis elegans* UNC-83–UNC-84, span the nuclear envelope lumen, and therefore connect the INM and ONM (reviewed in Gruenbaum *et al*, 2005). In vertebrates, it has been shown that nesprins interact with cytoskeletal elements, whereas SUNs interact with the nuclear lamina. These interactions are thought to regulate the correct positioning of the nucleus within the cell, but might also have a role in signal transduction (Fig 1B).

Another crucial function of the INM, which is not discussed in detail here but was recently reviewed in Akhtar & Gasser (2007), is the organization of chromatin (Fig 1C). The association of genes with the nuclear envelope has been linked to both the repression and activation of transcription. For example, several studies of the regulation of mating-type loci and telomeres in the yeast *Saccharomyces cerevisiae* have shown that, when silenced, these chromatin regions are preferentially located in close proximity to the nuclear envelope in an environment enriched with repressive factors (Akhtar & Gasser, 2007). In fact, the accumulation of repressive factors on silenced chromatin might even reinforce tethering to the nuclear envelope. By contrast, transcriptional activation has been linked to the translocation of genes to the nuclear periphery, specifically to regions containing NPCs, although such re-localization does not appear to be obligatory.

This review focuses on an additional function of the INM, which has emerged from recent insights. INM proteins engage in direct chromatin-independent interactions with transcription factors (Fig 1D), and the sequestering of transcription factors to the INM limits their transactivation or transrepression abilities. Here, we describe the examples of this function discovered so far, and discuss the roles of both the lamins and the integral INM proteins that participate in these interactions.

Lamins as transcription factor magnets

The most well-documented example of the principle of sequestering transcription factors to the nuclear envelope is probably that of c-Fos by lamin A/C (Ivorra *et al*, 2006). c-Fos, together with c-Jun, forms the transcription factor activating protein 1 (AP1), which participates in several crucial cellular processes including cell proliferation and

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Submitted 7 March 2007; accepted 17 August 2007

Fig 1 | Proposed parallel functions of the nuclear envelope in regulating gene expression. (A) Regulated access of transcription factors (TFs; curved arrows) through nuclear pore complexes. (B) Signal transduction through nuclear envelope lumen-spanning protein assemblies of nesprin/uncoordinated 84 (UNC84; light blue) and UNC-83–UNC-84 (dark blue). (C) Assembly of a repressive chromatin environment. (D) Sequestering of transcriptional regulators.

differentiation. Ivorra and co-workers isolated lamin A/C as a heterodimerization partner of c-Fos. In serum-starved quiescent cells, AP1 activity is low and c-Fos is localized to the nuclear envelope (Fig 2). This perinuclear localization of c-Fos strictly requires lamin A/C, as the addition of serum restores AP1 function and relocates c-Fos from the nuclear periphery to the nucleoplasm. Conversely, lamin A/C remains at the nuclear envelope and is therefore a negative regulator of AP1 through the sequestering of c-Fos to the INM. Interestingly, *Lmna*^{−/−} cells show enhanced proliferation (Ivorra *et al*, 2006), indicating that the lamin–c-Fos interaction regulates maintenance of the quiescent state. However, proliferation might also be enhanced by a lack of lamin A/C-dependent anchoring of the retinoblastoma (Rb) protein (Johnson *et al*, 2004; Pekovic *et al*, 2007), which is an intranuclear event that also involves the non-membrane lamin A/C-interacting protein, lamina-associated polypeptide 2α (LAP2α; Markiewicz *et al*, 2002).

The mature form of lamin A is generated through a series of post-translational modifications that include farnesylation and proteolytic processing to remove the farnesylated terminus. The unprocessed lamin A—or pre-lamin A—was found to associate with the sterol regulatory element-binding protein 1 (SREBP1; Capanni *et al*, 2005). SREBP1 is synthesized as a precursor protein embedded in the endoplasmic reticulum membrane. The depletion of cholesterol causes intramembrane proteolysis and the release of the DNA-binding amino-terminal section of SREBP1, which translocates to the nucleus and induces the expression of cholesterol metabolism genes such as peroxisome proliferator-activated receptor-γ (PPARγ; Fajas *et al*, 1999). Pre-lamin A and SREBP1 co-localize to the nuclear rim, which coincides with the downregulation of PPARγ expression (Capanni *et*

al, 2005). Therefore, it is thought that pre-lamin A limits the access of the transcription factor SREBP1 to the nuclear interior. However, there is evidence that the overexpression of pre-lamin A alone is able to repress the expression of PPARγ, even when localized to the nuclear interior (Capanni *et al*, 2005), which questions the necessity of SREBP1 sequestration at the nuclear lamina. By contrast, this might reflect a more general inhibitory effect of overexpressed lamins on RNA polymerase II transcription (Kumaran *et al*, 2002; Spann *et al*, 2002).

Of notable interest is the disease familial partial lipodystrophy, which is characterized by impaired lamin A/C processing. Pre-lamin A specifically accumulates in lipodystrophy cells (Capanni *et al*, 2005) and this phenotype seems to be directly correlated with altered PPARγ induction, because the forced accumulation of pre-lamin A in wild-type pre-adipocytes impairs their differentiation and reduces the amount of DNA-bound SREBP1.

Another transcription factor that might be sequestered by a lamin is octamer-binding transcription factor 1 (OCT1; Imai *et al*, 1997). OCT1 localizes to the nuclear periphery, which correlates with repression of the collagenase gene, a transcriptional target of OCT1. However, at present, it is unclear whether OCT1 localization is the cause or effect of gene repression—that is, whether OCT1 directly associates with the nuclear lamina or only with repressed genes that are located there. Physical interaction studies between OCT1 and lamins, and intranuclear localization of the repressed collagenase gene, might answer this question.

INM transcription factor scavengers

In addition to nuclear lamins, several integral INM proteins have been found to interact with transcription factors. Approximately 15

Fig 2 | Model of the interactions of transcription factor complexes at the nuclear lamina with lamin A/C (left) or pre-lamin A (right). c-Fos–c-Jun heterodimers activate a proliferative genetic programme. Sequestering of c-Fos by lamin A/C results in the suppression of this programme. Low cholesterol induces the nuclear access of sterol regulatory element-binding protein 1 (SREBP1) so that it can activate cholesterol metabolic genes. This activation is downregulated by the sequestering of SREBP1 by pre-lamin A. NPC, nuclear pore complex.

integral INM proteins have been fully characterized in mammalian cells so far and two recent genomic approaches (Dreger *et al*, 2001; Schirmer *et al*, 2003) have substantially increased the number of putative INM proteins. Integral INM proteins seem to have little in common (Schirmer & Gerace, 2005), although three INM proteins share a lamin-associated polypeptide (LAP), emerin and MAN1 (LEM) domain of approximately 40 amino acids (Lin *et al*, 2000), which has been found to function as a promiscuous protein–protein interaction platform. Most integral INM proteins are not specifically associated with NPCs and localize throughout the INM.

MAN1 is an integral INM protein with an amino-terminal LEM domain, two transmembrane domains and a carboxy-terminal RNA-recognition motif. Several studies have probed the interaction between MAN1 and Smad transcription factors (Bengtsson, 2007; Cohen *et al*, 2007; Ishimura *et al*, 2006; Lin *et al*, 2005; Osada *et al*, 2003; Pan *et al*, 2005). Smads are crucial regulators of transforming growth factor- β (TGF β), bone morphogenic protein (BMP) and activin signalling (reviewed in Massague *et al*, 2005). Activation of TGF β and BMP receptors at the plasma membrane leads to phosphorylation of receptor-specific R-Smads, which oligomerize with the common mediator Smad4, and subsequently translocate to the nucleus, where they bind to DNA and regulate transcription (reviewed Bengtsson, 2007). In both *Xenopus* embryos and human cells, MAN1 has been found to functionally antagonize TGF β and BMP signalling (Cohen *et al*, 2007; Ishimura *et al*, 2006; Lin *et al*, 2005; Osada *et al*, 2003; Pan *et al*, 2005). R-Smads have been shown to physically interact with MAN1 (Lin *et al*, 2005; Pan *et al*, 2005), indicating their direct inhibition by MAN1. The mechanism of this interaction is controversial (Bengtsson, 2007; Worman, 2006), although most authors propose that R-Smads are sequestered to the INM, thereby preventing transcription of their target genes (Fig 3A). However, the fact that most R-Smads are cytoplasmic in the absence of TGF β or BMP signalling, and not clearly at the nuclear envelope, argues against this (Pan *et al*, 2005), although a detergent-insoluble pool of R-Smads clearly co-localizes with MAN1 (Pan *et al*, 2005). Therefore, a consensus view

could be that MAN1 acts as a nuclear scavenger, sequestering R-Smads that illegitimately enter the nucleus. MAN1 also interacts with several other transcriptional regulators, including the transcription factor germ-cell-less (GCL), the BCL2-associated transcription factor (BTF) and the barrier-to-autointegration factor (BAF; Mansharamani & Wilson, 2005), although the significance of these associations is not yet understood.

In *S. cerevisiae*, the cellular import of amino acids is controlled by two homologous transcription factors, Stp1 and Stp2 (species-specific tRNA processing protein), which are synthesized as latent cytoplasmic factors (Andreasson & Ljungdahl, 2002). Amino-acid induction causes cleavage of Stp1 and Stp2, liberating the DNA-binding and transactivating domains (Abdel-Sater *et al*, 2004; Andreasson *et al*, 2006). The cleaved forms of Stp1 and Stp2 localize to the nucleus where they bind to specific promoters (de Boer *et al*, 2000; Nielsen *et al*, 2001). Mutations in amino-acid sensor independent (ASI) genes constitutively activate target promoters (Forsberg *et al*, 2001). *ASI1*, *ASI2* and *ASI3* each encode integral INM proteins (Boban *et al*, 2006; Zargari *et al*, 2007; Fig 3B). Asi1 and Asi3 are homologous proteins with five membrane-spanning domains and a C-terminal RING-finger like region. Asi2 has two transmembrane regions but lacks other functional domains. In *asi1* Δ , *asi2* Δ and *asi3* Δ mutant cells, uncleaved Stp1 and Stp2 can bind to their target genes and activate transcription (Fig 3B). This suggests that Asi proteins might also act as scavengers of transcription factors that have inadvertently entered the nucleus.

Activators of repression or repressors of activation?

The single spanning INM protein emerin is encoded by the *EMD* gene, which, when mutated, produces the X-linked form of Emery–Dreifuss muscular dystrophy (EDMD; Gruenbaum *et al*, 2005). The lamin-associated protein LAP2 β was originally identified as a single spanning INM protein with a nucleoplasmic binding region for lamin B and chromatin (Foisner & Gerace, 1993). Both emerin and LAP2 β associate with several transcriptional regulators, and this association invariably coincides with repression of the transcription factor target genes. In most instances, the mechanism of repression is not clear because it is uncertain whether the transcription factor acts as an activator or repressor of transcription—often transcription factors can do both. If the transcription factor acts as an activator, sequestering the transcription factor away from its target gene is a possible mechanism. If the transcription factor acts as a repressor, a model would be created of a repressive environment for the target gene at the nuclear periphery. An exception to this uncertainty is the β -catenin–emerin interaction (Markiewicz *et al*, 2006). β -catenin is a co-activator of Wnt-signalling-responsive genes, which are involved in a range of developmental and homeostatic processes. In the presence of Wnt, β -catenin accumulates in the nucleus where it forms active transcriptional complexes with transcription factors of the T-cell factor/lymphocyte enhancing factor (TFC/LEF) family. The level of β -catenin activity is therefore regulated by its rate of accumulation in the nucleus. The overexpression of emerin prevents nuclear accumulation of β -catenin and inhibits its transcriptional activity (Markiewicz *et al*, 2006). When emerin is mislocalized from the INM to either the cytoplasm or nucleoplasm, it fails to inhibit β -catenin activity, indicating that the correct localization of emerin is required for its repressive effect (Fig 3C). These data indicate that, at least in this case, emerin is not involved in chromatin-mediated repression, but rather in inhibiting β -catenin activity, possibly by stimulating its nuclear export.

Fig 3 | Model of interactions of transcription factors with inner nuclear membrane proteins. **(A)** In the absence of transforming growth factor- β (TGF β) or bone morphogenic protein (BMP) signalling, MAN antigen 1 (MAN1) acts as a nuclear scavenger that sequesters R-Smads that illegitimately enter the nucleus, thereby preventing transcription from their target genes. **(B)** The yeast amino-acid sensor independent (Asi)-complex prevents illegitimate expression of Stp1/Stp2 (species-specific tRNA processing factor)-responsive genes when amino-acid signalling is absent. **(C)** Overexpression of emerlin prevents nuclear accumulation of β -catenin and inhibits its transcriptional activity, presumably by increasing nuclear export of β -catenin. **(D)** The transcriptional capacity of E2F-DP3 (Early gene 2 factor-Differentiation regulated transcription factor polypeptide 3) is reduced when bound to germ-cell-less (GCL), which itself can associate with emerlin and lamina-associated polypeptide 2 β (LAP2 β). It is unclear whether transcriptional repression works through sequestering (left) the E2F-DP3-GCL trimeric complex, or if the GCL-E2F-DP3 actively represses target genes at the nuclear periphery (right). NPC, nuclear pore complex.

By contrast, both mechanisms of repression are still possible explanations for the function of the transcriptional regulator GCL, which was identified as an interaction partner of both emerlin (Holaska *et al*, 2003) and LAP2 β (Nili *et al*, 2001). GCL binds to the DP3 subunit of the heterodimeric transcription factor E2F-DP3, and the transcriptional activity of the E2F-DP3 heterodimer is crucial for cell-cycle progression. Overexpression of either LAP2 β or GCL reduces the transcriptional capacity of E2F-DP3 (Nili *et al*, 2001). It is uncertain whether this repression works through sequestering the E2F-DP3-GCL trimeric complex (Fig 3D, left) or whether the GCL-E2F-DP3 complex actively represses target genes at the nuclear periphery (Fig 3D, right). Both scenarios are possible because it is unclear whether GCL acts as a transcriptional activator or repressor. So far, repressed E2F/DP3

target genes have not been co-localized together with GCL at the nuclear periphery.

Finally, the transcriptional repressor BCL2-associated transcription factor (BTF), which was picked up in a yeast two-hybrid screen, was also identified as a binding partner of emerlin (Haraguchi *et al*, 2004). Under normal growth conditions, BTF is a nuclear protein; however, on induction of apoptosis, the protein accumulates at the nuclear periphery, presumably binding to emerlin. Overexpression of BTF causes cell death involving the inhibition of anti-apoptotic BCL2 proteins (Kasof *et al*, 1999). Importantly, EDMD-associated missense mutations specifically disrupt the binding of BTF to emerlin, suggesting that dysregulated nuclear access induces apoptosis. However, the precise repressor function of BTF is poorly understood, and it is not clear whether apoptosis coincides with increased or decreased repressor activity.

Concepts and perspectives

On the basis of the examples presented in this review, it seems clear that the nuclear envelope is actively involved in the modulation of gene expression by sequestering transcription factors that have entered the nucleus. Although the groups of nuclear envelope proteins and the transcription factors that they interact with are heterogeneous, interaction with the nuclear envelope proteins clearly restricts the access of some transcription factors to their target genes, and restrains their activating or repressing capacities. The spatial separation from their target promoters therefore appears to be sufficient for inactivation. In several cases it is not possible to discriminate between inactivation of the transcription factor and inactivation of the target gene at the nuclear periphery. Both *in situ* localization and *in vitro* analysis of inactive target genes are required to answer these questions.

We speculate that the tethering of transcription factors to the nuclear envelope might aid in fine-tuning pathways that are prone to fluctuations, such as growth factor and amino-acid signalling. Specifically, under non-inducing conditions, some transcription factors are kept latent in the cytoplasm away from their target promoters. However, it is possible that such cytosolic sequestering is not fully effective and that a few DNA-binding-competent transcription factors might illegitimately access the nucleus in the absence of an inducing signal. In these scenarios, the nuclear envelope might bind to and inactivate transcription factors to ensure that no transcription occurs when activating signals are absent. Therefore, in a biological systems context, sequestering might provide a means of enlarging the difference between the on and off states of a gene (Boban *et al*, 2006).

What happens to the pool of nuclear envelope-bound transcription factors? It is possible that, in some cases, association with the nuclear envelope causes further inactivation of a transcription factor by inducing nuclear export and/or degradation. This strategy might explain why some nuclear envelope proteins are known to regulate transcription factors, even though their co-localization at the nuclear envelope has not been documented. Another possibility is that nuclear envelope localization reflects a stable pool of nuclear envelope bound transcription factors. This pool might be released on activation by the correct signal, which could provide cells with a mechanism to react quickly to changing conditions without the need to import transcription factors from the cytosol.

Another crucial question is to what extent syndromes and disorders associated with mutations in lamins and INM proteins are caused by defects in the sequestering of transcription factors. In light of the many examples of INM-transcription factor interactions documented so far, and the fact that many more INM proteins remain to be characterized, it is likely that the regulation of transcription factor activity by lamins and INM proteins might be a more common phenomenon than previously anticipated. This increases the likelihood that this mechanism is important for understanding and eventually curing nuclear envelope-related diseases.

ACKNOWLEDGEMENTS

We thank B. Kalverda for critical reading of the manuscript. SH is supported by a long-term European Molecular Biology Organization (EMBO) fellowship.

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