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The Sky's the LEMit: New insights into nuclear structure regulation of transcription factor activity

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Abstract

The nucleoskeleton has been associated with partitioning the genome into active and inactive compartments that dictate local transcription factor (TF) activity. However, recent data indicates that the nucleoskeleton and TFs reciprocally influence each other in dynamic TF trafficking pathways through the functions of LEM proteins. While the conserved peripheral recruitment of TFs by LEM proteins has been viewed as a mechanism of repressing transcription, a diversity of release mechanisms from the lamina suggest this compartment serves as a refuge for nuclear TF accumulation for rapid mobilization and signal stability. Detailed mechanisms suggest that TFs toggle between nuclear lamina refuge and nuclear matrix lamin-LEM protein complexes at sites of active transcription. In this review we will highlight emerging LEM functions acting at the interface of chromatin and nucleoskeleton to create TF trafficking networks.

Introduction

The 1952 electron micrographs of the *Amoeba proteus* nuclear membrane demonstrated a fibrous lamina supporting the inner leaflet, an observation which "necessitate(d) a reinterpretation of the structure of this membrane"[1]. This observation launched seven decades of reinterpretation of the astonishingly complex nucleoskeleton that extends from the periphery (nuclear lamina) into the nucleoplasm (nuclear matrix). The nucleoskeleton profoundly influences transcription via the regulation of transcription factors (TFs) and reciprocally relies on TFs in order to achieve specificity[2]. At the nuclear periphery A and B-type lamins form tetrameric 3.5nm filaments which assemble into distinct but interacting semiregular meshworks[3]. This lamin framework delineates the transcriptionally repressive nuclear lamina compartment which is characterized by dense peripheralized heterochromatin interacting with several classes of lamina-associated proteins[4]. By contrast, the

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transcriptionally active nuclear matrix consists of A-type lamins solubilized by post-translational modifications working with lamin-associated proteins[5,6].

A key link between the nucleoskeleton and transcription is a unique family of seven genes encoding integral inner nuclear membrane proteins sharing a LEM domain (named after the founding members LAP2, Emerin, Man1)(Figure 1)[7]. Abnormalities associated with LEM protein function range from aging, cardiovascular disease, and cancer resistance, highlighting their critical role in signal transduction[8]. Three LEM subgroups reflect their unique domain organization. Group I LEM proteins contain a N-terminal LEM-domain and a C-terminal single-pass transmembrane domain (apart from certain LAP2 splice forms). Group II LEM-proteins contain two transmembrane domains, a DNA-binding MSC domain, and a N-terminal LEM domain. Group III LEM-proteins are highly divergent, lacking transmembrane domains and containing ankyrin repeats[9]. The ability of LEM proteins to directly bind lamins and DNA make them prime candidates for Lamina-Associated Domain (LAD) tethers, regions of heterochromatin that interact with the lamina[10]. However, numerous recent studies suggest a reinterpretation of LEM protein function that highlights new aspects of transcriptional regulation and disease relevance. Here we highlight recent work revealing the ancestral functions of these LEM proteins and how they have been co-opted to generate complex regulatory circuits on nucleoskeletal elements for localized TF function.

Ancestral LEM proteins tether chromatin and participate in nuclear structure

Group II LEM proteins are well conserved throughout eukaryotic evolution[9,11], and recent studies deepen our understanding of their ancestral functions. In protozoa, the LEM domains demonstrates weaker sequence conservation than the MSC domain, indicating that the DNA-binding capacity of the MSC domain predated the development of the LEM-domain with its own indirect DNA-binding function. Functional studies in the binucleate protist *Tetrahymena thermophila* identify two LEM2-like proteins that localize to the nuclear envelope with one accumulating in the macronuclei designated for degradation and the other localizing to the nuclear pore complexes of the micronucleus and inhibiting its degradation, reinforcing the LEM protein role in nuclear structural maintenance[12].

A key nuclear maintenance partner emerging with Group II LEM proteins is the Barrier-to-Autointegration (BAF) protein, a DNA binding protein conserved in metazoa and first identified for its role in blocking viral integration into the mouse genome[13]. Vertebrate LEM2 and BAF, working with Charged Multivesicular Body Protein 7(CHMP7), and ESCRT-III complex, enforce nuclear-cytoplasmic segregation in the settings of mitosis and envelope rupture[14–17]. BAF ranked as the top hit in a siRNA depletion screen of 1,295 genes to induce micronuclei formation when the mitotic spindle was disrupted. BAF cross-bridges low-complexity distal regions of chromatin by forming dimers coating the surface of chromosomes, condensing the chromatin locally to generate a diffusion barrier. In a telling experiment, dimerization-deficient BAF mutant fails to rescue knockdown of endogenous BAF, indicating the DNA compaction by BAF is a major mechanism of structuring the

nucleus[18*]. In a fascinating recent follow-up, LEM2 uses phase-separation onto microtubules via its low complexity region to coordinate the actions of BAF in chromatin cross-bridging with CHMP7 and ESCRT machinery in nuclear membrane repair during envelope reformation[19**] (Figure 2A). Together, it appears BAF co-evolved with the LEM domain to enforce appropriate nuclear-cytoplasmic compartmentalization, with BAF creating a diffusion barrier by compacting chromatin.

An intriguing question is what takes the place of BAF chromatin compaction and LAD-like formation in yeast where the LEM2 amino-terminal LEM domain acts to tether chromatin and centromeres to the nuclear periphery without lamins or BAF. The carboxy-terminal MSC domain anchors telomeres and epigenetically silences peripheral chromatin via recruitment of the HDAC-containing SHREC complex[20](Figure 2B). These LEM2 associated LADs appear to be pruned by the same ESCRT machinery LEM2 associates with for nuclear envelope repair[21]. Studies suggest lamina proteins Nur1 and Bqt4, which form complexes with LEM2, are vital to this process[20,22]. Super resolution microscopy reveals that Bqt4, LEM2, and Nur1 aggregate into distinct microdomains within the nuclear lamina, with LEM2 distributing both diffusely across the lamina and forming distinct puncta. Deletion of Bqt4 results in loss of the diffusely perinuclear LEM2 population with the Bqt4-dependent diffuse LEM2 population functioning to maintain heterochromatin silencing[23*]. This intriguing finding suggests that subpopulations of LEM-associated complexes exist within the nuclear lamina, allowing for greater compartmentalization and functional specialization.

In higher eukaryotes, lamina sequestration and repression of cardiac genes occurs in part through LAP2β and HDAC3[24]. HDAC3, which associates with LAP2β on the nuclear lamina, functions through a mechanism independent of its catalytic activity. Knockout of HDAC3 results in the mislocalization of cardiac genes into the nucleoplasm prematurely, leading to precocious differentiation that can be rescued by exogenous catalytically-dead HDAC3 mutants or HDAC3-LAP2β fusions, but not a LAP2β-binding deficient HDAC3 mutant. Given the diversification of the LEM protein family in these organisms, an open question is how Group I and Group II LEM proteins differ in LAD anchorage functionality, how the loss of the direct DNA-binding capacity of the MSC domain changes the functionality of Group I LEM proteins, and how LEM proteins in general work in concert with other lamina components to mediate a diversity of long-term and short-term lamina-chromatins interactions[25–28].

Nuclear Lamina: LEM proteins make a TF refuge, not a graveyard

While LEM proteins help form LADs that repress transcription, neither direct nor indirect LEM protein binding to chromatin provides the sequence specificity required for tight transcriptional regulation, directing attention to LEM protein interaction with sequence-specific TFs. Analysis of *Igh, Ikzf1, and Bcl11a* loci, which are peripherally localized in LADs in fibroblasts but nucleoplasmic and active in pro-B cells, reveals that flanking LAD chromatin sequences are sufficient for peripheral targeting of ectopic loci. The LAD flanking sequences were enriched in YY1 and CTCF binding sites, and knockdown of YY1 and lamin A/C (but not lamin A alone or CTCF) were sufficient to disrupt LAD

formation[29]. Notably lamin A/C disruption has previously been reported to disrupt LEM protein recruitment to the nuclear lamina[30]. These data suggest that transcription factors act in concert with the nuclear lamina, likely through a LEM protein bridge, to sequester genes to the nuclear periphery as a mechanism of transcriptional silencing. Similarly, the peripheral sequestration of non-DNA-bound TFs by LEM proteins exerts a predictably repressive function on signal transduction[31]. This relationship is typified by the repressive interaction between Man1 and SMAD transcription factors, as MAN1 scaffolds SMAD2/3 dephosphorylation by PPM1A[32–34].

However recent studies suggest LEM interactions with TFs are more than just repressive, but also serve as a dynamic nuclear refuge for the accumulation of TFs without the risk of inadvertent activation or degradation. For example, acetylated GLI1 is sequestered to the nuclear lamina by the LEM protein LAP2β[35**] (Figure 3). While this interaction represses GLI1 transcriptional activity, it also antagonizes GLI1 nuclear export. Without LAP2β expression, GLI1 fails to accumulate in the nucleus and cannot execute its transcriptional program. Inhibition of the proteasome with MG132 in LAP2β$^{-/-}$ cell results in the cytoplasmic accumulation of GLI1, while nuclear export inhibition by leptomycin B rescues the nuclear accumulation of GLI1. Therefore, LAP2β generates a nuclear reserve of inactivated GLI1 poised for subsequent activation by the inhibition of its nuclear export.

Dynamic LEM-lamina-TF interactions appear to hinge on post-translational modifications. Over a decade ago the release of c-Fos from the nuclear lamina was found to be dependent on its phosphorylation by lamina-associated and serum-stimulated Erk1/2[36,37]. More recently de-acetylation of GLI1was found to liberate it from LAP2β and allow accumulation on the nucleoplasmic LAP2 isoform LAP2α[35**]. These LAP2 splice-forms compete for a common zinc-finger binding site on GLI1, with LAP2β outcompeting LAP2α to bind acetylated GLI1 by a secondary interaction site at the acetyl moiety. Countering this repression, LAP2α catalyzes the deacetylation of GLI1 by the recruitment of HDAC1, facilitating the LAP2 hand-off mechanism. Overexpression of LAP2β, which represses GLI1 transcriptional activity at steady-state but generates a larger acetylated/inactivated lamina-bound reserve, allows more rapid recovery from deacetylation blockade compared to wild-type. These results illustrate how LEM proteins act as a refuge for TFs waiting to gain access to chromatin and can rapidly mobilize nuclear reserves[35**].

In addition to TF modifications, emerging data suggest post-translational modifications of the nucleoskeleton may be another mechanism controlling the egress of TFs from the lamina. The direct phosphorylation of lamin B1 by JNK at T575 has been suggested as a mechanism of Oct-1 release from the nuclear lamina[38,39] (Figure 3). Meanwhile, Emerin regulates the rapid mechano-responsiveness of the MRTF/SRF transcriptional complex in part by helping to induce nuclear actin polymerization. Disruption of Emerin localization by lamin A depletion resulted in a failure of MRTF to accumulate in the nucleus[40–42] (Figure 3). Finally, β-catenin uses the cytoplasmic Nesprins, a member of the mechano-sensing LINC complex for nuclear entry[43]. Wnt pathway activation induces the transient accumulation of β-catenin at the nuclear lamina prior transitioning to the nucleoplasm[44]. Emerin, known to act in association with the LINC complex, also induces β-catenin nuclear

export[45,46]. Yet to be elucidated in each of these cases is the mechanism and identity of the nuclear matrix recipient of these key TFs.

A further blind spot in our understanding is how the DNA compaction functions of BAF control LEM-TF interactions. LEM-bound BAF-dimers form femtomolar range DNA interactions and in principle should simply disengage all associated transcription factors[13]. Indeed all reported BAF-TF interactions are inhibitory[13,47,48]. Providing a potential solution, phosphorylation of BAF by Vaccinia-related kinases VRK1 and VRK2a at S4, with minor sites at T2 and T3, controls BAF nuclear-cytoplasmic distribution by disrupting its interaction with LEM-proteins, DNA, and TFs[49,50]. The Group III LEM-protein ANKLE2 balances VRK1/2 phosphorylation of BAF and PP2/PP4 dephosphorylation[50–52]. Recently the phosphorylation of BAF was reported to control interaction with E2F1 in Drosophila, with BAF antagonizing E2F1 nuclear accumulation [13,48]. In addition, BAF has been recently found to be SUMOylated at K6 by lamina associated SENP1/2 which promotes its DNA, Lamin A, and PCNA interactions without changing its LEM-protein interaction[53]. These data suggest that LEM-associated, stimulus-specific modifications of BAF provide a mechanism to drive TFs from the lamina refuge (Figure 3).

Nuclear Matrix: Nucleoplasmic structure enforces transcription

In addition to their established role in enforcing heterochromatin, recent studies support a vital role for LEM proteins in the establishment of the nuclear matrix. Solubilization of lamins depends upon both the phosphorylation of lamin A/C at S22 and S392 and the function of the nucleoplasmic LEM protein LAP2α[5,6,54]. LAP2α, a splice form of the LAP2 proteins, appears to have evolved in mammals following a retrotransposon domestication event, replacing its transmembrane domain with a unique coiled-coil domain[55] that binds and facilitates solubilization of lamins[54]. Genomic binding studies find that LAP2α and lamin A bind overlapping regions of chromatin in the nuclear interior[56] and depletion of lamin A results in increased nucleoplasmic genomic loci mobility[57], consistent with the formation of nucleoplasmic structural scaffolds for transcription. How lamin phosphorylation, LAP2α, and TFs influence each other remains an active area of investigation.

In addition to previous work showing the cell cycle regulatory retinoblastoma protein anchorage by LAP2α[5,58], recent work adds GLI1 to the list of LAP2α nuclear matrix transcriptional partners[35**] (Figure 4). Vicinal DNA-labeling via DamID indicates LAP2α and GLI1 co-bind hedgehog target genes, with FRAP measurements of GLI1 showing reduced mobility when associated with LAP2α. Loss of nucleoplasmic anchorage is correlated with a decrease in GLI1 transcriptional output and an increase in CRM1-binding (and subsequent nuclear export). The relative contributions of nuclear matrix anchorage to nucleoplasmic positioning versus scaffolding nucleoplasmic interaction surfaces remains to be determined.

An exciting recent report indicates that lamin A/C phosphorylation and LAP2α play a direct role in the regulation of transcription. The authors find that nucleoplasmic and soluble S22 phosphorylated lamin A/C co-binds binds active enhancers with the c-Jun transcription

factor[59**] (Figure 4). This confirms that the euchromatin regions previously found to bind lamin A/C and LAP2α are indeed bound by a subpopulation of phosphorylated lamin, with global run-on sequencing experiments confirming these are regions of active transcription. An intriguing finding was that the pathological lamin A/C mutant progerin itself does not show detectable levels of pS22, yet the regions bound by wildtype pS22 lamin A/C were altered in HGPS patient derived fibroblasts. This result supports the importance of lamin post-translational modifications and reinforces how LEM-lamina-matrix interactions are critical for disease prevention. Similar to GLI1, it is unclear whether c-Jun directs the nucleation of the nuclear matrix onto these regions or if the lamins bind preexisting sites that stabilize c-Jun chromatin accumulation. Studies into how c-Jun interacts with the nuclear matrix lamins, either through LAP2α or other scaffolding mechanisms, will likely shed light on how this dynamic structure influences transcription.

Concluding Remarks

LEM proteins have evolved at the interface of chromatin and nucleoskeleton to control chromatin structure and modulate the activity of sequence specific transcription factors, providing the local dynamism needed to adapt to changing cellular environments. The ability to epigenetically silence peripheralized chromatin in LADs has led to the development of a transcriptionally inert compartment which TF nuclear trafficking pathways use to store inactive TFs which can be rapidly deployed by post-translational modifications. Rather than being a graveyard for TFs, LEM proteins enable the lamina to serve as a vital refuge that modifies transcriptional dynamics. Future questions include determining if LEM-based TF peripheral sequestration contributes to LAD specificity, how kinases/deacetylases act with specificity to release TFs from the lamina, and how BAF contributes to TF antagonism. Progress in the field will depend on overcoming some of the major technical challenges associated with studying the dynamic nature of the compartment. These include identifying the significance of transient and changing physical chemical properties of TF-LEM interactions, the functional redundancy of LEM proteins, the possible existence of sub-populations of LEM proteins, and the pleiotropic effects associated with the perturbation of these core nuclear structural entities. However, the introduction of proximal DNA and protein labeling technologies, generation of state-specific antibodies, and the ability to assay local mobility by rapidly improving imaging technologies make these questions trackable for the first time. Similarly, the recent appreciation of phase-separated compartments within the nucleoplasm makes their interactions with the nuclear matrix all the more intriguing. While phase-separation efficiently segregates factors based on solubility, lamin-based structures in the nucleoplasm promise an unprecedented mechanism of structural stability while allowing for dynamic changes. The sky is the limit for how LEM proteins contribute to a panoply of biological processes and human diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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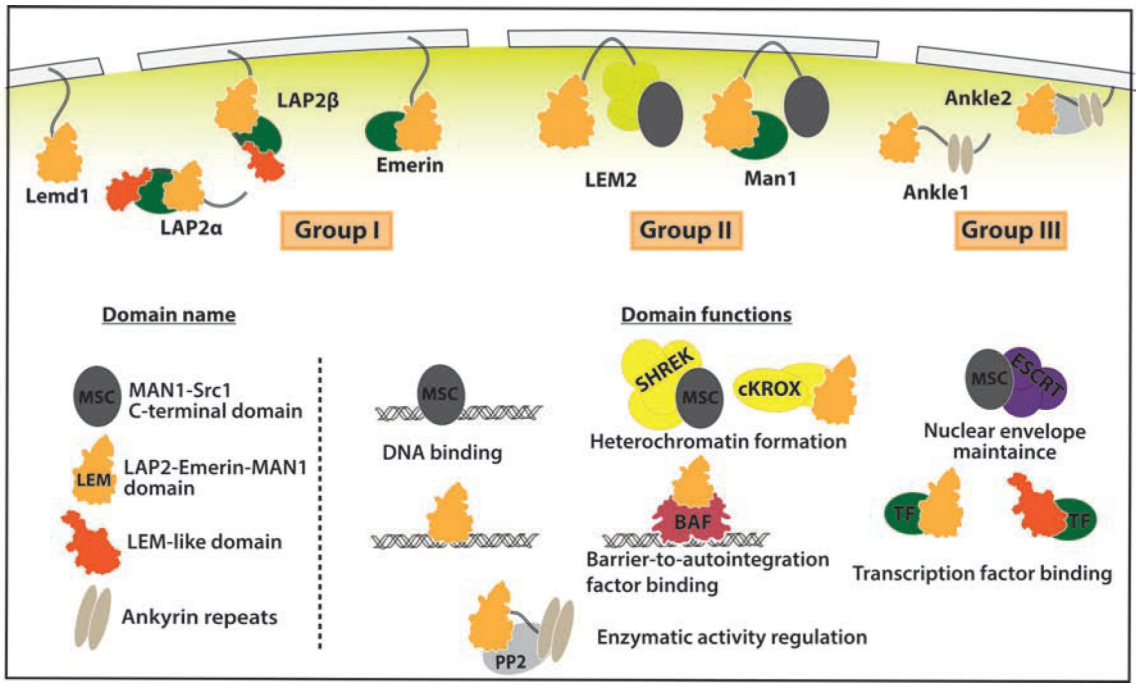

Figure 1. LEM family of nuclear lamina proteins

LEM proteins are classified into three groups: group I includes LAP2, Emerin and Lemd1; group II includes LEM2 and Man1; group III includes Ankle1 and Ankle2 (also named Lem4). Apart from their shared LEM domain, each group contains unique domains with distinct known functions. See text for details.

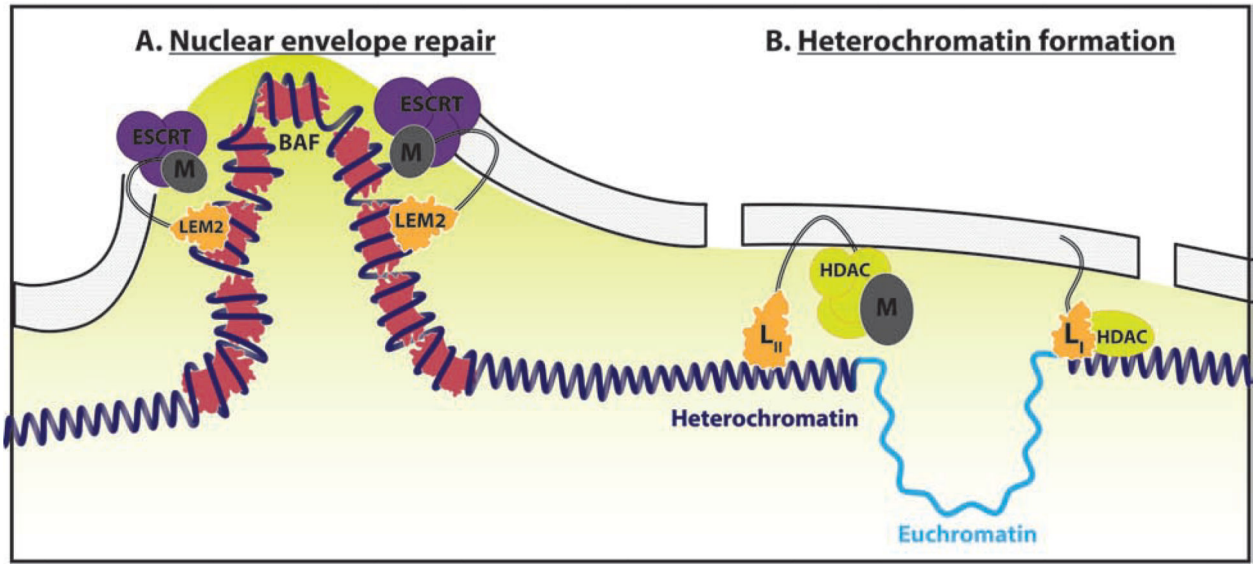

Figure 2. Ancestral LEM proteins tether chromatin and participate in nuclear structure

(A) Nuclear envelope repair coordinated by LEM2-CHMP7-ESCRTIII recruitment to BAF coated DNA. LEM2: orange, ESCRT: purple, BAF: pink. (B) Group I and II LEM proteins ($L_{I/II}$) tether and silence (yellow gradient) peripheral heterochromatin via the recruitment of HDAC complexes (yellow). LEM domain: orange, MSC domain: dark grey.

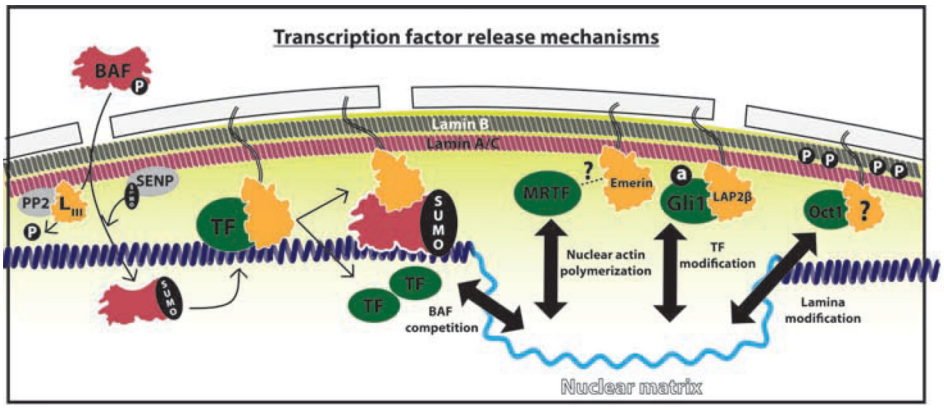

Figure 3. Nuclear Lamina: LEM proteins make a TF refuge, not a graveyard.

LEM proteins create a transcription factor (TF, green) refuge at the nuclear lamina (yellow gradient) which release TFs through diverse mechanisms. Left: Proposed model of BAF (pink) competition with TFs (green) for LEM protein (orange) and DNA interaction. BAF phosphorylation (P) is regulated by phosphatases (PP2) directed by Group III LEM proteins (L_{III}) while SUMOylation (SUMO) requires lamina-bound SENP1/2 (SENP). Right: Mechanisms of TF release from the nuclear lamina include nuclear actin polymerization by Emerin, TF modification such as GLI1 acetylation (a), and lamina modification such as B-type lamin phosphorylation (P).

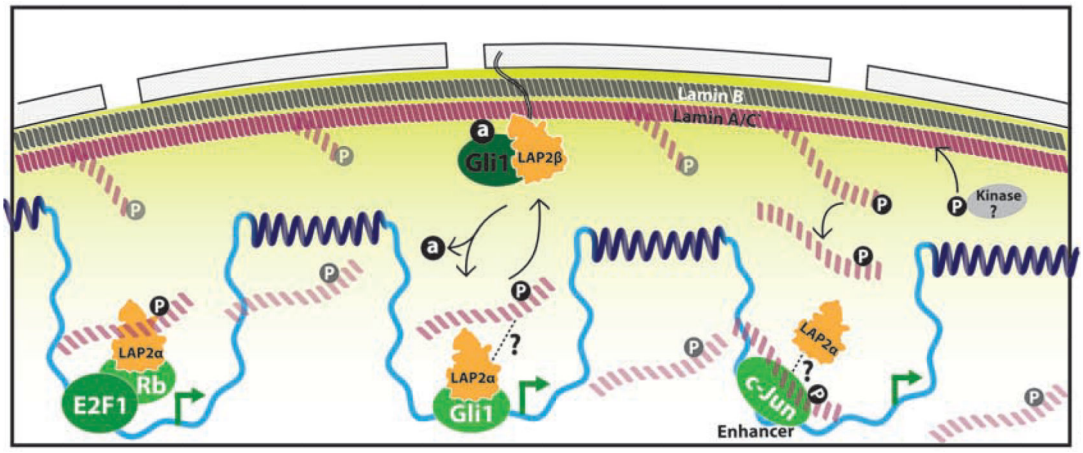

Figure 4. Nuclear Matrix: Nucleoplasmic structure enforces transcription.

Transcription factors (green) dock with nuclear matrix structures composed of depolymerized phosphorylated A-type lamins (P, pink) and LAP2 (orange) on euchromatin (light blue) to activate transcription (green arrow).