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Abbreviations used: nuclear envelope (NE), nuclear envelope transmembrane protein (NET), inner nuclear membrane (INM), outer nuclear membrane (ONM), endoplasmic reticulum (ER), nuclear pore complex (NPC), lamina-associated polypeptide 2 (LAP2), retinoblastoma protein (pRb), Emery-Dreifuss muscular dystrophy (EDMD).

Abstract
The nuclear envelope is a complex double membrane system that serves as a dynamic interface between the nuclear and cytoplasmic compartments. Among its many roles is to provide an anchor for gene regulatory proteins on its nucleoplasmic surface and for the cytoskeleton on its cytoplasmic surface. Both sets of anchors are proteins called NETs, embedded respectively in the inner or outer nuclear membranes. Several lines of evidence indicate that the nuclear envelope contributes to cell cycle regulation. These contributions come from both inner and outer nuclear membrane NETs and appear to operate through several distinct mechanisms ranging from sequestration of gene regulatory proteins to activating kinase cascades.

Introduction

The nuclear envelope (NE) is a highly organized double membrane system sequestering the nuclear contents from cytoplasmic activities. At the same time the NE must mediate all communication between the nucleoplasm and the cytoplasm. This is largely controlled by nuclear pore complexes (NPCs), >44 MDa structures which regulate trafficking of soluble macromolecules in and out of the nucleus [reviewed in 1]. However, direct connections between the cytoskeleton and the nucleoskeleton across the NE may provide an alternate mechanism for transducing signals between the cytoplasm and nucleus [reviewed in 2].

The two membrane components of the NE termed the “inner” and “outer” nuclear membrane (INM and ONM) are concentric and separated by ~50 nm in mammalian cells [30 nm in yeast; reviewed in 3]. The lumen created by this spacing is continuous with the lumen of the ER and the ONM is in fact both a part of the NE and of the ER with which it is continuous. Nonetheless, there are many distinctions between the protein content of the ONM and the ER, in terms of both ER proteins that are absent from the ONM [3,4] and ONM-specific proteins such as Syne/ Nesprin proteins. Nesprins interact either directly or indirectly with the cytoskeleton [reviewed in 2] and facilitate nuclear positioning necessary for cell polarization [5].

The INM of any given mammalian cell contains probably on the order of 100 different transmembrane proteins, many of which are tissue specific [6-8]. Very few of these proteins have been analyzed in detail, but most of those tested have been found to interact with chromatin and the intermediate filament Lamin polymer that underlies the INM [reviewed in 9,10]. The Lamin polymer and associated proteins are collectively referred to as the nuclear lamina, and have been shown to play critical roles in NE disassembly at the beginning of mitosis, nuclear shape and mechanical stability, nuclear anchoring/ migration within the cell, signaling cascades, as well as support of replication, transcription, and splicing [reviewed in 9,10].

Several studies also indicate a role for the NE in cell cycle regulation. It appears that this role can be achieved by many different mechanisms, ranging from NE functions in gene regulation to NE involvement in cytoskeletal organization to as yet undefined mechanisms. The consequences of this regulation cover the range from controlling entry into S-phase for stem cell proliferation to initiating withdrawal from the cell cycle and could explain NE functions in several debilitating diseases such as muscular dystrophies and the premature ageing syndrome Hutchison-Gilford Progeria [reviewed in 11,12].

The Nuclear Envelope in Mitosis

At the most simplistic level the NE can affect the cell cycle in mitosis. This statement may seem paradoxical since the NE is absent from higher eukaryotes during mitosis; however, defects in NE disassembly in prophase could have pleiotropic consequences. If the NE does not disassemble then tubulin cannot get in to assemble the mitotic spindle. Along a similar line, if NE contacts with the cytoskeleton are not dissolved then the mitotic scaffold may be disrupted or cytokinesis defective. If the NE only partially disassembles and sister chromatids remain in contact with any remaining NE then the segregation of chromosomes would be compromised. An early and possibly driving force in NE disassembly is the hyperphosphorylation of Lamins, which destabilizes the Lamin polymer [13,14]. Mutations in critical phosphorylation sites blocked entry into mitosis [15]. Thus failure to properly disassemble the NE can negatively impact on mitotic progression.

A second resolution to the paradox can be found in the question of what happens to NE proteins when the NE is no more? Rather than being turned over, these proteins appear to have separate functions in mitosis. Both NPC structural components (the Nup107-160 complex) and regulators of nucleo-cytoplasmic transport through the NPCs (Ran-GTP and Importinβ) have mitotic roles in spindle assembly and on kinetochores [16-18]. Lamins and several NETs also have been found on mitotic spindles and/ or the centrosome [8,19,20].

The nucleoskeleton is connected to the cytoskeleton via the LINC complex [21]. The basic units of this complex are SUN domain NETs in the INM and KASH domain proteins in the ONM. The SUN domain proteins bind lamins while the KASH domain Syne/ Nesprin proteins directly or indirectly bind to cytoskeletal components and centrosomes [reviewed in 2]. A recently identified KASH domain protein, KDP-1 in *C. elegans*, was found to be important for the timing of cell cycle progression between the end of S phase and entry into mitosis [22]; however, the mechanism of its action remains speculative.

Cell Cycle Regulation by Transcription Factor Sequestration

One mechanism by which NE proteins can affect cell cycle regulation is by sequestering transcription factors critical for initiating the cell cycle (Figure 1). The best-studied mechanism is the interaction of the retinoblastoma protein (pRb) with lamin A [23] and LAP2α [24]. pRb regulates the cell cycle at the G1/S transition by regulating the E2F family of transcription factors [reviewed in 25]. Hypophosphorylated pRb sequesters E2F early in the G1 phase of the cell cycle. In cells preparing to divide pRb becomes gradually phosphorylated at first by cyclin D/cdk 4 and 6 and later by cyclin E/cdk2. The phosphorylation releases E2F transcription factors so that they can activate genes involved in S-phase progression [26,27].

The interaction of pRb with Lamin A and LAP2α serves to both sequester the pRb/E2F complex and stabilize it from degradation by the proteasome [28]. Overexpression of LAP2α results in cell cycle arrest presumably by stabilizing the complexes to the point that E2F transcription factors are never released [24,29]. Knockdown of Lamin A yields a similar outcome in cell cycle arrest, but presumably from the opposite effect of destabilizing pRb complexes so they are degraded by the proteasome [30]. The importance of the Lamin A-LAP2α-pRb complex is underscored by observations that depletion of LAP2α in fibroblasts stimulates cell proliferation [29] and hyperproliferation of erythroid and epidermal progenitors was observed in mice with LAP2α disruption [31]. It is not clear, however, that this Lamin A-LAP2α-pRb interaction occurs at the NE: while most members of the LAP2 family are NETs, LAP2α is a soluble splice variant distributed throughout the nucleoplasm and though most Lamins are at the NE there are also nucleoplasmic pools [32].

Nonetheless, though less characterized, similar types of interactions occur for several NETs that due to their membrane spans are restricted to the NE. LAP2β, a membrane bound splice variant of the LAP2 gene, binds the transcriptional regulator Germ cell-less [33]. Transcription factors and other transcriptional regulators such as Germ cell-less, Btf, and Lmo7 have all been found to bind to the NET Emerin [reviewed in 34] while Smad transcription factors bind the NET MAN1 [35,36]. Phosphorylation of NETs may regulate such interactions as some Emerin residues are phosphorylated at particular stages in the cell cycle [37,38]. Thus it appears that

sequestering transcriptional regulators at the NE is a common mechanism by which NETs can regulate gene expression and correspondingly various aspects of the cell cycle.

Many New Tissue-Restricted NETs Influence Cell Cycle Progression

To identify additional NETs that contribute to cell cycle regulation, 39 novel NETs were screened for their ability to alter flow cytometry cell cycle/ DNA content profiles upon exogenous expression [39]. Eight had strong effects with seven increasing and one decreasing the 4N:2N DNA content ratio. A secondary screening of this subset found that these effects were lost or significantly impaired for two NETs when tested in cells lacking the p53 master regulator. Thus 75% of the NETs that affected the cell cycle did so by novel or less characterized pathways. These NETs, NET11/Sccpdh, NET31/Tmem209, Tmub1, Fam3c, Magt1, and Tmem126a, are generally uncharacterized proteins with no known functions. They are also highly restricted in expression: according to the BioGPS transcriptome database [40] Tmub1, Fam3c and Magt1 are all expressed more than 5x higher in certain blood cell types compared to the median value for over 80 tissues examined while Tmem126a is expressed in blood at roughly 50x higher than the median. These four NETs were all identified in a proteomic study of NEs isolated from blood [6]. In contrast, NET11/Sccpdh was preferentially expressed in brain and testis [40].

Of the NETs that required p53 for their effects on the cell cycle, one was widely expressed and the other more restricted to liver and fat cells [39]. The widely expressed NET59/Ncln has separately been linked to TGFß signaling pathways through an indirect interaction with Smad proteins [41]. Smads also interact with the NET MAN1 [35,36], which is also widely expressed. As the NET Emerin also intersects with signaling pathways (ß-catenin; [42]), widely expressed NETs may yield effects on the cell cycle through such interactions with well characterized signaling pathways.

NET4/Tmem53 was not only dependent on p53 but also on pRb for its effects. Knockdown of NET4/TMEM53 resulted in a decrease in phosphorylated pRb along with a doubling of p53 levels and a 7-fold increase in p21. These changes were all dependent upon active p38 MAP kinase [39]. This kinase is often associated with stress pathways [43]. The consequence of these changes was that cells withdrew from the cell cycle, becoming prematurely senescent. The preferential expression of NET4/Tmem53 in liver and fat cells and the other NETs in blood is consistent with the tissues where they were originally identified [6,10]. That so many NETs that were positive in the screen are tissue-specific suggests that they might participate in differentiation and/ or tissue regeneration through maintenance of satellite stem cell renewal in particular tissues.

Four of the eight NETs with effects in the screen have been tested for compartmentalization between the INM and ONM and interestingly NET4/Tmem53 and NET31/Tmem209 were both restricted to the ONM while NET59/Ncln and Magt1 appeared in the INM. This distribution is consistent with the hypothesis that some of these NETs will influence the cell cycle through ONM connections to the cytoplasm and others through INM regulation of gene expression.

Summary

Over a dozen different NE proteins have been linked to human diseases ranging from muscular dystrophies to neuropathies to premature aging syndromes [11,12]. Misregulation of the cell cycle is one of the three favored hypotheses for how NE

proteins can cause disease, possibly by blocking stem cell renewal. This hypothesis is supported by the observation that the Rb pathway is misregulated in the NE-linked Emery-Dreifuss muscular dystrophy (EDMD) and by disruption of the NET Emerin in mice [44,45]. As misregulation of pRb through NE defects caused hyperproliferation of erythroid and epidermal progenitor cells [31] the misregulation in EDMD could result in an early loss of muscle satellite cells. This is consistent with the timing of disease onset in late childhood.

Additionally, two Emerin mutations caused a near doubling in the length of the cell cycle [46]. While this points to a possible role of the cell cycle in EDMD pathology, the fact that four other EDMD mutations tested had no effect indicates that there must be multiple pathways to disease pathology. Alternatively, it may just be an issue of severity as knockdown of Emerin alone in *C. elegans* did not have notable effects on cell division whereas combined knockdown of Emerin and the NET MAN1 effectively blocked cell division [47].

The range of possible cell cycle links found for EDMD parallels the many ways that the NE has been found to intersect with the cell cycle (Table I). The fact that nearly 20% of new NETs tested affect the cell cycle [39] further suggests that we have just uncovered the proverbial tip of the iceberg for NE regulation of cell cycle progression.

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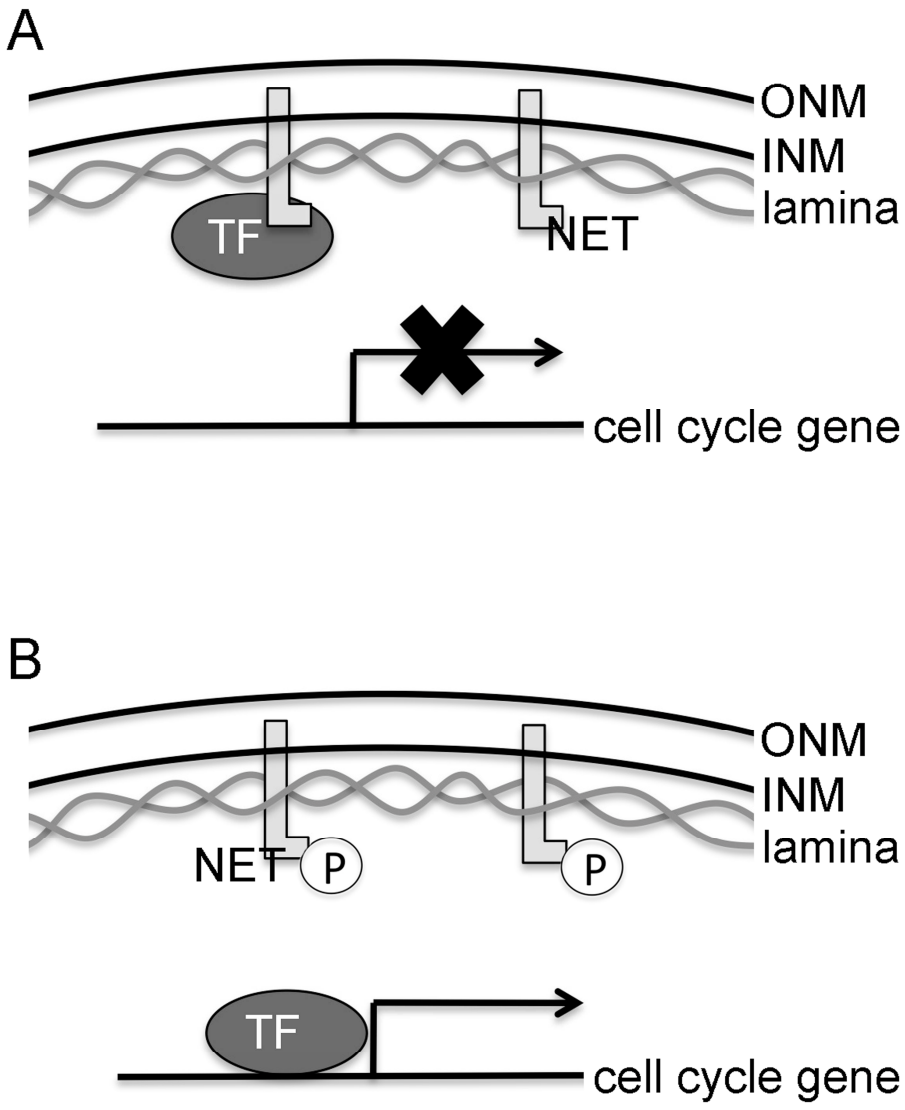
Table I. Examples of NE proteins with various cell cycle effects

NE protein	Up- or down-regulation	Cell cycle effect	Reference
Lamin A and LAP2α	downregulation of LAP2α	hyperproliferation of progenitor cells	[29-31]
Emerin and MAN1	downregulation	chromosome segregation defects	[47]
KDP-1	downregulation	timing between S-phase and mitosis	[22]
NET4/Tmem53	upregulation	G1 accumulation	[39]
	downregulation	cell cycle withdrawal	[39]
NET59/Ncln	upregulation	p53-dependent G2/M accumulation	[39]
	downregulation	defect in Smad signaling	[41]
NET31/Tmem209	upregulation	G2/M accumulation	[39]
NET11/Sccpdh	upregulation	G2/M accumulation	[39]
Tmub1	upregulation	G2/M accumulation	[39]
Fam3c	upregulation	G2/M accumulation	[39]
Magt1	upregulation	G2/M accumulation	[39]
Tmem126a	upregulation	G2/M accumulation	[39]
NET5/Samp1	downregulation	centrosome positioning defect	[19]
Ran	upregulation	aids spindle assembly	[17]
Importinβ	upregulation	aids spindle assembly	[18]

Figures and Legends



to Figures

Fig. 1.

NETs can affect the cell cycle by sequestering transcriptional regulators. A. Transcription factors (TF) are kept away from their cell cycle gene targets in the nucleoplasm by binding to NETs. This is similar to how overexpression of LAP2α causes cell cycle arrest. B. Release of the transcriptional regulator from the NET, in this case suggested to result from NET phosphorylation, enables transcriptional activation of the cell cycle gene target.

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