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Nuclear envelope links to inherited disease gave the conundrum of how mutations in near-ubiquitous proteins can yield many distinct pathologies, each focused in different tissues. One conundrum-resolving hypothesis is that tissue-specific partner proteins mediate these pathologies. Such partner proteins may have now been identified with recent proteome studies determining nuclear envelope composition in different tissues. These studies revealed that the majority of the total nuclear envelope proteins are tissue restricted in their expression. Moreover, functions have been found for a number these tissue-restricted nuclear envelope proteins that fit with mechanisms proposed to explain how the nuclear envelope could mediate disease, including defects in mechanical stability, cell cycle regulation, signaling, genome organization, gene expression, nucleocytoplasmic transport, and differentiation. The wide range of functions to which these proteins contribute is consistent with not only their involvement in tissue-specific nuclear envelope disease pathologies, but also tissue evolution.

Introduction

The nuclear envelope (NE) was historically viewed as little more than a physical barrier, like the walls of a mediaeval city (**Fig. 1**). Such cities typically had a double wall or moat for protection and accordingly the NE is comprised of two separate lipid bilayers, the inner and outer nuclear membranes (INM and ONM) with a lumen in between.[1] That the NE would have two "walls" vs. the single lipid bilayer of the plasma membrane reinforced the basic functional view scientists had of this structure to protect the all-important genome. It has been proposed variously that the original evolution of the NE was a fortuitous consequence of the growth of tubular ER or plasma membrane invaginations[2] and that it enabled increasing genome complexity by supporting regulation and accurate segregation of a larger genome in mitosis.

The physical support to this nuclear "wall" is provided by the intermediate filament lamin polymer that underlies the INM. Lamins were the first identified NE proteins due to their abundance at ~3 million copies per mammalian nucleus.[3] They form 10 nm wide filaments from the stacking of coiled-coil dimers in linear arrays,[4] more like the fibers of a rope than the building block structure of actin filaments and microtubules. Though differing thus from rigid city walls this structure is actually stronger because actin filaments and microtubules will rupture under stresses that leave intermediate filaments, which can stretch to three times their length, intact,[5] and so this structure may be of greater benefit to the nucleus as we now know it—a dynamic organelle under considerable mechanical pressures from chromatin on the inside and cytoskeletal connections on the outside.[6] Lamins were likely the original intermediate filaments and are highly conserved in evolution among higher eukaryotes,[7] but not so much as this stabilizing function: recent reports indicate that nucleated organisms previously thought to lack lamins such as *Dictyostelium* and *Trypanosoma brucei* have functional homologs with this coiled-coil based structure.[8,9]

The gates of the city are the nuclear pore complexes (NPCs), large macromolecular assemblies that form transport channels at places where the ONM bends in to fuse with the INM. NPCs are built from 30 core components, called nucleoporins or Nups that are present in multiple copies according to the 8-fold symmetry of the assembled structure.[10]

All the above appeared to be still consistent with the idea of the NE as just a protective barrier, but in a mediaeval city some of the most important activities, from the coordination of roads to the sentries to the markets and general commerce, took place at the walls or just inside the gates. Accordingly, a greater functional complexity for the NE began to be realized with the discovery that both the INM and ONM contain a variety of NE transmembrane proteins (NETs). The first NETs were identified starting in 1988 by microscopy screening, genetic and biochemical means, mostly based on associations with the lamin polymer. Novel NETs continued to be discovered at a pace of about one per year (reviewed in ref. 11) until their number grew exponentially with the application of proteomic approaches a little over a decade later.[12,13] The study of NETs and lamins in the past 20 years has now linked the NE to functions ranging from cell and nuclear mechanical stability to cell cycle regulation and stem cell maintenance, signaling cascades, genome organization and gene expression.

Lamins and several of the earlier discovered NETs have also been linked to many human diseases. These range from muscular dystrophies[14-18] to lipodystrophies,[19,20] cardiomyopathy,[21] neuropathy,[22] dermopathy,[23] osteopoikilosis (isolated, together with melorheostosis or as a symptom of Buschke-Ollendorff syndrome),[24] dystonia,[25-27] and premature aging syndromes.[28-30] The new NETs identified by proteomics may provide an answer to a conundrum regarding these diseases, namely how can mutations in near ubiquitous proteins in the NE cause diseases restricted to specific tissues? A potential resolution can be found in the "guilt by association" hypothesis that disease-causing mutations in relatively ubiquitous NE proteins might disrupt binding to as yet unidentified tissue-specific partner proteins to generate pathology in that particular tissue.[31] This idea is supported by observations that many disease-linked NE proteins appear to function in complexes and that few have specific enzymatic functions themselves that could result in pathologies. Apart from the structural functions of the lamins and NETs of the SUN and nesprin families, the proteins thus far mutated in NE diseases have few inherent functions. Only LBR, which is mutated in the bone disorder Greenberg skeletal dysplasia,[32] has been shown to have an enzymatic activity—that of a sterol C-14 reductase.[33] Other NETs linked to disease have no known enzymatic functions, but instead appear to influence a wide variety of activities through their binding partners, of which they have a great many.[11] Indeed, observations that Emery-Dreifuss muscular dystrophy (EDMD) can be caused not only by lamin A mutations,[15,17] but also by mutations in its interacting partners emerin[14] and, at least indirectly, nesprins[18] demonstrate that these proteins are part of larger complexes that yield disease when disrupted and support the "guilt by association" hypothesis. This idea is also supported by observations that NE-linked diseases tend to be genetically heterogeneous, with at least 19 variants described thus far for limb-girdle muscular dystrophy[34] and 30 for Charcot-Marie-Tooth disease.[35] If unidentified tissue-restricted components of large NE protein complexes do indeed mediate the tissue-restricted disease pathologies they could themselves potentially cause additional disease variants.

Nuclear Envelope Proteome Tissue Specificity

As a first step to attempt to identify candidate proteins that mediate tissue-restricted NE disease pathologies, new proteomic studies were undertaken on NEs isolated from different tissues. The first study determined the NE proteome of a lymphocyte-enriched peripheral blood leukocyte fraction.[36] Fluorescence activated cell sorting (FACS) revealed that the fraction was roughly 75% T-cells and helper T-cells. The cells from each blood donor were divided in two fractions. One was activated by treatment with phytohemagglutinin, because an enormous amount of dense peripheral chromatin can be observed by electron microscopy at the NE in the untreated cells and this largely dissipates upon such activation. Thus it was expected that some differences in protein composition at the periphery must occur to direct the visual differences in attached chromatin.

Indeed, not only were there many proteins identified that had not been found in the original proteomic studies[12,13] and which were preferentially expressed in blood or blood specific according to transcriptome data,[37] but differences were observed in NET composition even between the two states of the same cells from the same donors (**Fig. 2A**). Moreover, in keeping with the idea that some of these differing proteins would contribute to chromatin organization, visual screens identified different sets of blood-specific NETs that could promote chromatin compaction or alter spatial genome organization.[36]

A second study used identical conditions for extractions and mass spectrometry to investigate the NE proteome of skeletal muscle.[38] Whereas the lymphocyte nuclei are round and have a very large amount of dense peripheral chromatin, most of the muscle nuclei are ovoid, relatively flattened, and have an intermediate amount of dense peripheral chromatin. Moreover, there is a greater tendency for centromeres to be at the nuclear periphery in differentiated muscle cell nuclei,[39] further suggesting that there would be differences in NE INM protein composition. There are likely to be differences in the ONM as well because the lymphocytes have a single nucleus and a relatively small cytoplasm whereas muscle cells are syncitial with many nuclei in an individual cell. The muscle NE proteome also included many proteins that were not found in other NE proteomic studies[38] and, again, transcriptome analysis indicated that many of these proteins are either preferentially expressed in muscle or muscle-specific.

The liver NE proteome had been analyzed previously,[13] but could not be directly compared with the leukocyte and muscle studies because these later studies had many more biological and technical replicates using more sensitive mass spectrometers as well as using multiple sequential proteolytic digestions, which was found to increase identification of transmembrane proteins presumably lost otherwise because of aggregation of hydrophobic regions. Thus, a third study used these same conditions to re-examine the proteome of liver NEs.[40] The new liver NE proteome identified roughly 2½ times more proteins compared with the original study; however, these proteins for the most part were not ones found in the leukocyte and muscle studies. Instead, many were, like those found in the original study, preferentially expressed in liver according to the transcriptome data.[37]

The preferential tissue expression of many of the predicted transmembrane proteins identified in the three studies was directly confirmed by both RT-PCR and western blot where antibodies were available.[40] Moreover, staining of rat tissue cryosections with these antibodies demonstrated that those found by proteomics only in a particular tissue yielded the characteristic "rim" staining of the NE only in that tissue, while only background staining was observed for the tissues where it was not found by the mass spectrometry (**Fig. 2B**). This confirmed both their tissue-specificity and their qualification as NETs.[40] All in all, less than 20% of the total NE proteins identified and a similar number of the putative NETs identified were found in the NEs of all three tissues (**Fig. 2C**). This is especially remarkable given that none of the three tissues investigated represented a homogeneous population of single
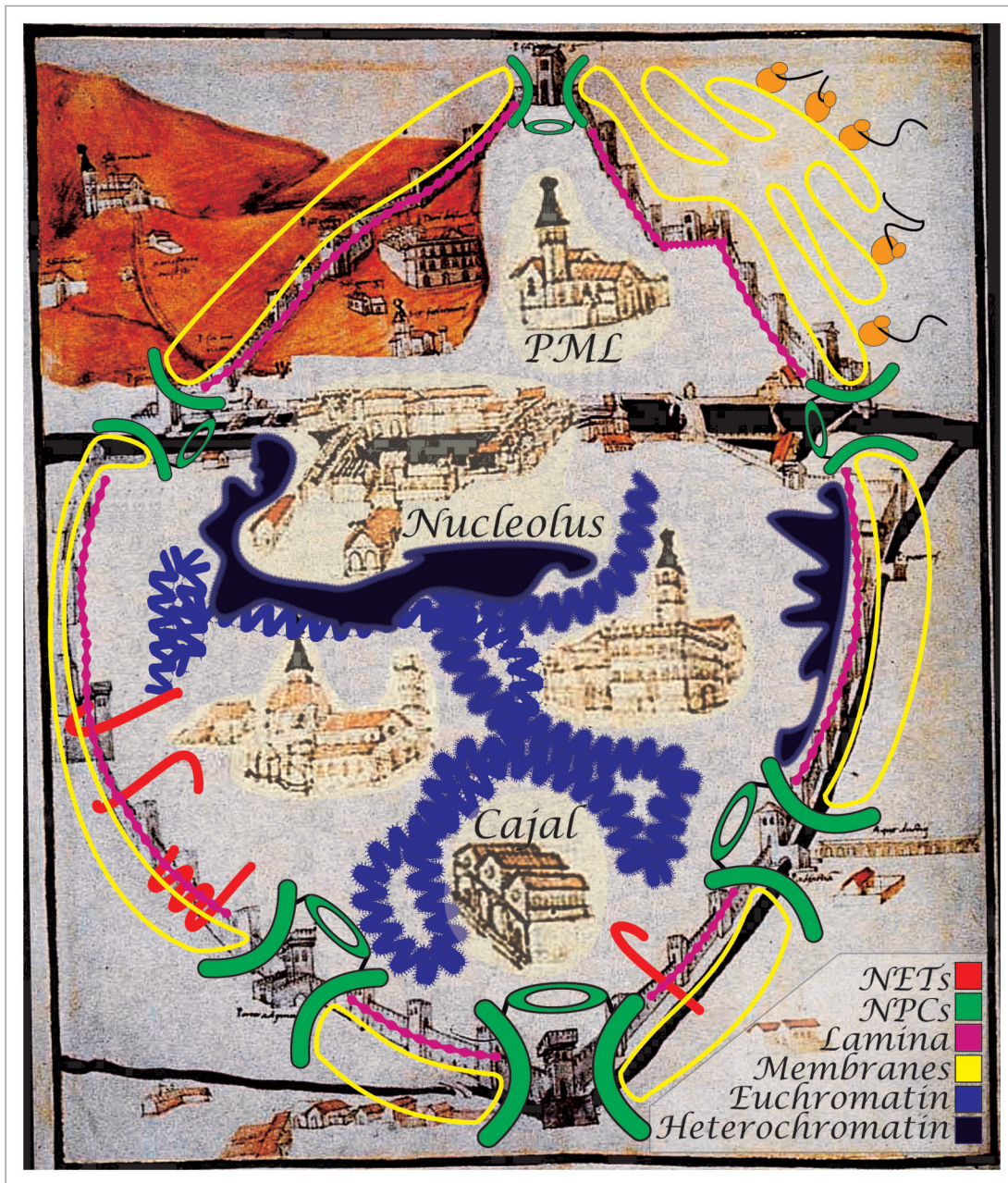
Figure 1. The mediaeval nuclear envelope. Historically the nuclear envelope (NE) was viewed as little more than a barrier, like the walls of a mediaeval city. The NE has a double membrane structure with inner (INM) and outer (ONM) membranes and the ONM is continuous with the ER. An intermediate filament lamin polymer underlies the INM, giving it stability, and is connected to the INM by several NE transmembrane proteins (NETs).

cell types. Blood leukocyte-enriched populations were >75% lymphocytes, which were mostly T-cells and helper T-cells but also included cytotoxic T-cells and B-cells, yet the populations also included 5–20% myeloid cells. Muscle contains connective tissue, nerves and blood vessels, not just myocytes. Similarly, liver may be comprised of about 80% hepatocytes, but it also contains nerves and blood vessels, blood cells and fat cells. Thus, it might have been expected that the cell types shared between different tissues would bias the results toward overestimating the proportion of shared proteins among the sampled tissues. Many of the newly identified NETs appear to be very specific to

the cell types and tissues in which they were found while others are expressed in a subset of tissues according to transcriptome data;[37] for simplicity we will use the term "tissue specific" henceforth.

The unexpectedly high degree of tissue-specificity observed raised the concern that contaminants from other organelles might be contributing to the tissue differences. Such potential contaminants can be estimated based on proteome studies of other organelles and GO-functional/ subcellular localization annotations from the Gene Ontology database.[41] The major membrane contaminants of NE preparations would be expected

from the ER that is continuous with the NE and from mitochondria that can stick to NEs and also be caught in NE invaginations. Both of these organelles have been extensively investigated and their compositions have been determined by proteomics.[42-44] Proteins identified in the NEs from different tissues that were known to associate with these potentially contaminating organelles accounted for slightly over 10% of all the proteins identified in the NEs; however, they were found in all tissues examined and so could not account for the tissue differences observed.[40] In the case of ER contamination it is possible that, having not been specifically analyzed for tissue differences, tissue-specific ER contaminants are under-represented in the analysis; however, the behavior of so-called contaminants was the same for both ER and mitochondria and the mitochondrial proteome was determined from several different tissues. In fact, tissue differences were observed in the mitochondria study,[43] but these were much smaller than those observed in the NE studies (**Fig. 2D**). Thus it is unlikely that the tissue differences observed in the NE proteome reflect tissue differences in expected contaminating organelles.

It is in fact more likely that the potential contaminants from these other organelles have separate roles in the NE as it has been estimated that 40% of proteins have multiple subcellular localizations.[45] This idea is supported by the proteomics data in that if the proteins reflected contamination from the other organelles their relative abundance in the NE preparations should reflect their relative abundance within the organelles. However, spectral counts (a semiquantitative measurement of protein abundance used in mass spectrometry based on the number of times a particular peptide is recovered) for the ER and mitochondrial proteins found did not reflect their relative abundance within those organelles. Thus it is likely that many of these potential contaminants are indeed bona fide NE proteins.

Consistent with the likelihood that the identification of the first NE proteins reflected their relatively higher abundance, the NETs that were more tissue-specific were identified with far lower spectral counts than the first characterized ones. Lamins A/C and B1 were identified with 3772 and 2301 spectra respectively and the number of spectra for the first NETs identified were 1826 for LAP1, 1473 for LAP2, 384 for LBR, 751 for SUN1, and 3184 for SUN2. Nurim and emerin were comparatively low with only 51 and 124 spectral counts respectively. In contrast, the vast majority of the new NETs identified and especially those identified in just one tissue had a spectral count lower than 20.[40]
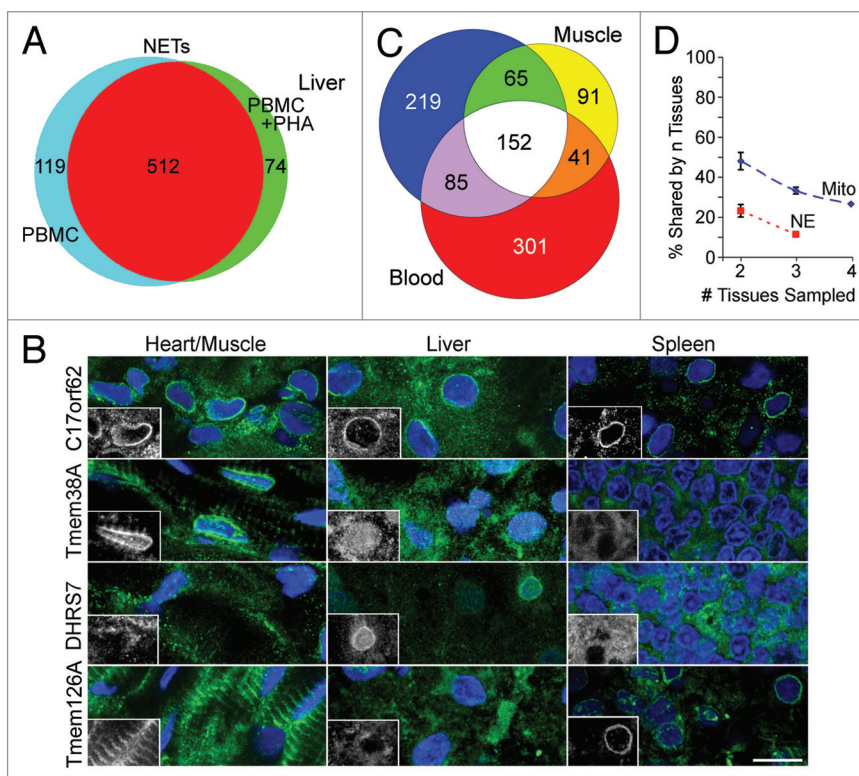
Figure 2. Nuclear envelope proteome composition. (**A**) Differences in NETs identified between unstimulated and PHA-activated states of the leukocyte NE. (**B**) Staining of rat tissue cryosections with antibodies to different NETs confirms their tissue specificity. C17orf62 was identified in all tissues and antibodies gave a nuclear rim staining confirming NE residence in all three tissues. In contrast Tmem38A antibodies only give a nuclear rim staining pattern for muscle where it was uniquely identified, DHRS7 antibodies for liver where it was uniquely identified and Tmem126A antibodies for blood where it was uniquely identified. Images taken with permission from Figure 5C in Korfali et al., 2012 Nucleus.[40] (**C**) Less than 20% of NETs identified in the three proteome studies of blood leukocytes, muscle and liver were found in all three tissues. Figure taken with permission from Figure 4A in Korfali et al., 2012 Nucleus.[40] (**D**) Comparison of NE tissue variation with mitochondrial tissue variation. The percentage of the total proteins identified that were found in multiple tissues is plotted against the number of tissues.

Tissue-Specific Contributions to Disease Pathology

The possibility that some of the newly identified tissue-specific NETs contribute to NE-linked disease pathologies is supported by some having already been linked to other similar inherited diseases (**Table 1**) and by the pathologies resulting from knockout in mice for others. Lamin A and the well-characterized and similarly widely expressed NETs emerin, nesprin1, nesprin 2, and the soluble α splice variant of the NET LAP2 have all been linked to neuromuscular disorders, muscular dystrophies or cardiomyopathies. In addition the more tissue-specific NETs identified by proteomics of muscle NEs DTNA, VMA21, RYR1 have also been linked to neuromuscular disorders, muscular dystrophies or cardiomyopathies. Thus these NETs could potentially mediate the tissue-specific pathologies of the diseases caused by the widely expressed NETs or potentially cause other variants of these diseases. Other muscle NETs like TMEM38A or Popdc2 are not linked to a disease thus far, but animal models underline their potential role in human muscular disease.

Table 1. Confirmed NETs associated with disease

Protein name	Gene name	ENSG number	Associated disease	OMIM number	Reference	Tissue identified in (see ref. 40)
VMA21 vacuolar H⁺ -ATPase homolog	VMA21	ENSG00000160131	Myopathy, X-linked, with excessive autophagy	%310440	46	muscle
Ryanodine receptor 1	RYR1	ENSG00000196218	Central core disease of muscle	#117000	53, 54	muscle
			King-Denborough syndrome, Malignant hyperthermia susceptibility 1	#145600	55	
			Minicore myopathy with external ophthalmoplegia	#255320	56	
Wolfram syndrome 1 (wolframin)	WFS1	ENSG00000109501	Wolfram syndrome	#222300	62	muscle
			Deafness, autosomal dominant 6/14/38	#600965	60	
			Wolfram-like syndrome	#614296	61	
Leucine rich repeat containing 8 family, member A	LRRC8A	ENSG00000136802	Agammaglobulinemia 5	#613506	67	blood
ATP-binding cassette, sub-family B (MDR/TAP), member 1	ABCB1	ENSG00000085563	Inflammatory bowel disease 13	#612244	149	blood
Epidermal growth factor receptor	EGFR	ENSG00000146648	Adenocarcinoma of lung, nonsmall cell lung cancer	#211980	150	liver
ALG2, α-1,3/1,6-mannosyltransferase	ALG2	ENSG00000119523	Congenital disorder of glycosylation, type Ii	#607906	151	liver and blood
Sequestosome 1	SQSTM1	ENSG00000161011	Paget disease of bone	#602080	152	liver and blood
Magnesium transporter 1	MAGT1	ENSG00000102158	Immunodeficiency with magnesium defect, Epstein-Barr virus infection and neoplasia	#300853	153	liver and blood
			Mental retardation, X-linked 95	#300716	154	
Transmembrane protein 70	TMEM70	ENSG00000175606	Mitochondrial complex V (ATP synthase) deficiency, nuclear type 2	#614052	66	blood and muscle
ER lipid raft associated 2	ERLIN2	ENSG00000147475	Spastic paraplegia 18, autosomal recessive	#611225	155	all tissues
Transmembrane protein 43	TMEM43	ENSG00000170876	Arrhythmogenic right ventricular dysplasia 5	#604400	156	all tissues
			Emery-Dreifuss muscular dystrophy 7	#614302	157	
Lamin B receptor	LBR	ENSG00000143815	HEM skeletal dysplasia	#215140	32	all tissues
			Pelger-Huet anomaly	#169400	158	
			Reynolds syndrome	#613471	159	
Thymopoietin	LAP2	ENSG00000120802	Cardiomyopathy, dilated, 1T	#613740	160	all tissues
Emerin	EMD	ENSG00000102119	Emery-Dreifuss muscular dystrophy 1	#310300	14	all tissues
LEM domain containing 3	LEMD3	ENSG00000174106	Buschke-Ollendorff syndrome	#166700	24	all tissues
Nesprin 1	SYNE1	ENSG00000131018	Spinocerebellar ataxia 8	#610743	161	all tissues
			Emery-Dreifuss muscular dystrophy 4	#612998	18	
Nesprin 2	SYNE2	ENSG00000054654	Emery-Dreifuss muscular dystrophy 5	#612999	18	all tissues

VMA21 was identified only in the muscle NEs and confirmed to target to the NE. Mutations in *VMA21*—a gene encoding an essential assembly chaperone of the vacuolar ATPase—that result in haploinsufficiency cause a myopathy with excessive autophagy characterized by intracytoplasmic autophagic vacuoles with sarcolemmal features.[46] Tmem38A was only identified in the muscle NE proteome, is both very preferentially expressed and abundant in muscle (194 spectra), and is in both the ONM and INM.[38,40] Both Tmem38A and its related but more widely expressed homolog Tmem38B appear to function as a counter ion channel for calcium release, which is very important in muscle contraction. Studies knocking out both proteins together resulted in embryonic cardiac failure and dysfunctional skeletal

muscle.[47] Though less severe, the phenotype of knocking out just the muscle-specific *TMEM38A* was also focused in muscle, with defects in vascular smooth muscle function resulting in hypertension in intact mice[48] and clear evidence of elevated Ca^{2+} pools in ex vivo muscle with a stronger initial contractile force followed rapidly by muscle fatigue.[49] Another protein contributing to this nexus[50] identified in the muscle nuclear membrane[38,40] and confirmed to target to the NE[51] is the ryanodine receptor 1 (encoded by *RYR1*) which serves as a calcium release channel. This protein also, unlike most of the tissue-specific NETs, was highly abundant with 235 spectra.[38,40] Mice carrying a homozygous targeted mutation in *RYR1* died perinatally with skeletal muscle abnormalities.[52] Mutations in

RYR1 are associated with three myopathies: (1) Central core disease, a mild congenital myopathy characterized by delay of motor developmental and mild proximal weakness;[53,54] (2) King-Denborough syndrome, a myopathy with dysmorphic features and malignant hyperthermia susceptibility;[55] and (3) minicore myopathy, a neuromuscular disorder pathologically defined by multiple areas of reduced mitochondrial oxidative activity along a limited extent of the longitudinal axis of the muscle fiber.[56] The popeye domain containing protein Popdc2, identified in only the muscle NEs and confirmed in the INM,[38] has been knocked out in multiple animal models. Morpholino-mediated knockdown of this NET in zebrafish resulted in aberrant development of skeletal muscle and heart[57] whereas *POPDC2* null mutant mice developed stress-induced bradycardia.[58] Finally, though not ye*t* tested for NE targeting, another NET identified only in the muscle NEs, DTNA (dystrobrevin), when mutated results in left ventricular non-compaction—a rare, unclassified cardiomyopathy.[59]

Not all disease-linked NETs identified in muscle make such clean associations with muscle disease: though only identified in the muscle NEs, mutations in the gene encoding WFS1 cause Wolfram syndrome characterized by optic atrophy, deafness,2 and/or diabetes.[60-62] However, this is not necessarily inconsistent with the matched tissue hypothesis because the NE proteome of eyes and ears was not determined. Indeed, transcriptome data indicate that while WFS1 is highly expressed in muscle, it is even more highly expressed in retina.[37] The case of WFS1 is worthy of further note respecting the issue of subcellular localization. WFS1 was previously reported to localize primarily in the ER,[63] but is clearly also in the NE. Though WFS1 may have multiple cellular localizations and only be at the NE in certain tissues, the ER targeting was found by overexpression in heterologous HeLa and HepG2 cells[63] that were not derived from tissues that express WFS1, and we have reported previously that heterologous overexpression of NETs can yield different subcellular localizations depending on the cell type used.[64] Different subcellular localizations have clearly been shown for Tmem70, which was originally found to localize to mitochondria and be mutated in patients with mitochondrial complex V deficiency.[65] Tmem70 was found in both muscle and leukocyte NEs and according to transcriptome data are preferentially expressed in skeletal muscle, brain and blood.[37] Not surprisingly, thus, it has also been linked to a neonatal encephalocardiomyopathy.[66]

Another tissue specific NET involved in a tissue specific disease was LRRC8A (Leucine-rich repeat-containing protein 8A) that was only found in the blood leukocyte NEs. A mutation of *LRRC8A* resulted in a truncated protein inhibiting B-cell development.[67] Thus muscle-specific NETs can cause muscle disease and blood-specific NETs can cause blood disease.

While the examples above make a reasonable case for some of these new tissue-specific NETs contributing to the pathologies of the diseases linked to widely expressed NE proteins, it should be noted that there are other possible mechanisms to achieve the tissue-specific pathologies. First of all, we have focused just on the NETs, but it is just as likely that soluble tissue-specific proteins that interact with the NE proteins mutated in disease could mediate the specific tissue pathologies. An example that could potentially explain emerin mutations causing EDMD is its binding to tissue-specific transcription factors. Emerin binds to the Lmo7 transcription factor that is expressed at high levels in muscle and heart and regulates a variety of muscle-related genes.[68] Lmo7 has also been shown to be important for heart development in zebrafish,[69] and so could easily be involved in EDMD pathology where heart is typically affected in addition to skeletal muscle. Emerin has also been shown to interact with Btf,[70] a death-promoting transcriptional repressor expressed at high levels in blood.[37] Not surprisingly, these soluble tissue-specific proteins were also identified in the NE data sets along with many other potentially relevant transcriptional regulators. Yet another mechanism to achieve tissue-specificity is through tissue-specific splice variants. Mutations causing EDMD have also been identified in nesprin1α and nesprin2β—both small muscle specific isoforms of these NETs.[18] We still do not know the full range of splice isoforms encoded by the *SYNE* (nesprin) genes, but based on those identified thus far it is likely that there will be many more. Finally, there could be differences in how the metabolic and physical function of different cell types intersects with the loss of a common NE function. However, countering this point is that even for the different lamin A-linked muscular dystrophies, where one could argue pathology is directed by the physical stress of muscle usage, there are differences in the specific muscle groups affected in the different diseases.[71] Nonetheless, a combination of any of the above factors could work synergistically to direct pathology to a particular tissue. For example, though emerin and LAP1 are both near ubiquitously expressed, there are tissue differences in the actual level of expression. It was recently found that these two proteins work together to support skeletal muscle maintenance such that they could contribute together to muscle disease.[72]

Tissue-Specific Contributions to Cell Cycle Regulation

The basic regulation of the cell cycle through its stages (G_1, S, G_2, and M) is common for cells in all tissues. Nonetheless, cells in different tissues distinguish themselves by length, frequency, directionality and cause of induced cell divisions. These parameters can differ for cells in the same tissue or even the same cell type at different stages of differentiation such as in the layers of an epithelium. In fact the best-studied example of a NE link to the cell cycle is a complex that when disrupted in mice causes hyperproliferation of the progenitor layers of epidermis.[73] This complex is formed from lamin A and LAP2α binding together to the retinoblastoma protein (pRb), a key cell cycle regulator.[74,75] LAP2α is a soluble splice variant of the NET LAP2β. Disruption of this complex resulted in reduced pRb levels and a reduced capacity to undergo cell-cycle arrest in response to DNA damage[76] or accumulation of pRb and G1 arrest[77] depending on the cell type used. These results are consistent with the idea that the Lamin A/LAP2α complex both sequesters and stabilizes pRb so that, depending on the total milieu of cell cycle controls in a particular cell/ tissue type, different outcomes can be achieved.

Though other mechanisms for how NE proteins can influence the cell cycle are less worked out, such influences are likely relevant to some NE-linked diseases. A lamin A mutant that causes EDMD yielded defects in myogenic differentiation when expressed in a mouse in vitro differentiation system, apparently because myoblasts become unable to exit the cell cycle which is a necessary prerequisite to form myotubes.[78] Additionally, transcriptional fingerprints obtained from both emerin and lamin A-linked EDMD patients revealed that pRb pathways as well as MyoD pathways are impaired, suggesting disruption of cell cycle regulation and myogenic differentiation impairs muscle regeneration in the disease.[79] This is also consistent with a doubling in the length of the cell cycle observed in tissue culture cells expressing some emerin mutations known to cause EDMD.[80]

One can actually envision many possible ways that NE proteins could affect cell cycle regulation. (1) The first is by direct interaction with cell cycle regulators such as has already been shown with pRb. In general, sequestration at the NE (and thus away from target genes) of any transcriptional regulator involved in the cell cycle would be expected to have profound effects. (2) A second mechanism by which the NE could influence the cell cycle is involvement in the transmission of signaling cascades from extra or intracellular signals into the nucleus. This could occur via NETs involved in signaling (see signaling section below) or by direct transport through the NPC. (3) Though the NE of higher eukaryotes is disassembled during mitosis, due to the many interactions of lamins and NETs with chromatin, failure to properly break these contacts could activate a checkpoint from lagging/ stuck chromosomes (see below). (4) NE proteins can also affect the success of mitosis through separate functions when the NE is disassembled. Lamin B and NPC-associated proteins contribute to spindle function during mitotic chromosome segregation.[81,82] Tissue-specific NETs may also contribute as a subset of these is enriched at the spindle poles during mitosis (see later cytoskeletal section).[38,83] (5) Finally, NETs could intersect with various cell cycle regulatory pathways or activate these pathways through mechanical stress or cancer activation. This latter mechanism is supported by recent findings with the tissue-specific NET4/Tmem53.[84]

NET4/Tmem53 was linked to the cell cycle in a FACS-based screen of 39 newly identified NETs from the liver and leukocyte proteomic studies.[13,36] Comparing the 2N DNA peak (representing the G_1 phase of the cell cycle) with the 4N peak (representing G_2/M) from FACS profiles between cells transfected with various NETs and untransfected cells in the same population revealed that NET4/Tmem53 expression promoted an accumulation of the 2N or G_1 cell population.[84] This effect was dependent on the master cell cycle regulator/ tumor suppressor protein p53 as re-testing exogenous expression of this NET in p53^{-/-} cells not only lost the G_1 accumulation effect, but actually switched it to a partial G_2/M accumulation. The cell cycle effect was also lost in pRb deficient cells. Correspondingly, knockdown of *TMEM53* resulted in a doubling of p53 levels, a 7-fold increase in p21, and a dramatic reduction in pRb phosphorylation without changes in overall levels of pRb. These effects are all consistent with a well-characterized pathway of signal propagation toward cellular senescence and indeed cell cycle withdrawal was confirmed as the endpoint for both NET4/Tmem53 overexpression and knockdown. Testing for factors upstream of p53 revealed a dependency for all these effects on the p38 MAP kinase (mitogen-activated protein 14, MAPK14) that is involved in oncogenic activation and cellular stress.[84] As NET4/Tmem53 is preferentially expressed in liver, it is possible that it is used for an added layer of cell cycle control that may be needed because liver is both a highly regenerative organ and the toxin sink for the body and this combination could easily lead to cancer transformation if the cell cycle is not both tightly controlled and responsive to stress. Alternatively, p38 activation could result from NET4/Tmem53 detecting physical stress or mechanical strain during liver regeneration.

The FACS-based screen also identified seven other NETs (NET11/Sccpdh, NET31/Tmem209, NET59/Ncln, Tmub1, Fam3c, Magt1, Tmem126A; **Table 2**) with an increased accumulation of 4N (G2/M) cells.[84] Further testing revealed that the effect of all these NETs except for NET59/Ncln was independent of p53, suggesting that they are more likely to be affecting M phase of the cell cycle or operate through a completely novel mechanism. As no significant mitotic abnormalities were observed during the screen, it seems most likely that these cells arrest in G_2 in a p53-independent manner[85] opening the exciting possibility that they activate a differentiation program through the regulation of cell cycle exit. This fits with the fact that, like NET4/Tmem53, most of these NETs were very tissue specific[37] and thus might be expected to have tissue-specific cell cycle effects. It is therefore not too surprising that in a separate study NET31/Tmem209 was found to function together with the nucleoporin Nup205 to increase nuclear levels of c-myc and this may explain the role of increased NET31/Tmem209 levels specifically in lung cancers.[86] That roughly 20% of the new NETs tested had effects on the cell cycle suggests that these types of tissue-specific modifying functions may be quite prevalent.

As noted above, failure to properly disassemble the NE in prophase or reassemble it in telophase could prevent successful mitosis. NE disassembly is driven by phosphorylation of lamins, NETs and nucleoporins to release their associations with chromatin.[87-91] Failure to fully disengage NE proteins from chromatin could result in blocking of microtubule attachments to kinetochores resulting in lagging chromosomes and aneuploidy. Correspondingly, NE reassembly is driven by dephosphorylation of these NE components; however, unlike disassembly where lamins play a driving role, the NETs and nucleoporins appear to dominate reassembly. This is because many NETs bind chromatin and DNA (reviewed in ref. 92) so that they can direct the membranes in which they are inserted to the mitotic chromosomes. The NET Lem4 (ANKLE2) promotes the dephosphorylation of the chromatin protein BAF by inhibiting BAF's mitotic kinase NHK-1/Vrk-1 and simultaneously recruiting its phosphatase PP2A.[89,93] The widely expressed NETs Lap2β, emerin and MAN1 through binding BAF have been shown to be instrumental in reforming the NE by reinstating chromatin-NE interactions.[94] However, the new tissue-specific

Table 2. Novel NET functional groupings

NET	Liver spectra	Muscle spectra	Resting leuk spectra	Activated leuk spectra	Reference
Cell Cycle					
NET4/Tmem53	3	0	0	0	84
NET11/Sccpdh	118	6	2	4	84
NET31/Tmem209	537	34	17	11	84
NET59/Ncln	75	0	16	12	84
Tmub1	31	0	2	5	84
Fam3c	0	0	6	5	84
Magt1/IAG2	7	0	4	2	84
Tmem26a	0	0	17	0	84
Signaling					
NET59/Ncln	75	0	16	12	108
NET25/LEM2	18	47	14	12	109
NET39/Ppadc3	3	0	0	0	110
NET45/Dak	1	0	6	1	111
NET13/Smpd4	644	101	19	20	112
NET37/KIAA1161	5	6	0	0	113
Cytoskeleton					
NET5/Tmem201	105	163	10	2	83,126
KLHL31	0	4	0	0	38
Tmem214	100	22	10	13	38
Wfs1	0	40	0	0	38
Gene Organization					
STT3A	130	8	14	20	36
TAPBPL	4	0	6	5	36
NET29/Tmem120A	2	0	0	0	96
NET39/Ppadc3	3	0	0	0	96
NET5/Tmem201	105	163	10	2	96
NET45/Dak	1	0	6	1	96
NET47/TM7SF2	106	5	0	6	96

NETs are likely to contribute as much to this process because their nucleoplasmic regions tend to have high isoelectric points for binding the negatively charged DNA[95] and several of these tissue-specific NETs have been found to reposition chromosomes (see later chromosome repositioning section).[96]

Tissue-Specific Contributions to Signaling

The NE has to pass signals from a variety of signaling cascades to the genome to trigger changes in gene expression in response to various cellular and extracellular cues. In most cases signals were thought to pass into the nucleus through the NPC by regulated transport of activated transcription factors or other regulators that would activate transcription factors already in the nucleus. However, several widely expressed NETs have now been linked to a handful of signaling pathways. Analysis

of changes in transcription profiles between heart from a wild-type or an emerin-null mouse revealed emerin connections to 10 signaling pathways, including Wnt and TGFβ pathways and MAPK and JNK kinase cascades[97,98] and in skeletal muscle myoD and pRb pathways were affected.[99] One possible explanation of emerin's effect on a plethora of pathways, as well as on myogenic differentiation, is disruption of miRNA expression in emerin-null cells.[100] Emerin was also shown to bind β-catenin and through it affect the Wnt signaling pathway.[101] This crosstalk between emerin and β-catenin influences adipogenesis[102] so that, though widely expressed, emerin plays significant roles in multiple differentiation pathways. Interestingly, ONM resident nesprin-2 interacts with α-catenin and together they form complexes with emerin and β-catenin.[103] The widely expressed NET MAN1 has separately been shown to affect Smad/BMP/TGFβ signaling.[104-106] The initial model from this data was that

MAN1 binding to Smads simply sequestered this part of the signaling pathway at the nuclear periphery, away from targets in the genome, thus inhibiting BMP/ TGFβ signaling. However, from recent work it turns out that MAN1 directly competes with the transcription factor FAST1 for binding to Smads and then additionally recruits the phosphatase PPM1A to inactivate the bound Smads, thus delivering a double whammy knockout blow to the signaling pathway.[107] A less direct intersection with TGFβ signaling pathways appears to exist for one of the new NETs that was found to influence cell cycle progression: NET59/Ncln has been reported to form an ER-based complex with NOMO that antagonizes Nodal signaling and so affects TGFβ pathways.[108]

Several other NETs identified in the NE proteomics studies have since been linked to various signaling pathways (**Table 2**). NET25 (Lem2), a paralog of MAN1, was also widely expressed, but was found to be required for efficient myoblast differentiation. NET25 is able to complement emerin's role in myogenesis, and, like emerin, it negatively regulates the ERK1/2 pathway.[109] NET39 also affects a common kinase signaling pathway, but is very tissue-restricted in expression being highly expressed in heart and skeletal muscle[37] and strongly induced during C2C12 mouse myogenic differentiation.[110] NET39 is a member of the LPP family of membrane lipoprotein phosphatases. Its knockdown promoted myogenesis and its overexpression inhibited C2C12 differentiation.[110] NET39 was further found to interact with mTOR and it is thought to act on this signaling pathway in C2C12 myogenesis.[110] Another tissue-specific NET, NET45/DAK is highly liver specific and a member of the evolutionarily conserved family of dihydroxyacetone kinases. It has been shown to interact with MDA5, a cytoplasmic sensor of viral RNA. MDA5 activates expression of interferon-β and is essential for the innate immune response. Overexpression of DAK negatively regulated MDA5-induced interferon-β expression and its knockdown increased interferon-β levels upon viral infection.[111]

Very dissimilar signaling pathways have been linked to other NETs. NET13/SMPD4 is a member of the sphingomyelin phosphodiesterase family. Such proteins are linked to many different lipid signaling pathways.[112] Though far from tissue-specific, NET13/SMPD4 is extremely variable in expression being absent from roughly half of tissues in a large transcriptome study.[37] NET37 is preferentially expressed in muscle and a member of family 31 glycosidases. It is highly expressed in skeletal muscle and upregulated during C2C12 myogenic differentiation. Its knockdown reduces C2C12 differentiation potential by half and its catalytically dead mutant failed to rescue this phenotype, consistent with an important role for this INM-anchored glycosidase in myogenesis.[113] NET37 interacts with the IGF-II precursor and is required for IGF-II secretion. Through this it positively regulates the Akt pathway in C2C12 differentiation.[113] As more novel tissue-specific NETs are tested it seems likely that more such signaling pathways will be uncovered. Notably, unlike most of the original identified NETs, many of these appear to have enzymatic functions to contribute to these processes.

Possible Tissue-Specific Contributions to Cytoskeletal Organization and Mechanical Stability

The intermediate filament lamins and the widely expressed NET SUN and nesprin proteins have been shown to contribute to nuclear mechanical stability in experiments measuring resistance to applied mechanical forces.[6,114] The transmembrane SUN proteins in the INM bind to the lamin polymer via their nucleoplasmic region while their lumenal region is part of a connection to the lumenal region of transmembrane nesprins in the ONM. Together this nexus is called the Linkers of the Nucleoskeleton to the Cytoskeleton or LINC complex (**Fig. 3**).[115] The cytoplasmic regions of most nesprin isoforms include an actin-binding site to connect to the cytoskeletal actin microfilaments. There is also indirect evidence that LINC might be able to also connect to other cytoplasmic filaments as nesprin 3 binds plectin,[116] which can indirectly connect to all cytoplasmic filaments, and microtubule motors have been found to associate with some nesprin isoforms[117,118] (**Fig. 3**). Some have also proposed that emerin be considered as an additional LINC complex component.[119] The LINC complex is involved in a number of cellular functions including nuclear positioning, mechanotransduction, cell division and the organization of the cytoskeleton (reviewed in ref. 119). Disruption of LINC complexes induces an overall loss of mechanical stiffness across the cytoskeleton.[120] LINC complex components and possibly the complex itself are additionally important for telomere positioning through SUN proteins[121] and for association of the centrosome with the nuclear membrane through emerin.[122]

Although LINC is highly conserved, it is possible that tissue specific proteins interact with the complex and there is even some tissue specificity from orthologs and splice variants within the core LINC components. There are five SUN proteins of which SUN1 and 2 are widely expressed while SUN3, 4, and 5 are testis specific. There are four nesprins with many splice variants, and those shown to be involved in EDMD are muscle specific.[18] The nesprin effects in muscle dystrophy could be due to mechanical instability or also to disruption of nuclear positioning under the neuromuscular junction. Both a dominant-negative nesprin mutant and nesprin 1 and 2 double knockout mice fail to recruit synaptic nuclei to the neuromuscular junction in skeletal muscle.[18,123] In skeletal muscle, levels of nesprin1 in general are highest in synaptic nuclei,[124] further supporting tissue specificity of function.

Transmembrane Actin-associated Nuclear Lines or TAN lines (**Fig. 3**) are a subset of actin filaments that direct nuclear positioning in migrating cells through connections that involve LINC complex proteins nesprin2-giant and SUN2.[125] A potential tissue-specific component has recently been added to the TAN lines in that NET5/Samp1 was found to contribute to stabilizing the interaction between the TAN lines, LINC proteins and the nuclear lamina.[126] This function is assisted by the fact that NET5/Samp1 has been shown to bind lamin A/C, emerin, SUN1, and SUN2.[127] NET5/Samp1 also may play a role in aspects of cell polarity as its knockdown results in an increase
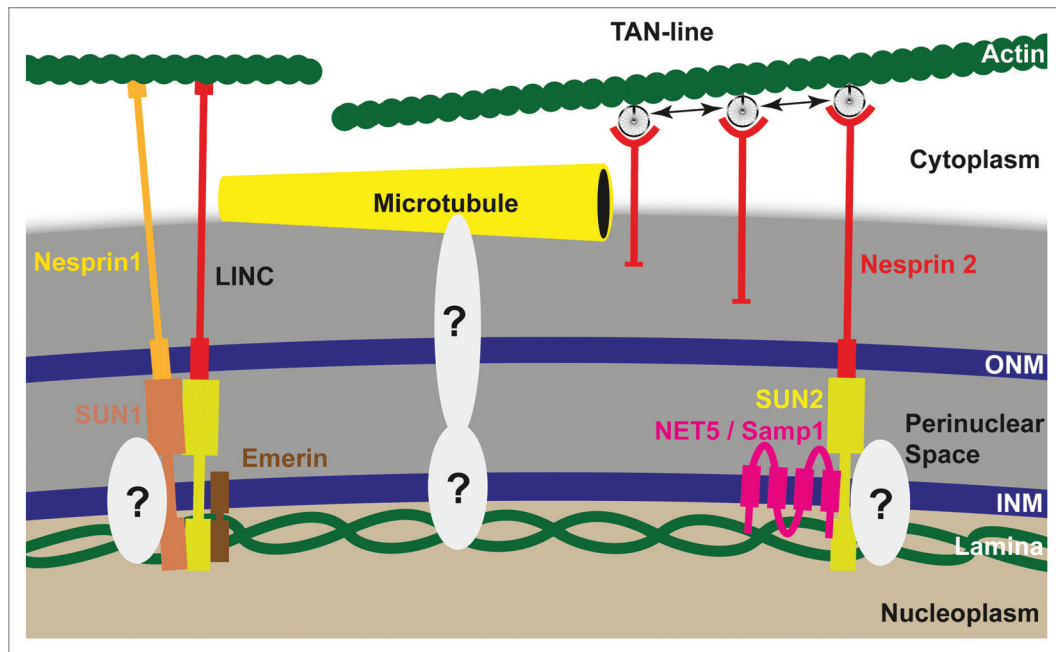
Figure 3. Nucleoskeleton and nuclear envelope connections to the cytoskeleton. The LINC complex is comprised of SUN and nesprin NETs that connect the inner (INM) and outer (ONM) nuclear membranes across the nuclear envelope lumen. From the INM SUN proteins connect to the intermediate filament lamin polymer and from the ONM the nesprins connect to actin microfilaments and possibly indirectly to other filament systems. Emerin has been proposed to function together with the LINC complex as might other as yet unidentified proteins among the tissue-specific NETs. A similar complex connects to TAN-lines that operate like train tracks on which the nucleus migrates to be in line with the leading edge during cell movements. The somewhat tissue-restricted NET5/Samp1 has been shown to function together with this complex as, again, may other tissue-specific NETs. Some muscle NETs tracked with microtubules at the nuclear surface suggesting the possibility of another complex like LINC directed specifically for microtubules.

in the distance between the NE and the centrosome.[83] These different aspects of NET5/Samp1 function may be directed in part by different tissue-specific splice forms observed using NET5 antibodies.[96] Several NETs identified in the muscle NEs were found in a visual screen to affect aspects of cytoskeletal organization (**Table 2**).[38] Of particular note, two of these muscle NETs appeared to track with microtubules at the nuclear surface, suggesting that there may be another type of LINC complex formed by different NETs that is more specific for microtubule connections. Given the promiscuous interactions of SUN domain proteins and nesprins,[120] it is likely that tissue-specific expression of their isoforms as well as potential interactions with tissue-specific NETs, as already shown for NET5/Samp1, may play an important role in the spatial and temporal control of nucleo-cytoskeletal coupling.

Tissue-Specific Contributions to Genome Organization and Gene Expression

It has been clearly shown that specific chromosomes, chromosome regions, and chromatin domains have preferred positions in the interphase nucleus and it is thought that this may function to optimize gene regulation. For example, both microscopy and biochemical approaches indicate that the gene-poor (and mostly transcriptionally inactive) chromosomes tend to be at the nuclear periphery while gene-rich (and mostly transcriptionally active) regions locate to the interior (reviewed in[128]). Though most chromosomes follow this general tendency for gene poor chromosomes to be at the periphery, the spatial organization of genes and chromosomes can also be tissue or cell-type specific. Particular genes or chromosomes tend to be found in the nuclear interior in one cell type while being at the periphery in another (reviewed in ref. 128).

The NE is thought to be one of the major drivers of chromosome and gene positioning within the interphase nucleus due to the fact that mutations in or loss of NE proteins (i.e., lamins and NETs) can result in altered spatial chromosome organization (reviewed in ref. 128). The effects of widely expressed NE proteins on chromosome positioning likely reflects the general tendency for gene poor chromosomes to be at the periphery driven by interactions between lamins and core histones[129] and several NETs that bind silenced chromatin. For example LBR binds heterochromatin protein 1 (HP1)[130] and LAP2β binds the transcriptional repressor germ-cell less[131] and histone deacetylase 3 (HDAC3).[132] Accordingly, recent studies have shown these proteins to function in generic aspects of spatial genome organization through an interaction with lamin B1, LAP2β, and HDAC3[133] and general peripheral positioning of heterochromatin through LBR.[134]

The mechanism for achieving tissue-specific patterns of spatial genome organization is expected to also function through affinity tethering, but the players have until recently remained obscure. However, with the identification of so many tissue-specific NETs a visual screen was recently engaged that found
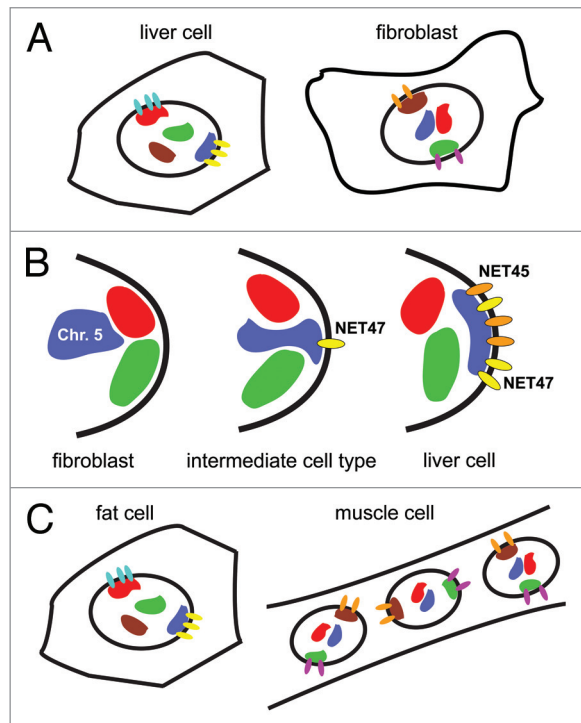
Figure 4. Tissue-specific radial chromosome organization can be mediated by tissue-specific NETs. (**A**) Distinct spatial chromosome arrangements can be achieved by the differential expression of tissue-specific NETs that have been shown to each reposition partially distinct yet overlapping sets of chromosomes to the nuclear periphery. The red and blue colored chromosomes are at the nuclear periphery in liver cells because liver-specific NETs that have affinity to these chromosomes are expressed. These liver-specific NETs are not expressed in fibroblasts resulting in a more internal localization of the same chromosomes. (**B**) Affinity principle of NET-mediated chromosome positioning. Human chromosome 5 is preferentially internal in fibroblasts but in a cell type such as heart where NET47 is weakly expressed might have weak affinity for the periphery. In liver, where NET47 and NET45 are both strongly expressed, chromosome 5 would have a stronger affinity for the periphery. (**C**) The same principle could apply during differentiation where the same progenitors can develop into muscle or fat cells, each of which has differences in the milieu of NETs expressed and differences in the pattern of radial gene and chromosome positioning.

that several extremely tissue-specific NETs could reposition chromosomes to the nuclear periphery (**Table 2**).[96] Each of these NETs affected just a subset of chromosomes and different NETs affected different combinations of chromosomes, suggesting that each NET has different binding sites on DNA/chromatin that can function synergistically so that in combination they can achieve a threshold of affinity to move an entire chromosome to the periphery. For example, in liver cells only a liver-specific subset of NETs is expressed (NET45 and NET47 and possibly others). This particular combination of NETs is not expressed in other cell types such as fibroblasts. This results in capturing of a particular chromosome set at the nuclear periphery in liver cells but not in fibroblasts (**Fig. 4A and B**). As several tissue-specific NETs were also found for other tissues such as muscle, one could imagine that as progenitor cells choose their eventual fate between a muscle and fat cell lineage that changes in NET

composition could likewise engineer a particular pattern of spatial genome organization specific to that tissue that could also contribute to its differentiation (**Fig. 4C**). Though it is not yet known whether these tissue-specific NETs bind DNA, chromatin proteins or transcriptional regulators sitting on particular genes, secondary structure predictions indicate that many have coiled coils or leucine zippers that could be used in interactions with transcriptional regulators and some, such as NET5/Samp1,[127] have zinc fingers that could be used for direct binding to specific DNA sequences.

The effects of this repositioning on gene expression are still under investigation, but there are several intuitive mechanisms whereby gene expression could be changed through gene positioning. (1) Local propagation of silencing to new genes recruited to the same general position due to the local abundance of silencing enzymes, for example the affinity of HDAC3 for LAP2β.[132] This mechanism could also work in the opposite direction with propagation of activation as the protein originally identified as NET43, now known as hALP1, only has a predicted transmembrane span in certain species, but in humans where it lacks this it can be recruited to the NE through binding to SUN1 at the end of mitosis where it facilitates the decondensation of chromatin.[135] (2) Increasing the effective concentration of a particular transcriptional regulator through compartmentalization could also alter gene expression. The NE represents ~1/30th the volume of the nucleus, so LAP2β recruitment of germ-cell less[131] could make its local concentration 30-fold higher than the actual concentration in the whole nucleus. Similarly, transcription factors could be sequestered away from targets in the genome as was shown for MAN1 and Smads.[104,105] (3) Steric factors blocking access to the DNA for transcriptional activators or alternatively in the structure of tethered chromatin—still unknown—could enable greater access to the local epigenetic silencing enzymes or transcriptional repressors. Testing these various possibilities is hindered by the fact that, when genes move in a physiological context such as differentiation, many additional changes occur within the time frame researchers are able to sample such as the pattern of transcriptional regulators, epigenetic marks on the chromatin, the transcriptional state of the gene, etc. The inability to modulate gene position without the myriad physiological and developmental changes has resulted in the exclusive use of artificial tethering systems to address this question with resulting contradictory findings (reviewed in ref. 128). We anticipate that the recent identification of endogenous players in this process—the tissue-specific NETs that alter chromosome and gene positioning—will in the next years enable clear answers to this question.

Tissue-Specific Contributions to Nucleo-Cytoplasmic Transport

The NPCs are arguably the largest protein complex in the cell and were originally weighed in at ~125 MDa by cryo-electron microscopy studies.[136] However, proteomic studies that identified roughly 30 core component proteins only account for a mass of ~40 MDa for the yeast NPC and ~60 MDa for the mammalian

NPC.[10,137] This discrepancy has generally been attributed to the addition of transport receptors and their substrates that transiently associate with the NPC core structure in the process of translocating through the NPC central channel, but it is also possible that there are as yet unidentified NPC components some of which may be tissue specific.

The first indications of tissue-specificity in the NPC came from observations of tissue-specific expression of transport receptor variants (reviewed in ref. 138). Chief among these is the importin-/karyopherin-α family, which is encoded by multiple genes that each produces multiple tissue-specific splice variants. Some of these splice variants have been shown to play important developmental roles,[139] and this is likely due to their favoring transport of developmentally important nuclear regulators.

It was not long after the first reports of tissue-specific transport receptors that the transmembrane nucleoporin gp210 (also called POM210) was cloned in a study comparing uninduced to induced mesenchyme.[140] This was the first demonstration of cell type and developmental specificity in expression of a core component of the NPC. This developmental and tissue-specificity was later confirmed in a wider study in mouse that directly compared it to other nucleoporins POM121 and Nup62 that were expressed in all tissues examined.[141] More recently it was found that gp210 is important for both myogenic and neuronal differentiation.[142] This followed on work showing that Nup133 is important for neural differentiation in mice[143] and that Nup358/RanBP2 also changes during myogenesis.[144] This latter study is perhaps the most telling as here it was found that Nup358/RanBP2 levels increase during myogenesis concomitant with a change in the physical architecture of the cytoplasmic filaments of which Nup358/RanBP2 is a primary component.[144] This suggests that differences in the physical/mechanical needs of differentiated muscle require either a sturdier cytoplasmic face to the NPC or that a requirement for a higher metabolic load enlists more filaments to capture cargos. Tissue-specific variants of Nup358/RanBP2 and POM121 have also been observed as well as altered expression and splicing for Nup98/96.[145-147]

It remains to be seen whether any of these new NE proteins are tissue-specific components of the NPC, but it is noteworthy that
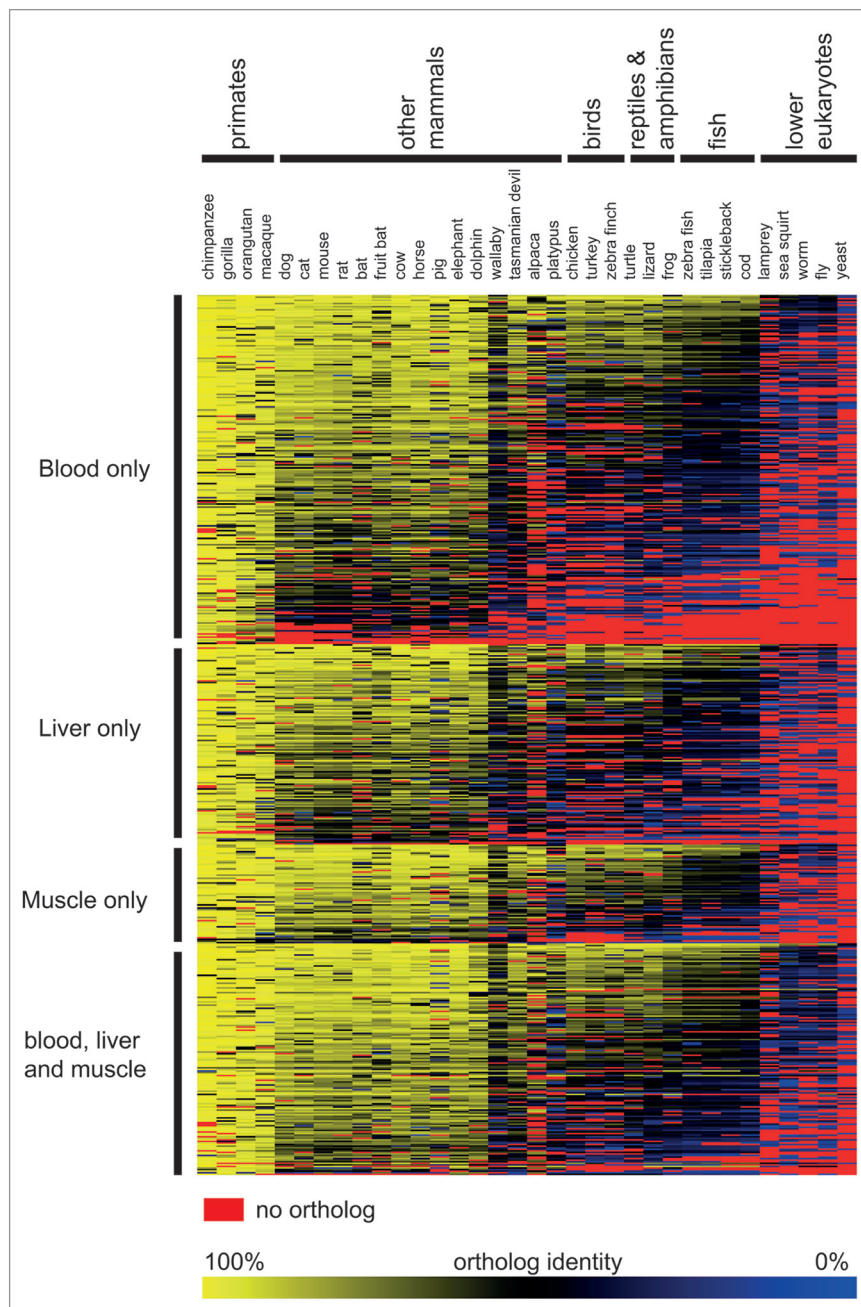
Figure 5. Evolutionary conservation of tissue-specific and widely-expressed NETs. Orthologs based on ENSEMBL annotations are plotted as a heat map for new NETs identified in the various tissue proteomic studies. NETs identified only in the blood NEs, only in the liver NEs, or only in the muscle NEs are clustered as well as a group of NETs identified in all three. The color-coding from yellow to blue indicates decreasing sequence identity of the orthologs and red indicates no ortholog was present in a particular organism. There is clearly more conservation among the NETs that were found in all three tissues, but even more interestingly clear breaks in the conservation of NETs through evolution can be observed. Some loss can even be found between humans and other primates, another break occurs between primates and other mammals. Then within mammals some additional breaks can be observed, particularly with regards marsupials. A larger loss of orthologs occurs going into reptiles and fish and birds are even more remote from humans. Finally, the lower eukaryotes have very few NET orthologs.

stretches of phenylalanine-glycine (FG) motifs in unstructured regions containing prolines are a characteristic feature of many nucleoporins and the NE data sets were strongly enriched for these motifs.[95,148] Moreover, the third mammalian transmembrane nucleoporin NDC1 was not found in the NPC proteomics,[10,137] but was identified in the NE proteomics studies.[13,36,38,40] Together these observations argue for at least a reasonable possibility that additional uncharacterized nucleoporins and transport receptors can be found in these data sets.

Potential Tissue-Specific Contributions to Tissue Differentiation and Evolution

Any of the above-discussed functions linked to tissue-specific proteins could have effects on overall tissue differentiation and function. Tissue-specific NETs that play roles in cell cycle regulation could, if defective, reduce the abundance of a particular cell type within a tissue and the ability of the tissue to regenerate when damaged. Defects in signaling, spatial genome organization and gene regulation from tissue specific NETs could modulate tissue-specific gene expression and the overall metabolism supported by the altered genes. Loss of a tissue-specific NET contributing to cytoskeletal organization could yield defects in the establishment of cell polarity, affecting the efficiency of polarized secretion in the tissue.

In all of the above-mentioned cases, the tissue could conceivably carry defects while retaining the outward appearance of normalcy. The transcription factors driving differentiation and gene expression would still be there, the core of the LINC complex would still provide basic cell mechanical functions, basic nucleo-cytoplasmic transport would still occur. We postulate that the roles of tissue-specific NETs is to fine tune cell functions so that, for example, in some NE-linked diseases loss of an interaction with a tissue-specific NE protein could yield subtle defects that only become pronounced when a muscle is repeatedly stressed or a fat store is called upon to manage a heavier metabolic load. Any athlete can attest that the metabolic and physical loads we normally place on muscle in an average day are minimal compared with the kind of demands placed on the same muscle groups in an athletic competition. Accordingly, the timing of clinical presentation in many NE-linked diseases—when children begin to become more active physically or upon puberty when higher usage, metabolic or endocrine loads would be placed on the tissue—is consistent with this idea of defects in tissue-specific NETs resulting in failure to achieve optimal gene expression or metabolism while initially giving the appearance of normal development.

It is intriguing to speculate that this fine-tuning by tissue-specific NETs helped drive organism complexity. Comparison of the evolutionary conservation of all the NETs identified in the various proteomic analyses revealed that the more tissue-specific NETs were the least evolutionarily conserved.[40] Thus, the tissue-specific NETs evolved as organisms began to distinguish more complex tissues and tissue functions. Interestingly, when searching for orthologs in a wide range of eukaryotes whose genomes have been fully sequenced and annotated, we observed enormous variation even in closely related organisms (**Fig. 5**).

The red on the heat map indicates no ortholog to the human NET and some human NETs did not even have orthologs in other primates. A sharp drop in the organisms having orthologs of NETs occurs when leaving primates to other mammals with another sharp drop between mammals such as dog, horse and dolphin and mammals such as wallaby, alpaca and platypus (**Fig. 5**). Yet another sharp drop is observed with birds, and, interestingly, there is more ortholog identity between humans and fish and reptiles than between humans and birds. Very few NET orthologs were found in lower eukaryotes including some of the most commonly used model organisms of worms, flies and yeast (**Fig. 5**). While these lower eukaryotes are excellent to study the core components of these systems such as the core LINC complex or core NPC, the full functional complexity will only be able to be properly addressed in human tissues.

This evolutionary analysis also indicates some interesting outliers that might be worth investing in as model systems to study family complexity. Because of their association in the LINC complex one might think that nesprins would co-evolve with SUN proteins. However, based on ENSEMBL data, in fish nesprins have twice the normal number of orthologs in mammals while SUN proteins have half the number. Chickens on the other hand have jumped from the normal mammalian number of 4–6 SUN protein genes to 16. Understanding what advantage trebling the number of SUN proteins has to chickens when other gene families have not similarly expanded may help to understand fully the functions of this interesting protein family.

The three tissues from which these NE proteomes were determined were chosen because they have widely differing characteristics; however, there is still a need for other tissues to be examined. Interestingly, when testing NET expression in various tissues skin was lacking in many of the more widely expressed of the novel NETs.[40] We anticipate that there are likely to be large numbers of additional tissue-specific NETs found in skin, brain and germ cells based on this analysis; however, for any tissue linked to disease it makes sense to engage proteomic analyses to identify its most tissue-specific NETs.

Conclusions

Our view of the NE has in the past 20 years evolved from that of little more than a physical wall to a dynamic structure perhaps even more complex than the plasma membrane in its responses in signaling and its variety of functions. Though this review has focused on the more tissue-restricted NETs with less characterized enzymatic functions, there were also many well characterized membrane proteins identified at the NE that have functions in ion transport, membrane biogenesis, proteolysis and dozens of other functions also relevant to this organelle. In retrospect, this is not surprising when considering that the NE must integrate signals from all over the cell and even from outside the cell to rapidly respond to a wide range of stimuli with changes in gene expression, protein and mRNA degradation, initiation or escape from the cell cycle, nuclear size, nuclear and cell migration, etc. The various tissue NE proteome data sets are rich with information that can be applied to all these functions.

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