Guilt by Association

THE NUCLEAR ENVELOPE PROTEOME AND DISEASE*

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The discovery that many inherited diseases are linked to interacting nuclear envelope proteins has raised the possibility that human genetic studies could be assisted by a fusion with proteomics. Two principles could be applied. In the first, the proteome of an organelle associated with a genetically variable disease is determined. The chromosomal locations of the genes encoding the organellar proteins are then determined. If a related disease is linked to a large chromosomal region that includes a gene identified in the organelle, then that gene has an increased likelihood of causing the disease. Directly sequencing this allele from patient samples might speed identification compared with further genetic linkage studies as has been demonstrated for multiple diseases associated with the nuclear envelope. The second principle is that if an organelle has been implicated in the pathology of a particular disorder, then comparison of the organelle proteome from control and patient cells might highlight differences that could indicate the causative protein. The distinct, tissue-specific pathologies associated with nuclear envelope diseases suggest that many tissues will have a set of disorders linked to this organelle, and there are numerous as yet unmapped or partially mapped syndromes that could benefit from such an approach. *Molecular & Cellular Proteomics 5:1865–1875, 2006.*

In the last 10 years, it has become clear that mutations in a small group of nuclear envelope (NE)[1] proteins are responsible for at least 15 inherited human diseases (Table I). The diverse range of tissue-specific clinical phenotypes associated with these disorders and the fact that many of the proteins in question are widely expressed have led to a renewed interest in the organization and function of the NE.

The NE (Fig. 1) is a double membrane structure consisting of two lipid bilayers separated by a regular spacing of around 50 nm. The outer nuclear membrane (ONM) is continuous with the endoplasmic reticulum (ER), and its cytoplasmic face is similarly studded by ribosomes (1, 2). To what extent it resembles more distal ER is unclear, but the ONM harbors some distinctive proteins of its own that are involved in tethering the NE to the cytoskeleton (3, 4). The space between the outer and inner membranes forms the lumen, a soluble compartment that is also continuous with the ER. The outer and inner membranes are linked at nuclear pores, large multiprotein complexes that span the entire NE, forming gated channels through which all transport in and out of the nucleus occurs (5). The inner nuclear membrane (INM) hosts a unique complement of integral proteins that interact with chromatin and provide attachment points for the nuclear lamina, a fibrous network composed principally of the intermediate filament lamin proteins (6, 7). Although lamins also occur in the nucleoplasm, the vast majority are assembled into the lamina, which is closely associated with the INM and is thought to provide strength and support to the NE (8–10).

THE NUCLEAR ENVELOPE AND MUSCULAR DYSTROPHY

In 1994, positional cloning studies identified a gene encoding a novel transmembrane protein dubbed emerin that was responsible for X-linked Emery-Dreifuss muscular dystrophy (11). Emerin was subsequently localized to the INM (12, 13), an unexpected finding as emerin is ubiquitously expressed: how could mutations in a universal integral NE protein cause a disease predominantly affecting skeletal and cardiac muscle and tendons? A further surprise came when mutations in *LMNA* (an alternatively spliced gene that produces lamin A, lamin C, and other variants) were shown to cause not only autosomal variants of Emery-Dreifuss muscular dystrophy (14, 15) but two additional inherited disorders affecting other muscle groups: type 1B limb-girdle muscular dystrophy (16) and familial dilated cardiac myopathy with conduction defects (17). It was subsequently found that emerin and lamin A/C interact strongly and that some *LMNA* mutations lead to the loss of emerin from the NE (18, 19), providing an explanation for why emerin and lamin A/C mutations could cause similar diseases. However, because the mutated proteins are both widely expressed and functions of the NE were thought to be similar in all cell types, it was less easy to explain their specific pathology in muscle diseases.

An attractive model to account for the involvement of the NE in muscle disorders is the mechanical stress hypothesis: the lamina functions to maintain the structural integrity of the nucleus, so mutant lamins could lead to fragility in the NE (20, 21). The high forces generated in tendons and contractile organs such as skeletal and cardiac muscle might damage

    [1] The abbreviations used are: NE, nuclear envelope; ER, endoplasmic reticulum; ONM, outer nuclear membrane; INM, inner nuclear membrane; NET, nuclear envelope transmembrane protein; LBR, lamin B receptor; LAP, lamina-associated polypeptide; MudPIT, multidimensional protein identification technology; 2-D, two-dimensional.

TABLE I

Inherited diseases associated with the nuclear envelope

OMIM, Online Mendelian Inheritance in Man database at the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov); #, the disease is caused by mutations in the protein shown; *, mutations are associated with the disease, but a causal link has not been conclusively demonstrated; AD, autosomal dominant; AR, autosomal recessive; CNS, central nervous system; HEM, hydrops-ectopic calcification-moth-eaten.

Disease	Synonym	Mutated NE Protein	Inheritance	Phenotype	OMIM	Reference
Emery-Dreifuss muscular dystrophy, type 1/2/3	EDMD1 EDMD2 EDMD3	Emerin Lamin A/C Lamin A/C	X-linked AD AR	Early contractures (stiff/fixed joints) of elbows, Achilles tendon, neck and spine. Progressive weakness in upper arms and lower limbs. Dilated cardiomyopathy with conduction abnormalities.	#310300 #181350 #604929	11,12 14 15
Limb girdle muscular dystrophy, type 1B	LGMD1B	Lamin A/C	AD	Progressive weakness of shoulder, upper arm, hip and upper leg muscles with later development of dilated cardiomyopathy with conduction abnormalities.	#159001	16
Dilated cardiomyopathy with conduction defect	CMD1A	Lamin A/C Lamina-Associated Polypeptide 2α	AD	Ventricular dilation and impaired systolic function. Sudden death due to cardiac pump failure may occur after conduction abnormalities. No skeletal muscles affected.	#115200 *188380	17 66
Dunnigan-type familial partial lipodystrophy	FPLD2	Lamin A/C	AD	Loss of subcutaneous fat from limbs and trunk with simultaneous accumulation in face and neck. Insulin resistance and diabetes mellitus.	#151660	26,27
Seip syndrome	BSCL2	Lamin A/C	Unknown	Adipose tissue absent from early infancy. Hypertriglyceridemia, hyperglycemia, diabetes mellitus, mild mental retardation, cardiomyopathy and dark, rough skin patches.	*150330	115
Lipoatrophy with diabetes, hepatic steatosis, hypertrophic cardiomyopathy and leukomelanodermic papules	LDHCP	Lamin A/C	Unknown	Generalized lipoatrophy with acute accumulation of fat in liver. Accompanied by skin pigment abnormalities and cardiomyopathy.	#608056	116
Mandibuloacral dysplasia, type A/B	MADA MADB	Lamin A/C ZMPSTE24	AD AR	Postnatal growth retardation, craniofacial anomalies (especially crowding or loss of teeth), skeletal malformations, mottled skin pigmentation, stiff joints and autoimmune hair loss. Partial lipodystrophy, insulin resistance and diabetes.	#248370 #608612	117 118
Restrictive dermopathy	RD	Lamin A/C ZMPSTE24	AR	Abnormally rigid and translucent skin, joint contractures and pulmonary hypoplasia. Impaired fetal body movements lead to deformity. Early neonatal death due to respiratory insufficiency.	*150330 *606480	29
Charcot-Marie-Tooth disorder, type 2B1	CMT2B1	Lamin A/C	AR	Progressive deterioration of motor and sensory nerves leading to atrophy of limb muscles and numbness/sensory problems. Nerve conduction velocities not affected.	#605588	28
Pelger-Huet anomaly	PHA	Lamin B receptor	AD	Neutrophil nuclei in heterozygotes have fewer segments and course chromatin, with no effects on normal health. Homozygotes are also prone to epilepsy and skeletal abnormalities, e.g. polydactly and metacarpal shortening.	#169400	64
Greenberg / HEM skeletal dysplasia	GSD/ HEM	Lamin B receptor	AR	Widespread tissue edema in fetus. Disorganized bone structure, short limbs and conversion of cartilage to bone. Early in utero lethality.	#215140	36
Buschke-Ollendorff syndrome	BOS	MAN1 / LEMD3	AD	Skeletal defects include multiple spots of increased bone density (osteopoikilosis) and bands of sclerosis in a flowing pattern (melorheostosis). Sometimes accompanied by joint contractures, skin lesions, muscle atrophy, hemangiomas, and lymphedema.	#166700	30
Hutchison-Gilford progeria syndrome	HGPS	Lamin A/C	De novo / AD	Childhood onset of premature ageing including growth retardation, baldness, facial hypoplasia, delayed tooth formation, aged skin, osteoporosis, atherosclerosis, arthritis. Teenage mortality due to cardiovascular disease.	#176670	33,34
Atypical Werner syndrome	AWS	Lamin A/C	AD	Adult onset of premature ageing. Hard, tight skin, cataracts, subcutaneous calcification, premature arteriosclerosis, diabetes mellitus, premature ageing of face.	*150330	35
Torsion dystonia	DYT1	TorsinA	AD, 30-40% penetrance	Prolonged, involuntary muscle contractions induce abnormal posture and twisting or repetitive movements in arms and legs. Caused by CNS dysfunction rather than neurodegeneration.	#128100	31,32

weakened nuclei in these tissues, leading to malfunction and disease. In support of this idea, lamin A/C-deficient cells have reduced viability in response to mechanical strain, and their nuclei are more fragile and less able to withstand tension and compression forces (10, 22, 23). This parallels *in vitro* studies indicating that lamin A forms the most stable interactions of the various lamin subtypes and that differing degrees of mechanical stability may be required for individual cell types (24, 25).

In addition to muscular dystrophies, mutations in NE proteins are now known to cause a range of other diseases with distinct tissue-specific pathologies (see Table I). These include lipodystrophies (26, 27), neuropathy (28), dermopathy (29), osteopoikilosis with melorheostosis (30), dystonia (31, 32), and premature aging syndromes (33–35). The mechanical stress hypothesis is clearly not adequate to explain the pathologies in these other tissues, such as the abnormal white fat distribution observed in lipodystrophy patients (26, 27), the bone abnormalities seen in Buschke-Ollendorf syndrome (30) and Greenberg skeletal dysplasia (36), or the deterioration of motor and sensory nerves in Charcot-Marie-Tooth disorder (28).

Another popular disease model proposes that mutations in NE proteins might disrupt interactions between the NE and chromatin, leading to defects in gene regulation (38). Inter-phase nuclei are highly organized with gene-poor chromosomes and heterochromatin associated with the nuclear periphery in most cell types investigated (39, 40). Lamins and other NE proteins interact with chromatin-modifying factors such as heterochromatin protein 1 and core histones (for a review, see Ref. 41), which may influence gene expression by maintaining a compartment of silenced DNA around the nuclear periphery (42–44). The disruption of peripheral heterochromatin has been reported in a number of NE-associated diseases, consistent with chromatin regulation being an important disease mechanism (45–47).

Lamins and other NE proteins have also been shown to bind to a growing number of transcriptional regulators including the retinoblastoma protein (48, 49), sterol response element-binding protein (50, 51) and germ cell-less (52, 53). These results suggest that NE proteins may have a direct role in the regulation of specific genes, an idea that is strengthened by recent work uncovering the misexpression of target genes downstream of these regulators in emerin and lamin A/C mutant cells (54).

A defective NE could therefore lead to disease by causing nuclear fragility under mechanical stress, by disrupting chromatin organization or transcriptional regulation, or by a com-
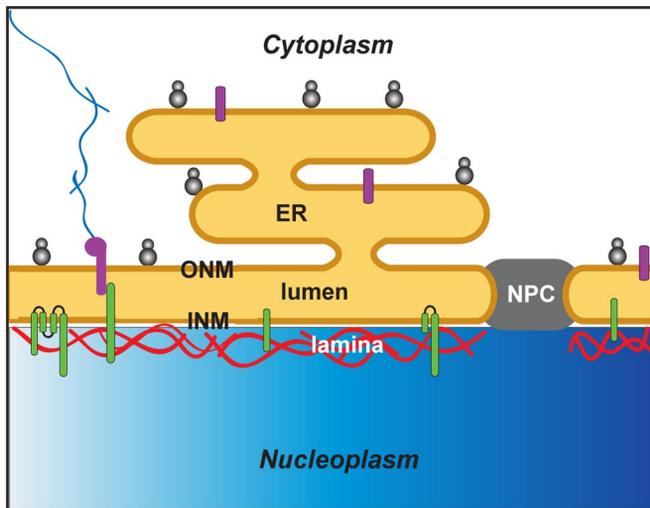
Fɪɢ. 1. **Structure of the nuclear envelope.** The nuclear envelope is composed of a double lipid bilayer punctuated with nuclear pore complexes (*NPC*). The INM harbors a unique set of integral proteins (*green*) with varying numbers of transmembrane spans. Many of these interact with the underlying nuclear lamina (*red*), a meshwork of intermediate filaments that provide strength and support to the membrane system. The ONM and lumen are common to the ER. Ribosomes and a shared set of integral membrane proteins (*purple*) are associated with the ONM and ER. The ONM also has some unique components that are believed to connect the cytoskeleton (*blue filaments*) to the nuclear envelope. These proteins (known as synes/nesprins) appear to be retained in the ONM by an interaction with INM factors (known as SUNs) that bridges the luminal space, thus linking the cytoskeleton to the nuclear lamina.

bination of the above mechanisms. Recent work also suggests that *LMNA* mutations may disrupt the DNA damage response pathway, providing a possible explanation for the premature aging diseases Hutchison-Gilford Progeria syndrome and atypical Werner syndrome (55). Other disease models for which there is currently less evidence include faults in cell differentiation pathways, defects in the functions of the contiguous ER (such as lipid regulation and the storage or release of Ca^{2+}), or aberrant cell cycle regulation (for a review, see Ref. 56).

ENTER PROTEOMICS

None of these models could explain all the diverse tissue pathologies as the mutated NE proteins are broadly expressed. Moreover proximal mutations in *LMNA* cause distinct diseases that affect different tissues, and phenotypic variation in these disorders suggests that additional factors are involved that have yet to be identified (57–60). It was therefore considered that an improved fundamental understanding of NE organization might lead to a grand unifying disease hypothesis, and comprehensive identification of the protein complement of the NE was the first crucial step.

Although 29 structural components of the nuclear pore complex had been identified in yeast and mammals by proteomic analysis (61, 62), only around 10 mammalian NE trans-

membrane proteins (NETs) were known by 2001 (63). Discounting those associated with the nuclear pores, these were the lamin B receptor (LBR), lamina-associated polypeptides (LAP1 and -2), emerin, MAN1, nurim, and synes/nesprins 1 and 2. Of these, LBR (36, 64), emerin (11), MAN1 (30, 65), and LAP2 (66) have been linked to human disease (Table I).

Although many of the proteins associated with nuclear pores have a clear and defined function, relatively little is known about the other NE proteins. LBR exhibits sterol C-14 reductase activity and additionally binds to B-type lamins, DNA, histone H3/H4, and heterochromatin protein 1 (for a review, see Ref. 41). Its sterol reductase activity is reduced in Greenberg skeletal dysplasia, but whether this is related to the disease mechanism has not been shown (36). The syne/nesprin genes have multiple splice isoforms, some of which encode very large proteins (800–1000 kDa) of the ONM. Together with the subsequently discovered SUN proteins of the INM, these are thought to form a complex spanning the entire NE, thus linking the nuclear lamina to the cytoskeleton (see Fig. 1; for a review, see Ref. 67). Most NETs (such as emerin, LAP1, LAP2, and MAN1) have no associated enzymatic activity to date, yet have multiple binding partners that do act as enzymes. This has led to the suggestion that they principally serve as hubs for the assembly of multiprotein complexes whose functions are defined by their partners. For example, MAN1 appears to down-regulate transforming factor-β superfamily signaling by sequestering activated R-SMAD signal receptors to the NE (68). As this pathway is involved in bone morphogenetic protein signaling, its disruption by mutations in MAN1 could be the cause of the skeletal defects observed in Buschke-Ollendorf syndrome patients (30). Thus, identifying the full complement of NE proteins could highlight candidate partners with the requisite enzymatic activities to bear on the wide range of NE diseases.

Following its success with the nuclear pores, proteomics was the method of choice to extend the list of NETs. However, one major problem needed to be surmounted: the continuity between the rough ER and the ONM causes biochemically isolated NEs to be contaminated by ER membranes, thus hindering the identification of novel NETs. The first proteomic analysis of the mammalian nuclear membrane used comparison between biochemical extractions to help identify true NE proteins, relying on the fact that the lamina and associated INM proteins are resistant to extraction with salt and non-ionic detergents and that integral membrane proteins can be enriched by chaotrope extraction. 2-D gels were used to resolve the protein fractions remaining in separate salt, Triton X-100, and chaotrope (carbonate/urea) extractions of NE preparations. Protein spots were then excised from each gel and digested with protease, and the resulting peptides were identified by MALDI-TOF mass spectrometry. The authors considered that proteins identified in all three fractions would be good candidates for novel NETs. This comparative approach was successful in identifying seven of the 10 NETs known at

the time, two new splice isoforms of LAP2, and two novel integral membrane proteins (LUMA and SUN1, a homologue of *Caenorhabditis elegans* UNC-84A) that were shown to reside in the NE (69).

The use of proteomics to identify new NE proteins was therefore a significant advance. However, identifications were not obtained for about 25% of the protein spots excised from the 2-D gels in this study, raising the possibility that a number of NETs remained to be identified (63).

The arrival of multidimensional protein identification technology (MudPIT) provided an opportunity to surmount this problem as it avoids 2-D gels altogether (70, 71). In this high throughput technique, the entire protein fraction under analysis is initially digested into peptides without prior electrophoretic separation. These peptides are then resolved by combined reverse phase and cation exchange chromatography and eluted into an ion trap tandem mass spectrometer. The use of tandem mass spectrometry frequently allows protein identifications to be made from single peptides by using SEQUEST analysis against a database of protein sequences (72, 73). MudPIT thus provides several advantages for identifying membrane proteins, which are often of low abundance and poorly resolved on 2-D gels (74).

The second proteomic analysis of the NE combined MudPIT with an *in silico* subtractive approach to eliminate ER proteins and other contaminants from the analysis. A microsomal membrane fraction was prepared containing fragments of ER, mitochondrial membranes, and cytoskeletal proteins that normally contaminate NE preparations. Both NE and microsomal fractions were analyzed using MudPIT, and proteins appearing in both fractions were excluded from the final dataset. This approach was successful in identifying all of the 13 previously characterized NETs expected and an additional 67 putative NE proteins with predicted transmembrane spans (75). Eight of these new NETs were initially shown to localize to the NE using tagged constructs, demonstrating the effectiveness of this new technique (75). Two more NETs were subsequently demonstrated to reside at the NE (76, 77), and over a dozen more that have been tested in our laboratory thus far colocalize with NE markers.[2]

PROTEOMICS AND DISEASE

The identification of so many novel NETs was unexpected and seemed to complicate rather than facilitate the development of a grand unifying disease hypothesis because there were no common themes in their predicted functions. In fact, most of the novel NETs had either no similarity to known proteins or were related to protein families of unknown function. Those containing homology to known functional domains were quite varied in their predicted roles, which ranged from a sugar hydrolase to phosphatases and aminopepti-

dases (see supplementary Table VII in Ref. 75). Moreover some NETs had multiple predicted functions, *e.g.* NET45 was annotated with both kinase and phosphatase-like domains, and NET57 was predicted to have ubiquitin ligase, zinc finger, and endonuclease regions. NET37 had a predicted sugar hydrolase function that was the only role related to a known pathway for muscle disease. However, the defects in glycosylation associated with a number of muscular dystrophies (including limb-girdle muscular dystrophy type 2I) all occur in the cytoplasm (78); so unless NET37 is in the ONM, this could not logically serve as the basis for an NE disease mechanism.

That so many additional muscle-related NE diseases were identified rapidly after Emery-Dreifuss muscular dystrophy is, at least in part, due to the "guilt by association" approach. The logic of this approach devolves from certain characteristics of the NE diseases.

(i) The same NE protein can cause multiple similar diseases. For example, mutations in lamin A/C cause several muscle disorders including Emery-Dreifuss muscular dystrophy, limb-girdle muscular dystrophy, and multiple types of cardiomyopathy (79).

(ii) Multiple interacting NE proteins can also cause similar diseases or even variants of the same disease. For example, emerin and lamin A/C interact with one another (19), and both cause variants of Emery-Dreifuss muscular dystrophy (11, 14, 15). Similarly mutations in LBR and MAN1 are associated with several bone disorders (30, 36).

(iii) Many diseases that can be caused by mutations in an NE protein are genetically variable. For example, there are at least 15 recognized types of limb-girdle muscular dystrophy (80) and over 20 variants of Charcot-Marie Tooth disease (81) that each map to different chromosome regions. For a number of these disease variants, a gene has yet to be determined.

Genetic mapping frequently reaches a point where a chromosome region containing hundreds of genes is clearly linked to a disease. However, mapping this disease to a smaller chromosome region requires additional recombination events that might need a new pedigree or another generation to identify genetically. To avoid this wait, clues in gene annotations can sometimes highlight a logical candidate within a chromosome region. For example, good candidates for investigation would be related proteins and partners in the same subcellular compartment. This approach has now been successfully applied to several NE diseases as was clearly stated in the linking of cardiomyopathy to mutations in *LMNA* (82). Just as the muscle diseases related to the NE increased rapidly due to guilt by association, the identification of *LMNA* mutations linked with the premature aging syndrome Hutchison-Gilford progeria led to the discovery of *LMNA* mutations associated with atypical Werner syndrome (35).

In principle, this approach could be widely applied, but it requires that proteomic datasets describing organellar composition be merged with current mapping data from human

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[2] W. E. Powell, V. Lazou, N. Korfali, and E. C. Schirmer, unpublished data.

geneticists. In the case of the information obtained in the NE proteome, there are two ways to focus on potential disease candidates.

The first is to concentrate on a few proteins by identifying ones that are more likely to interact with, or be related to, those already associated with disease. Five of the novel NETs identified in the subtractive proteomic study are related to some of the previously characterized NETs (75).

(i) NET9 is likely to be derived from a duplication of the *LAP1C* gene; however, this knowledge yielded little insight as, although first characterized in 1988 (83), no function has yet been identified for LAP1C. Nonetheless both LAP1C and NET9 (renamed LULL1) were recently shown to interact with torsinA (84), a protein that causes a form of inherited dystonia (31). This makes both proteins candidates for the guilt by association approach. In fact, torsinA itself could have been identified in this manner because it was present in the liver NE dataset. However, it was subtracted from the final results because it also appeared in microsomal membranes. Subsequently torsinA was confirmed to reside in both the NE and ER (85) and shown to cause the disease early onset torsion dystonia by accumulating in the NE of neuronal cells (84). This highlights a limitation of the subtractive approach: it disregards proteins that occur in multiple cellular locations.

(ii) NET47 has similarity to LBR, mutations in which are responsible for Greenberg skeletal dysplasia (36) and the Pelger-Huet anomaly syndrome (64). The relationship to a protein already linked to NE disease makes this a reasonable candidate for the guilt by association method.

(iii) NET25 and NET66 both contain LEM domains. L̲AP2, e̲merin, and M̲AN1, from which the LEM domain obtains its name (86), are all directly linked to disease (11, 30, 65, 66). The NETs carrying LEM domains are therefore good candidates for guilt by association because LEM domain proteins have been shown to interact with one another, and all thus far tested have been shown to bind to lamins, including NET25, now renamed LEM2 (76, 87).

(iv) NET53 harbors spectrin repeats and has some similarity to the syne/nesprin family, although it is clearly distinct from syne/nesprin 1 or 2. This family of proteins is involved in nuclear positioning in muscle syncytia in *C. elegans* and under the neuromuscular junction in mice (3, 88). NET53 has subsequently been named nesprin 3 and appears to link the cytoskeleton to the NE via plectin (77). Thus, it could be involved in structural aspects of muscle and important in muscle diseases.

The second way to highlight potential disease candidates is a high throughput analysis of the entire dataset. This was attempted by determining the genomic position of all the NETs identified in the liver NE proteome. These were then compared with large chromosomal regions linked to muscular dystrophies, dystonias, and neuropathies for which a causative gene was not yet identified. This information was taken from multiple databases in which researchers had entered their results (75). The percentage of the genome over which all these linked diseases were mapped was calculated, and the proportion of NETs that occurred in these chromosome regions was determined. The incidence of NETs occurring within large chromosomal regions linked to these diseases was roughly twice the frequency expected for random genes (*i.e.* the percentage of the genome covered by these linked regions). This does not clearly link any of these NETs to the diseases: it merely indicates that within the set of NETs there is a greater than random chance that some will be linked to the diseases (75). A flaw with the initial analysis was that it relied solely on the accuracy of the databases, and it was subsequently discovered that several of the diseases listed in these databases had recently been linked to other genes but not yet updated in the database. It is for this reason that the effective application of this method requires close interaction with expert human geneticists who are aware of the latest developments in their field. Nonetheless reanalysis of the data after removing the recently mapped diseases yielded an even stronger association between the NE and the remaining diseases. This was particularly interesting as some of the diseases were variants of disorders already linked to the NE (*e.g.* limb-girdle muscular dystrophy and Charcot-Marie Tooth disease).

TISSUE SPECIFICITY OF NETS

Further analysis of the NE proteomic datasets has refined our hypothesis because there appears to be considerable variation in the composition of the NE among different cell types. One of the first clues that the NE might change in different tissues came from the observation that lamin subtypes vary in their expression patterns. The A-type lamins are not present in early embryos or stem cells but are expressed in most differentiated cells (89–91). Moreover the relative levels of A-type lamins and of the other principal lamin subtypes B1 and B2 vary in distinctive patterns among different cell types (92, 93). The other clue was the discovery a few years before the subtractive proteomic study of the NET UNCL, which had varying expression levels in different tissues. As no *UNCL* mRNA was detected in liver (94), it was no surprise that UNCL was absent from the liver proteomic dataset (75).

To gain further insight into this question, advantage was taken of a high throughput "transcriptome" dataset generated at the Genome Foundation of the Novartis Research Institute. Transcript levels of mRNAs obtained from 72 human and 61 mouse tissues were compared using DNA arrays (95). This database contains expression information for most of the novel putative NETs identified in the subtractive liver NE MudPIT study and provides a numerical comparison of relative expression levels in different tissues. The ratios obtained by dividing the highest expression level by the lowest across this range of tissues were extremely high for the new NETs with most exhibiting greater than a 10-fold difference between
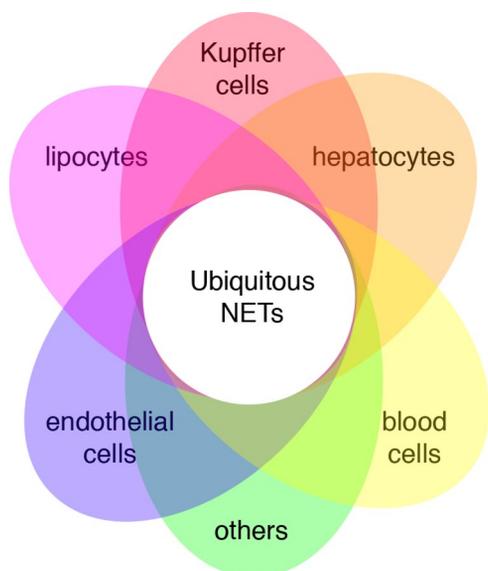
FIG. 2. **Tissue specificity of nuclear envelope transmembrane proteins.** Shown is a Venn diagram representing a hypothetical expression pattern of NETs in the liver. A central core of NETs (*white*) are broadly expressed, whereas others are restricted to specific cell types. Some of these cell types are unique to liver, but others may in essence occur in multiple tissues such as the lipocytes that are similar to adipocytes and fatty tissue. If each of the six groups of liver cells shown expressed seven unique NETs, then over half of the total of 80 NETs currently identified in liver would be cell-type specific. The identification of the full complement of NETs, including those involved in diseases of other organs, is likely to require the analysis of other tissue types.

FIG. 3. **A tissue-specific partner model for nuclear envelope diseases.** An intriguing aspect of NE disorders is that proximal mutations in lamin A lead to disease phenotypes in distinct tissues despite the fact that lamin A is widely expressed. This could be explained with the hypothesis that mutations in ubiquitous NETs have altered interactions with factors that are restricted to the affected organs. For example as depicted here, two tissue-specific complexes with overlapping binding sites interact with wild type (*WT*) and mutant lamin A (represented by the *red bar*) in heart and fat cells. Mutant I causes lipodystrophy by disrupting the binding of the adipocyte-specific complex (*blue/cyan*) while not affecting the interaction of the cardiac complex (*green/yellow*) with lamin A due to slight differences in the residues critical for binding each partner. The proximal defect in mutant II selectively disrupts the interaction of lamin A with the heart-specific complex, leading to cardiomyopathy. Mutant III inhibits the binding of both partners causing disease in multiple tissues as seen in Hutchison-Gilford progeria syndrome, Mandibuloacral dysplasia, and Seip syndrome.

tissues (96, 97). Nearly 20% of the putative NETs exhibited variation greater than 50-fold with the highest ratio at just over 300-fold. This degree of variation was surprising in a subcellular compartment that most had viewed previously as being highly conserved in different tissues. Moreover some of those NETs with particularly high degrees of variation exhibited background levels of expression in certain cell types.

This high degree of variation indicated that some of the NETs might be viewed as being tissue-specific. Strikingly the largest clustering of these NETs with preferential tissue expression patterns tended to be from cell types represented in liver (97), the material used to purify nuclear membranes for the subtractive proteomic study. The indication that several of these NETs were expressed in specific cell types provides an explanation for the far greater number of NETs identified in the liver NE MudPIT study compared with the earlier comparative MALDI-TOF analysis. In the earlier study, only one cell type was represented because NEs for analysis were purified from a cultured murine neuroblastoma cell line (69). In contrast, the rodent liver used in the subtractive study contained hepatocytes, Kupffer cells, lipocytes, endothelial cells, blood cells (contributed by the extensive vasculature as the tissue was not perfused), and other cell types (75). Therefore, if each cell type in liver contributed a set of unique NETs, it could account
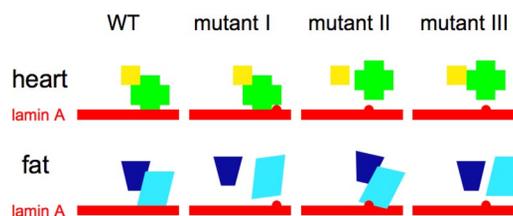
in part for the discrepancy between the two studies in the number of NETs identified. For example, if seven unique NETs were identified from each of six major cell types found in liver, then over half of the total of 80 NETs identified in the subtractive MudPIT study would be unique to individual cell types (Fig. 2).

TISSUE-SPECIFIC PARTNER MODEL FOR NE DISEASES

This emerging view of the NE proteome lends itself to a new model to account for the variation in NE disease phenotypes. The tissue specificity of NE diseases could be explained with the hypothesis that the widely expressed proteins underlying them have altered interactions with factors that are restricted to the affected tissues. Thus, a particular mutation in lamin A/C causing cardiomyopathy could disrupt an interaction with a tissue-specific partner in cardiac muscle while failing to disrupt an adipocyte-specific complex that binds at the same general site because of slight differences in the critical residues for binding each partner (Fig. 3). Conversely a second mutation in the same general binding site could selectively disrupt the adipocyte complex causing lipodystrophy, and a third mutation might disrupt both complexes causing a multivariant disease (such as Hutchison-Gilford progeria or Seip syndrome). Such partner proteins could be NETs or soluble factors with many potential functions, providing a possible link between this model and the gene expression and mechanical stability hypotheses.

Candidates for soluble partners were also identified in the MudPIT dataset from the subtractive study as many are tightly bound to lamins and NETs. Indeed a number of nucleoplasmic proteins that are known to interact with lamins and other

NETs were also present in this dataset (97): among these was the transcriptional regulator germ cell-less, which (as its name suggests) is preferentially expressed in the testis and regenerating cells (52). Other transcriptional regulators and chromatin-binding proteins found in this dataset may also partner with NETs to confer specific activities at the NE.

The principle of tissue-specific protein complexes at the NE among NETs is phylogenetically conserved. In *C. elegans*, the NET UNC-83 is required for nuclear migration and is dependent upon an interaction with the NET UNC-84 for its targeting to the NE (98). Although UNC-84 is ubiquitously expressed, UNC-83 is only found in tissues where nuclear migration occurs. However, mutations in either *unc-83* or *unc-84* yield the same phenotype. The idea that altered interactions with partner proteins are likely to be important for NE diseases is further supported by a recent structural study indicating that most mutations in lamin A map to the protein surface (99). These disease mutations are therefore more likely to disturb the binding of lamin A to other proteins than to destabilize the lamin protein itself. Support for this model also comes from the large number of binding partners that have been identified for emerin, which cover the entire length of the protein (100). Moreover six different monoclonal antibodies against emerin fail to recognize the protein in spleen cells, although each can detect it in other cell types, and emerin is clearly present in spleen as shown by Western analysis (101). This indicates that in some cell types nearly the entire surface of emerin is masked by binding to other proteins.

In addition to variation in the complement and levels of proteins present in the NE between cell types, there may also be variation in the post-translational modifications that these proteins carry. For example, a ubiquitously expressed NE protein could be modified in one cell type by a serine phosphorylation that blocks an interaction with a transcriptional repressor, whereas in another cell type the absence of this modification could enable the interaction. It has long been known that changes in the phosphorylation state of lamins drive the disassembly and reassembly of the lamin polymer in mitosis (102–106), but much less has been done to investigate interphase phosphorylation. It is certain that lamins are phosphorylated throughout interphase (107), but it is not clear whether this directs localized disassembly for nuclear growth or whether it regulates interactions with other proteins. In contrast, NET modification is clearly important for regulating protein interactions: LBR phosphorylation has been shown to regulate its binding to chromatin (108), and phosphorylation of emerin disrupts its interaction with barrier-to-autointegration factor (109). Moreover emerin exhibits at least four different patterns of phosphorylation through the cell cycle, and these are altered in Emery-Dreifuss muscular dystrophy patients (110). The application of the power of mass spectrometry to post-translational modifications at the NE in different cell types will likely lead the way to discovering how NET interactions are regulated. New NE MudPIT datasets from our laboratory are consistent with such tissue variation in protein modifications.[3]

CONCLUSIONS

In addition to the specifics discussed above, this approach merging proteomics with guilt by association could be adapted in many ways to identify further disease alleles. In cases where a complex rather than an organelle has been associated with a particular disorder, this approach could be used to identify the components of multiprotein complexes. For example, partners of emerin could be co-immunoprecipitated from different tissues to compare complexes in those tissues where emerin is linked to disease with those tissues where it is not. A related proteomic approach that could be taken to speed identification of new disease candidate genes would be to compare the organellar proteome or multiprotein complex from control and patient cells. In cases where an organelle or complex had been associated with a particular disorder, this might highlight differences that could identify the causative gene. The principle limitation of this approach is access to the appropriate patient material, *e.g.* to avoid measuring quaternary effects tissue samples would ideally include a time course of disease progression from a carrier in a pedigree starting before presentation of clinical symptoms. Despite these difficulties, the increasing sensitivity of proteomic methods will likely make this kind of approach viable in the near future.

This fusion of proteomics, the guilt by association approach, and the tissue-specific partner disease model has important implications for future proteomic studies. The similarity of the lipocyte cells in liver to adipocytes has highlighted a group of NETs specific to fat cells (97) that are candidates for guilt by association in other inherited lipodystrophies (111–114). The liver dataset could also be important for hepatic diseases. However, the MudPIT results from liver may not be the most appropriate dataset for identifying NETs involved in disorders of most other tissues. Clearly it will now be necessary to analyze the NE proteome from further organs and cell types, such as muscle or nerves, to identify new disease candidates.

The future benefit of such approaches will rely heavily on the interaction of human geneticists with proteomics laboratories. To facilitate such interactions, on-line databases could be set up to share organellar proteome data and to organize this according to the tissues analyzed, essentially a map of subcellular protein localizations throughout an organism. Between the frequent divergence to additional linkages uncovered during genetic mapping studies and the many currently unmapped or partially mapped inherited disorders, there are a great many diseases yet to be linked to a specific gene. Now is the perfect time to apply the powerful tool of organellar

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[3] E. A. L. Fairley, N. Korfali, S. K. Swanson, L. Florens, and E. C. Schirmer, unpublished data.

proteomics with the guilt by association approach to speed the identification of disease genes.

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