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Extremely Long-lived Nuclear Pore Proteins in the Rat Brain[+]

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Abstract

To combat the functional decline of the proteome, cells use the process of protein turnover to replace potentially impaired polypeptides with new functional copies. Here we found that extremely long-lived proteins (ELLPs) did not turn over in post-mitotic cells of the rat central nervous system. These ELLPs were associated with chromatin and the nuclear pore complex, the central transport channels that mediate all molecular trafficking in and out of the nucleus. The longevity of these proteins would be expected to expose them to potentially harmful metabolites putting them at risk of accumulating damage over extended periods of time. Thus, it is possible that failure to maintain proper levels and functional integrity of ELLPs in non-proliferative cells might contribute to age-related deterioration in cell and tissue function.

Functional deterioration and accumulation of damage to the proteome is an inevitable consequence of cellular aging. This damage is largely repaired through protein turnover where potentially impaired polypeptides are replaced with new, functional copies. These turnover mechanisms are particularly important in post-mitotic cells, such as neurons, because they cannot dilute potentially toxic species through cell division. As such, nearly all proteins within the human proteome are recycled in less than a few days (1, 2). However, a few exceptional cases of extremely long-lived proteins (ELLPs) with half-lives on the order of months have been identified (3, 4), including eye lens crystalline, collagen and myelin basic protein (MBP), a key structural component of myelination of nerve cells (4). Additional ELLPs probably remain to be discovered. For example, a recent study showed that a subset of nuclear pore complex (NPC) proteins, which form the transport channels responsible for mediating all nuclear trafficking (5), are present but no longer expressed in differentiated cells (6). Furthermore, in the nematode, C. elegans, all NPCs present in the adult animal are assembled during embryogenesis. Thus at least a subset of nucleoporins (Nups), are not, or are only very slowly, replaced during adulthood. However, because worms have a relatively short life span of a few weeks, it remains unclear if NPC components remain incorporated in the nuclear membrane over years, particularly in the central nervous system of mammals, which contain non-dividing cells that are as old as the organism itself (7).

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To explore this question, we performed pulse chase labeling of whole rats with the stable isotope 15N followed by mass spectrometry to monitor global protein turnover on a timescale of years (the average life span of a lab rat is 2 years). Two female Sprague Dawley rats and their progeny were fed a 15N-enriched algal cell diet, and at 6 weeks all progeny rats were switched to a 14N diet. Fully 15N labeled (t=0) rats were immediately sacrificed and their tissues (brain, liver, plasma, skeletal muscle, heart, kidney, lung, duodenum) harvested. Nuclei from liver, an organ that turns over within 4–6 months, and brain were purified, digested with trypsin, and analyzed by MudPIT (multidimensional protein identification technology) LCLC-MS/MS (multidimensional liquid chromatography-tandem mass spectrometry). At time=0, we calculated 15N isotopic protein labeling efficiency of >98% and identified more than 3,400 fully 15N proteins (20,754 peptides) and only 9 14N proteins (14 peptides). Two additional animals were sacrificed at 6 and 12 months and 15N/14N ratios were determined for more than 3,500 unique proteins. Only 7 heavy (15N) proteins (11 peptides) were found in the liver after 6 months, consistent with the relatively rapid turn-over of hepatocytes. In contrast, the brain contained a large number of heavy peptides (92 peptides) even after 12 months (Fig. 1B, Fig. S1E). These peptides corresponded to 25 proteins and included MBP and histones, the latter having reported half-lives of ~220 days in mouse brain (8), confirming the validity of our approach (Fig. 1A). All the other heavy proteins identified were components of the two essential core modules of the NPC, the pentameric Nup205 complex and the nonameric Nup107-160 complex (6) (Fig. 1B and Table 1). This represents an essential intracellular protein machine with protein components in excess of a year in age.

Detailed analysis of 15N spectral counts and 15N/14N MS1 ratios revealed that in contrast to the stable scaffold, the peripheral Nups and components of the central transport channel were devoid of heavy peptides, suggesting they were completely replaced after 6 months (Fig. 1C). Thus, unlike other large protein complexes, such as the proteasome or ribosome, in which all components have similar turn-over values (1, 2), the individual components of NPCs have very different lifetimes. This supports the idea that NPCs are built to last the entire lifespan of the cell and are not completely removed and assembled anew in post-mitotic cells. Rather, in the absence of the key regulators that orchestrate the complex NPC disassembly process in mitosis, NPC maintenance in non-dividing cells relies on the non- or extremely slow- exchange of scaffold and rapid replacement of peripheral Nups.

A lack of protein turnover exposes the proteome to an increased risk of aberrant chemical modifications and oxidative damage during aging, and thus might represent an Achilles heel of protein homeostasis in post-mitotic tissues. Indeed, healthy rats exhibit age-dependent decline of NPC function (6). Our results may thus provide a molecular explanation for the observed NPC deterioration and suggest that ELLPs represent a diverse class of proteins that regulate essential cellular functions and could be linked directly to the decline of the aging proteome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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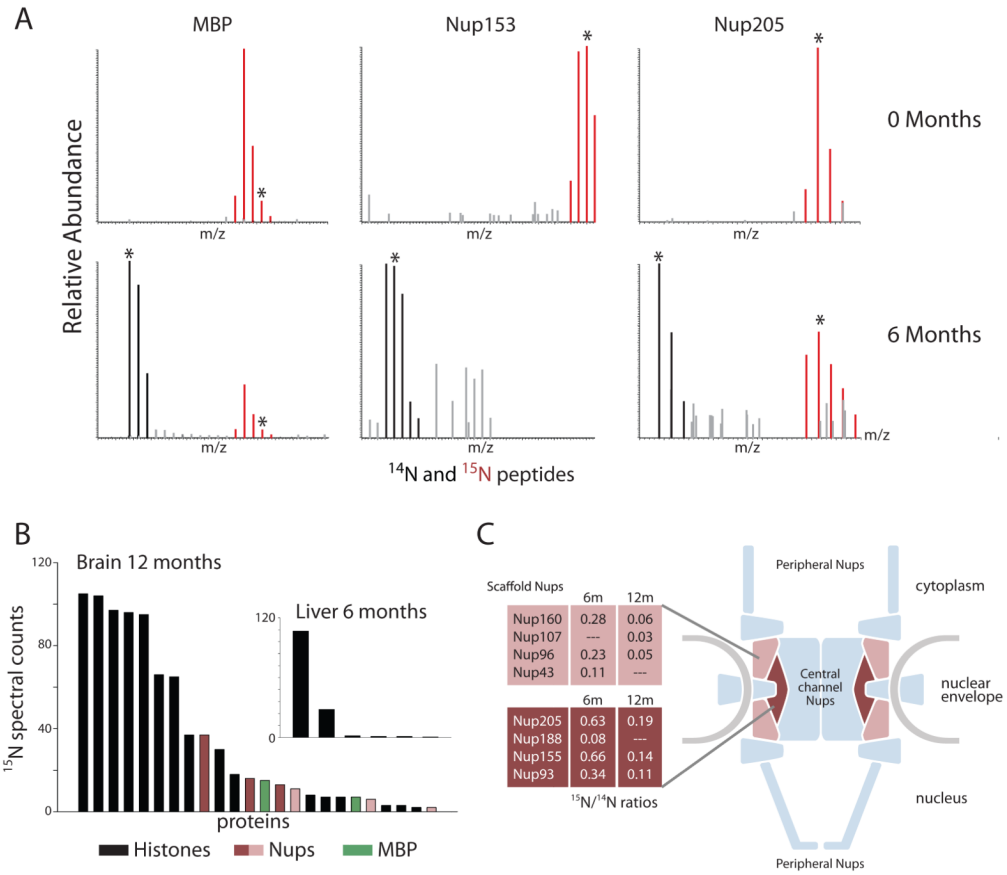

Fig. 1. Identification of NUPs and histones as extremely long-lived proteins in mammalian brain

(A) Raw MS1 scans (indicated M/Z ranges) at 0 and 6 months. Distinct peptides for indicated proteins; red indicates ^{15}N peptide peaks, black ^{14}N, grey other peptides, and asterisk peaks were successfully identified by MS/MS.

(B) Rank ordered distribution of ^{15}N MS/MS spectral counts (485) grouped as proteins (25) from 12 month brain nuclei and 6 month liver nuclei (inset); histones (black), NUPs (red/light red), and MBP (green).

(C) Schematic of NPC. Relative MS1 peak quantitation for each Nup with heavy peptide hits indicated as ^{15}N /^{14}N ratios when possible.