A mosaic of old and young nucleoporins

Takeshi Shimi and Hiroshi Kimura

Some nucleoporins, the nuclear pore complex (NPC) components, have exceptionally long lifetimes. In this issue, Toyama et al. (2019. J. Cell Biol. https://doi.org/10.1083/jcb.201809123) report that NPCs are maintained by a slow piecemeal replacement of NPC components in dividing and terminally differentiated cells and by whole-pore exchange in quiescent cells.

A snapshot of life taken at a given point in time provides us with a view of the steady state, but life is dynamic, constantly exchanging the components at equilibrium. In aging, the equilibrium irreversibly shifts to gain or lose life components in the extreme, which is accompanied by functional declines at multiple biological scales, including organ, tissue, organelle, cell, and protein. To understand the process of aging, knowing what changes or what remains unchanged with age is of utmost importance. Postmitotic cells in the brain and the heart are as old as the organism, and some regulatory proteins in their nuclei, such as histones, have an extremely long lifetime (1). Recently, Martin Hetzer and his colleagues identified nuclear proteins, including some nucleosomal histones and nuclear pore complex (NPC) components, as long-lived proteins (LLPs) in the rat brain by 15N metabolic pulse-chase analyses (2). These core histones (H3.1/2 and H4) and scaffold nucleoporins (Nups) in the NPC (Nup93, Nup107, and Nup205) are relatively immobile compared with other histones (e.g., H2B) and Nups (e.g., Nup133 and Pom121), respectively, in dividing cells, as determined by fluorescence recovery after photobleaching (3, 4). Therefore, protein stability of LLPs could be linked to the molecular exchange, even if taking into consideration protein synthesis and degradation. However, protein levels of LLPs are not necessarily correlated with their lifetime. The protein level of H3.1/2 becomes low relative to that of H3.3 in neuronal and glial chromatin with age (5, 6). In the case of Nup107, the protein level remains unchanged during differentiation of mouse C2C12 myoblasts to myotubes (7).

Despite the stability of LLPs, there is growing evidence for an age-dependent decline in nuclear function. Cells in the brain and heart are exposed to oxidative stress and accumulate DNA damage in their nuclei. DNA damage in gene promoters causes a decrease in protein expression in the aged cortex and cultured human neurons, which show reduced base-excision DNA repair (8). Mutations and genome rearrangements accumulate with age in mouse heart, and DNA damage caused by oxidative stress causes a significant increase in cell-to-cell variation in gene expression (9). In addition, aging impairs nuclear identity. Aged neurons exhibit a defect in nuclear transport and a loss of nuclear permeability barrier (7). Such defects could result in the aggregation of cytoplasmic proteins within the nucleus, which has been observed in cells from Parkinson’s disease patients (10). These findings led to the hypothesis that LLPs are vulnerable to damage accumulation and age-dependent decline in function (2, 7). In this issue, Toyama et al. determine the cellular distribution of LLPs and propose a molecular mechanism for maintaining protein complexes during aging.

Using the recombination-induced tag exchange system in C2C12 cells, Toyama et al. (11) monitored the replacement of histones H2B, H3.1, H3.3, and H4; the long-lived Nup93 and Nup96; the intermediate-stable Nup133; and the short-lived Nup Pom121 in dividing myoblasts, nondividing quiescent myoblasts, and nondividing terminally differentiated myotubes. While all four histones were turned over within 1–2 d in dividing myoblasts, histones H3.1, H3.3, and H4 persisted for 7 d in myotubes and quiescent myoblasts. These old histones concentrated in heterochromatin foci enriched with H3K9me3, which is consistent with the lack of histone turnover observed in heterochromatin in dividing yeast (12). Chromatin immunoprecipitation followed by sequencing showed the incorporation of both old and new H3.3 in transcriptionally active regions devoid of H3K9me3 in myotubes. Therefore, H3.1 and H4 in heterochromatin appear to be extremely stable in nondividing cells, even though H2A and H2B in the same nucleosome are still exchanged.

The mosaicism of old and new proteins in the same protein complex was further demonstrated in NPCs in nondividing cells. All Nups investigated by Toyama et al. (11) turned over at similar rates within 2 d in dividing myoblasts. In myotubes, however, Nup93 and Nup96 showed very little turnover, whereas Nup133 and Pom121 turned over moderately and rapidly, respectively, supporting the view that NPCs are composed of a mosaic of old and new Nups. In quiescent myoblasts, newly synthesized Nup93 and Nup96 were incorporated into their NPCs at significant rates. Structured illumination microscopy revealed that new Nup93/Nup96-containing NPCs were formed at a location separate from old Nup93/Nup96-containing NPCs in an endosomal sorting complex–dependent manner, keeping the total NPC density unchanged. Furthermore, using multi-isotope imaging mass spectrometry combined with scanning electron microscopy, Toyama et al. (11) found a mosaic of old and new NPCs in the mouse brain. These findings indicate two modes of NPC mosaicism in quiescent cells at the single NPC and the whole nucleus levels.
A single NPC becomes a composite of old and new protein components by piecemeal replacement in dividing and terminally differentiated cells and the whole nucleus possesses old and new NPCs by whole-pore exchange typically observed in quiescent cells (Fig. 1).

Toyama et al. provided strong evidence in support of the idea that age mosaicism of histones in the nucleosome and Nups in the NPC is a feature of long-term maintenance of nuclear function and integrity (11). LLPs in these nuclear structures likely play important scaffolding roles, keeping the structure’s identity. A question is whether there is any difference in function between the old and new protein–protein complexes. In the case of nucleosomes, the old H3.1s in heterochromatin show specific modifications, which can maintain chromatin state even if other nucleosome components (i.e., H2A and H2B) are dynamically exchanged. It would be interesting to examine whether there are links between the function and the structure of the old (mosaic) and new NPCs. Age mosaicism of the old and new protein–protein complexes could maintain nuclear structures and functions to prolong lives. Potential relations between age mosaicism and age-dependent decline has yet to be clarified, which could be a challenge for future research.

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1. Commerford, S.L., et al. 1982. Proc. Natl. Acad. Sci. USA. 79:1163–1165.
2. Toyama, B.H., et al. 2013. Cell. 154:971–982.
3. Kimura, H., and P.R. Cook. 2001. J. Cell Biol. 153:1341–1353.
4. Rabut, G., et al. 2004. Nat. Cell Biol. 6:1114–1121.
5. Piña, B., and P. Suau. 1987. Dev. Biol. 123:51–58.
6. Maze, I., et al. 2015. Neuron. 87:77–94.
7. D’Angelo, M.A., et al. 2009. Cell. 136:284–295.
8. Lu, T., et al. 2004. Nature. 429:883–891.
9. Bahar, R., et al. 2006. Nature. 444:1011–1014.
10. Woulfe, J., et al. 2010. Brain Pathol. 20:589–597.
11. Toyama, B.H., et al. 2019. J. Cell Biol. https://doi.org/10.1083/jcb.201809213
12. Aygün, O., et al. 2013. Nat. Struct. Mol. Biol. 20:547–554.