The importance of long-lived proteins: not just nuclear anymore

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Summary

The significance of mitochondrial long-lived proteins (mitoLLPs) to tissue health has remained mysterious for over a decade. In this issue of *Developmental Cell*, Krishna et al. (2021) demonstrate that mitochondrial lifetimes are highly heterogeneous and that mitoLLPs promote respiratory capacity by facilitating supercomplex assembly within the electron transport chain.

Main text

Age-related changes in protein homeostasis (proteostasis) underlie the dysfunction of multiple tissues across species. Therefore, determining precisely how changes in proteome integrity throughout life contribute to tissue dysfunction with age, is crucial for our ability to promote long-term health (Hipp et al., 2019). One area of emerging significance for our understanding of the relationship between proteostasis and ageing is that of protein lifetimes. During the last decade, several studies have demonstrated that the turnover rates of individual proteins can vary substantially, with some long-lived proteins (LLPs) persisting for weeks or even months in the nucleus and mitochondria of mouse and rat brain tissues *in vivo* (Price et al., 2010; Toyama et al.,

2013; Fornasiero et al., 2018). While LLPs in the nucleus have been shown to exhibit a high degree of lifetime mosaicism, both across and within cells, and to act as key scaffold proteins for the formation of nuclear pore complexes, the importance of mitoLLPs for mitochondrial function and tissue health, and the degree of lifetime heterogeneity that exists in mitochondria within cells and between tissues, remains unknown.

To determine the degree of lifetime heterogeneity within mitochondria *in vivo*, Krishna et al. (2021), in this issue of *Developmental Cell*, took advantage of MIMS-EM imaging (a technique that facilitates visualisation of protein, organelle and cellular turnover *in vivo*) to establish that aged mouse muscle and brain tissue have long-lived mitochondria with limited turnover. Interestingly, the authors also found that even within the same cells and tissues, mitochondria can exhibit different longevity and turnover rates indicating substantial mitochondrial age mosaicism. This phenomenon was also observed by the authors using human neurons *in vitro,* where the protein turnover of a mitochondrial ATP synthase subunit, ATP5C1, was monitored.

Next, the authors performed an unbiased *in vitro* analysis of proteome turnover using SILAC, a heavy isotope labelling method, to determine whether mitochondria contain LLPs in neurons and myotubes derived from differentiated human embryonic stem cells and mouse C2C12 myoblasts, respectively. These experiments revealed the presence of many LLPs in both neuronal and myotube mitochondria, with the overall protein turnover observed in neurons slower than in myotubes. Remarkably, neuronal and myotube mitochondrial proteins have longer lifetimes than proteins within other organelles, such as the endoplasmic reticulum and lysosomes. The authors noted that mitochondrial proteins are longer lived than the average proteome (40% of neuronal and myotube mitochondrial proteins detected in this study are LLPs) and that mitoLLPs include integral components of the ETC and import machinery. Interestingly, in both myotubes and neurons, ETC Complexes I and IV are composed of both LLPs and short-lived proteins (SLPs), whereas Complexes III and V are composed entirely of LLPs.

Accumulating evidence suggests that ETC complexes can exist as individual complexes (ICs) as well as higher-order super complexes (SCs) that may be crucial for maximizing respiratory capacity (Letts and Sazanov, 2017). Hence, to probe for the presence of LLPs in both ICs and SCs, Krishna et al. performed a detailed proteomic

analysis of the ETC using molecular biology and existing structural data. Due to technical issues with maintaining neuronal cultures, they decided to continue their experiments with only myotubes for this study. The authors established that mitoLLPs show distinctive turnover patterns within ICs (Figure 1). Specifically, Complex I possesses both stable and LLPs in the membrane arm and SLPs in the peripheral arm, Complex III is composed entirely of LLPs and Complex IV shows a heterogeneous turnover profile with some highly SLPs (such as NDUF4A, COX6B1) and several highly LLPs, such as COX7C. Strikingly, ETC proteins were found to exhibit differential stability in SCs compared to ICs, particularly in CI and $CIII_2$ (the obligate dimer of CIII), where LLPs show increased longevity in the SCs. In addition, mitoLLPs were also found to be core components of SC formation, forming most of the contact sites between the different ETC complexes within the respirasome (SC $I+III_2+IV$). Of particular importance is the protein COX7C, one of the longest-lived proteins in Complex IV, which also forms vital interactions with the three longest-lived proteins in Complex I (MTND5, NDUFB9 and NDUFB7) by directly binding to them within the context of the respirasome (Figure 1). This implies that LLPs mediate one of the most important contact points for respirasome formation (Letts and Sazanov, 2017).

The long lifetime of proteins within SCs could be essential for maintaining quaternary structure in the ETC. Therefore, the authors hypothesized that mitoLLPs may be critical for preserving SC formation and insulating ETC activity against conditions that cause a decrease in the transcription of genes encoding ETC components. In support of this, the authors demonstrate that COX7C is crucial for SC assembly, complex IV activity, and continued ETC integrity in response to reduced *COX7C* mRNA levels. Furthermore, reduced COX7C levels were found to perturb metabolic homeostasis and reduce health in myotubes (as measured by myotube thickness), suggesting that the long lifetime of COX7C may be critical for the maintenance of tissue health.

In summary, the work by Krishna et al. expands our understanding of the roles of LLPs within the cell and suggests that LLPs are integral to the formation and function of key complexes, not just in the nucleus, but also in mitochondria. This work also raises the intriguing prospect that cells have evolved mitoLLPs to complement existing mechanisms that maintain mitochondrial function in response to environmental stress (Song et al. 2021) and that mitoLLPs may help to delay the decline in ETC activity and

increase in ROS levels that occurs in ageing tissues (Su et al. 2015; Jang et al. 2018).

While these findings have advanced our understanding of the potential interplay between mitoLLPs and long-term tissue health, several key questions remain. Specifically, why are mitochondrial lifetimes so heterogeneous across tissues and within cells and what is the functional significance of this? Furthermore, how does the heterogeneity in mitochondrial turnover arise and what is the relevance of this to ageing and disease, are mitochondrial LLPs particularly susceptible to damage accumulation and does this contribute to the decline of mitochondrial function with age? Lastly, do mitochondrial LLPs provide resistance to environmental stresses and promote tissue health *in vivo*, and how is the lifetime of mitochondria and mitochondrial LLPs altered in long- and short-lived animals? Addressing these questions will further unravel the complex interplay between proteostasis, mitochondrial function and ageing, and will reveal new strategies to promote long-term tissue health.

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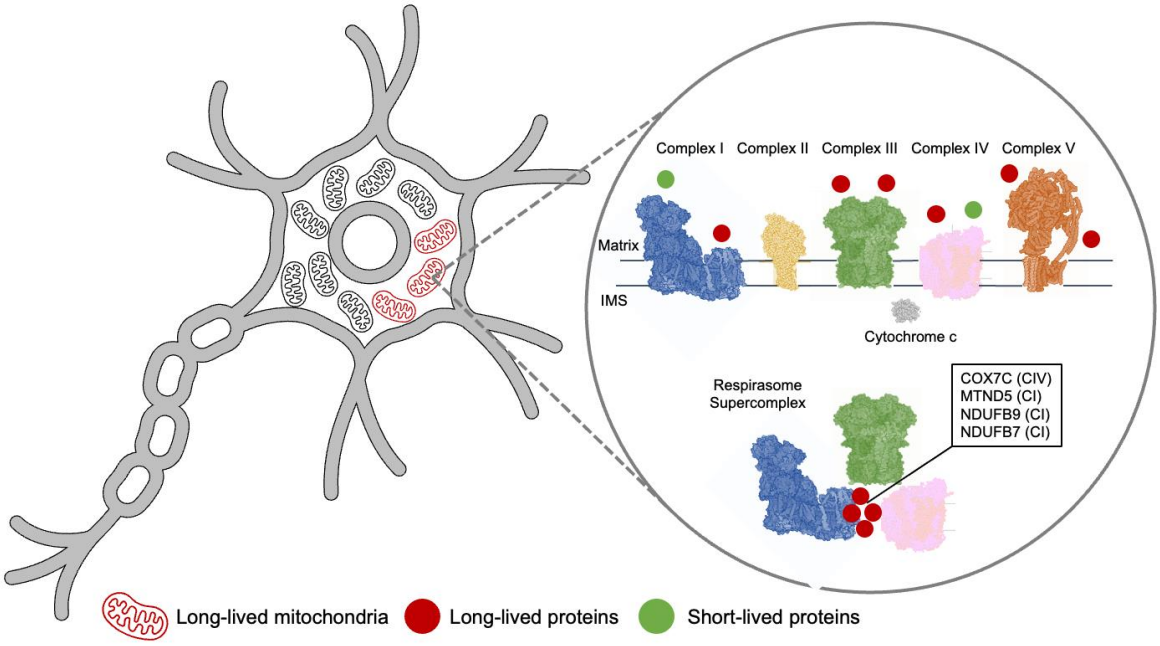

Figure 1. Long-lived proteins in the electron transport chain

Mitochondrial lifetimes are heterogeneous *in vivo*, with older mitochondria clustered within distinct regions of mouse cerebellar neurons. Both long- (red) and short-lived (green) proteins are found to be enriched within different sub-regions of ETC complexes, with long-lived proteins forming crucial contact sites within the respirasome super complex. Images are representative cartoons adapted from Letts and Sazanov, 2017, with the composition of short and long-lived proteins within specific ICs and the Respirasome SC depicted.