An IRON-clad Connection between Aging Organelles

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Lysosome (vacuole) and mitochondria decline interdependently during aging through an unclear mechanism. In this issue of *Cell*, Hughes et al. (2020) show that defective vacuole-mediated cysteine compartmentalization in aging yeast leads to iron limitation and mitochondrial dysfunction.

The past two decades have transformed our understanding of mitochondria and lysosomes. No longer merely the "recycling bins" and "powerhouses" of cells, lysosomes and mitochondria, respectively, have emerged as central regulatory hubs of cellular metabolism and signaling (Pagliarini and Rutter, 2013; Perera and Zoncu, 2016). The crosstalk between these two organelles appears to play a critical role in cellular physiology, and the interdependent decline of lysosomal and mitochondrial function is implicated in aging and age-related multisystem diseases, including neurodegenerative diseases (Deus et al., 2020; Ferguson, 2019). However, the molecular mechanisms for the functional inter-organelle crosstalk are unclear. Previously, Hughes and colleagues discovered that, during yeast replicative aging, an earlier loss of vacuole (yeast lysosome) acidification leads to age-related mitochondrial dysfunction (Hughes and Gottschling, 2012). Building upon this observation, in this issue of *Cell*, Hughes et al. (2020) demonstrate that the age-related, vacuole-mediated mitochondria inhibition is due to intracellular iron limitation. The authors further identified that this iron limitation is caused by a decline in vacuole-mediated cysteine compartmentalization and cysteine toxicity, generating oxidative stress. The work reveals an unappreciated interplay between cysteine and iron beyond their roles as substrates of iron-sulfur cluster (ISC) biogenesis. Interestingly, a similar iron-dependent relationship between dysfunctional endolysosomes and mitochondria was recently shown in mammalian cells, where it contributes to neuroinflammation in a lysosomal storage disease-mouse model (Yambire et al., 2019).

It has long been appreciated that a decline in mitochondrial function is associated with age-related impairment of multiple organs. Mitochondrial deterioration might be particularly detrimental to long-lived, postmitotic cells including neurons in the brain (neurodegenerative diseases) and sensory cells (age-related hearing loss). However, there is no common mechanism explaining this mitochondrial dysfunction during aging (Sun et al., 2016). For instance, mtDNA instability during replication error and cumulative mitochondrial damage have been suggested to contribute to the aging process. Alternatively, inefficient mitophagy might lead to accumulation of dysfunctional mitochondria—this model is particularly relevant in early-onset Parkinson disease mutations in mitophagy genes PINK1 and Parkin. In addition, although the long-held mitochondrial free radical theory of aging remains unproven, oxidative damage in the mitochondria might still be the culprit. Here, Hughes et al. (2020) provide a new mechanism of age-related mitochondrial decline, in which oxidative stress caused by a toxic level of cysteine leads to limitation of bioavailable iron during yeast aging (Figure 1). Interestingly, perturbation of iron homeostasis has been implicated in neurodegeneration through brain iron accumulation. In light of this new study, further efforts are needed to determine if the iron dyshomeostasis is also functionally linked to cysteine neurotoxicity in age-related neurodegeneration.

In this new study by Hughes et al. (2020), the authors first demonstrated that vacuole-mediated mitochondria inhibition is due to intracellular iron limitation. By using genetic screens to probe how vacuole acidification maintains mitochondrial function in yeast, the authors found that increasing intracellular iron (through either overexpression of plasma membrane low-affinity iron permease *FET4* or iron supplementation) restored mitochondrial function in cells with lysosome acidification defects due to V-ATPase depletion. Consistently, V-ATPase loss causes intracellular iron limitation (illustrated by activation of ISC-responsive transcription factor Aft1), ISC protein instability, and defects in mitochondrial ISC-containing respiratory chain (RC) complex functions. The authors then hypothesized that vacuole-mediated amino acid homeostasis might be responsible for iron limitation. This hypothesis is based on their previous finding that mitochondrial dysfunction due to loss of vacuole acidity can be suppressed by overexpression of AVT1, a V-ATPase-dependent vacuolar neutral amino acid importer. Here, by systematically screening all amino acids, the authors highlighted that cytoplasmic cysteine accumulation is necessary and sufficient for limiting intracellular iron availability in V-ATPase deficient cells. To further explore the mechanism, the authors used a more sensitive reactive oxygen species (ROS) indictor and demonstrated that cysteine accumulation leads to an oxygen-dependent oxidative stress that ultimately contributes to iron limitation. Finally, the authors further established that this cysteine-mediated iron deprivation is responsible for vacuole-mediated mitochondrial dysfunction in aged yeast. Together, Hughes et al. (2020) presented a comprehensive mechanism that showed vacuole-mediated cysteine

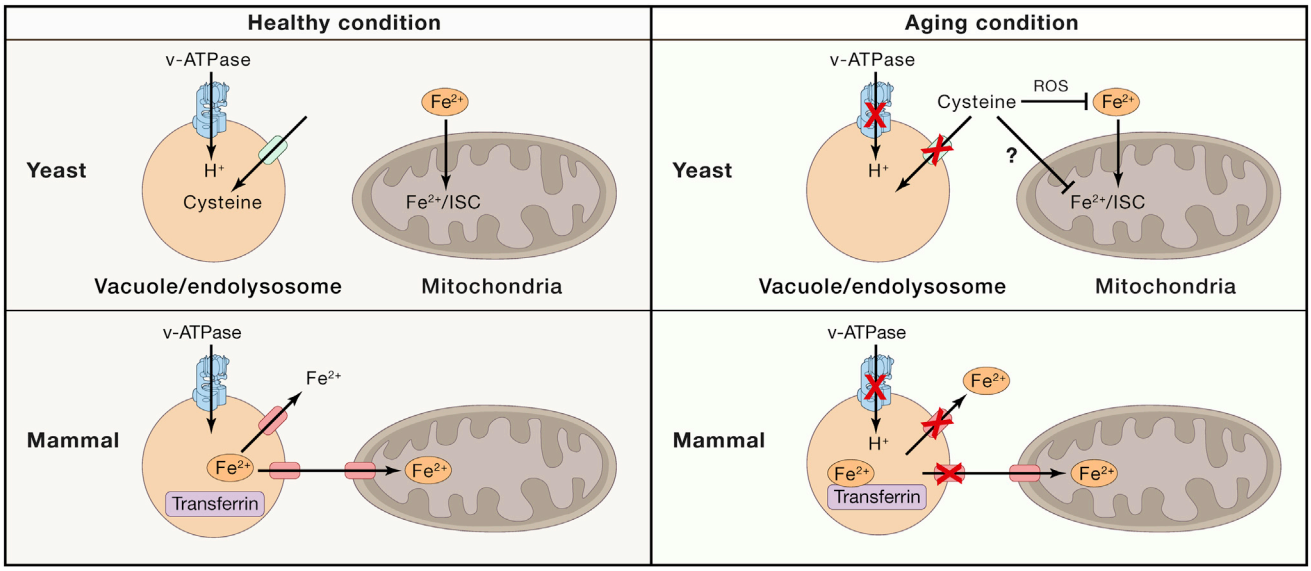

Figure 1. Iron Dyshomeostasis and Lysosomal-Mitochondrial Defects during Aging in Yeasts and Mammals

Decreased activity of lysosomal V-ATPase during aging leads to mitochondrial dysfunction in both yeast and mammals. The mechanism is unclear. Hughes et al. (2020) discovered that in yeast, vacuole acidification-mediated cysteine reuptake prevents its inhibition on cellular iron availability through oxidative stress. In mammals, acidification-mediated iron release from transferrin and subsequent delivery from endolysosomes to mitochondria provides a direct link between the two organelles. Despite subtle difference bewteen species, iron dyshomeostasis might be conserved as a functional link between lysosomes and mitochondria during aging.

compartmentation is critical for supporting iron bioavailability and mitochondria function. This regulation is perturbed during aging in yeast.

This landmark study represents an exciting area of research at the interface between cell biology and metabolism. In particular, it gets at the broad question of how the cellular compartmentation and metabolic biochemistry of nutrients and micronutrients can coordinately regulate the cellular homeostasis. Hughes et al. (2020) gives one example of how different nutrients could functionally interact with each other in the subcellular compartments, showing here that vacuole-mediated cysteine uptake supports mitochondria iron bioavailability. Functional characterization of the molecular details awaits future exciting research. For instance, it would be interesting to investigate whether cysteine-mediated iron inhibition acts through the mitochondrial ISC pathway, in particular frataxin, a chaperone of ISC biosynthesis that was recently shown to be modulated by oxygen tension (Ast et al., 2019).

Another question that remains to be explored is how these cellular processes of nutrient metabolism are conserved during evolution across different species. For instance, in mammals, extracellular iron bound to transferrin is primarily taken up by the transferrin receptor and enters the endosomal lumen through endocytosis. And iron is then released from transferrin in the acidic endosomal lumen and directly delivered to mitochondria via a "kiss-and-run" mechanism at the endosome-mitochondria contact site (Figure 1). Compared to that in yeast, this iron handling pathway in mammals provides a more direct way for endolysosome-mitochondria crosstalk to regulate iron metabolism (Yambire et al., 2019). A better understanding of iron metabolism in a cell-biological context in different organisms will be of great importance in future studies.

Finally, Hughes et al. (2020) highlighted iron metabolism as one type of the functional links of inter-organelle communication between lysosomes and mitochondria. Proximity and sometimes physical interaction of these organelles might be critical for the communication that supports cellular homeostasis. As mentioned above, iron delivery in mammalian cells might occur at the endosome-mitochondria contact. A direct physical tethering between organelles mediates inter-organelle lipid transfer, and defects in the proteins at the contact sites are implicated in neurodegenerative diseases (Kumar et al., 2018). Future work is needed to identify additional examples of metabolic and signaling inter-organelle crosstalk and their contributions to physiology. In conclusion, the mechanistic understanding of lysosome-mitochondria crosstalk centered around cysteine and iron during aging in Hughes et al. (2020) sheds exciting new light on the interface between cell biology, metabolism, and aging pathophysiology.

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A Single Transcription Factor Drives *Toxoplasma gondii* Differentiation

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Microbes that cause persistent infections (e.g., herpes viruses) do so by switching from fast-growing lytic states to slow-growing latent states. Waldman et al. have identified a single transcription factor that governs the switch between the lytic and latent forms of *Toxoplasma gondii*, a parasite that causes a persistent brain infection.

To establish lifelong colonization of their host, persistent pathogens often undergo dramatic changes to alter their metabolic state, rate of replication, and immunogenicity. *Toxoplasma gondii* is an obligate intracellular protozoan parasite that establishes a lifelong infection in the brain and skeletal and cardiac muscle of many mammals, including humans (Remington and Cavanaugh, 1965). This lifelong persistence allows *T. gondii* to reactivate and cause life-threatening disease in those with acquired immune deficiencies (Porter and Sande, 1992). To persist, *T. gondii* switches from its fast-replicating form, the tachyzoite, to its slow-replicating and encysted form, the bradyzoite. *In vivo*, bradyzoite-filled cysts are primarily found in neurons and myocytes, consistent with *in vitro* data suggesting that *T. gondii* may have higher rates of spontaneous differentiation and encystment within these cell types (Ferreira da Silva et al., 2008, 2009; Lüder et al., 1999; Swierzy and Lüder, 2015). Differentiation in other cell types, such as fibroblasts, can be induced *in vitro* by exogenous stressors such as alkaline pH, heat shock, small molecules (Compound 1), and nutrient starvation, suggesting that the tachyzoite-to-bradyzoite transition may be a general stress response (Lüder and Rahman, 2017) (Figure 1A). In this issue of *Cell*, Waldman et al. (2020) upend the current model of differentiation by identifying a single transcription factor that governs the switch between tachyzoites and bradyzoites (Figure 1B).

The current model of differentiation is built upon prior work that defined a family of parasite transcription factors (ApiAP2s) that modulate differentiation. Some of the ApiAP2s (AP2IV-3) induce the expression of bradyzoite-associated genes while others (AP2IV-4 and AP2IX-9) act as repressors of bradyzoite gene expression (Hong et al., 2017; Radke et al., 2018). However, no single ApiAP2 transcription factor is capable of completely ablating differentiation, leading to a model involving many regulators that influence the tachyzoite-bradyzoite switch but no single master regulator. In contrast to this model, Waldman et al. (2020) identify a single gene, bradyzoite-formation deficient 1 (BFD1), that is both necessary and sufficient for differentiation *in vitro*. To find this master regulator, Waldman and colleagues utilized alkaline pH-induced differentiation in conjunction with a CRISPR/Cas9 screen targeting genes with nucleic acid or DNA binding domains and parasites expressing a stage-specific bradyzoite fluorescent reporter. Parasites that lack BFD1 fail to encyst under several stress conditions, and transgenic parasites that constitutively express a stabilized BFD1 protein show high levels of spontaneous differentiation in unstressed conditions. Consistent with BFD1 being a transcription factor that regulates bradyzoite differentiation, BFD1 binds to the promoter regions of many bradyzoite-specific genes as well as to the AP2IX-9 promoter (Figure 1B).

