Mitochondria and Lysosomes: New Friend Youth Buddies (NFYB).

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Title of previewed paper: NFYB-1 regulates mitochondrial function and longevity via lysosomal prosaposin

Introduction.

Mitochondria are defined as the “powerhouse” of eukaryotic cells by coordinating physiology, energy metabolism and signal transduction (Siekvitz and Potter, 1955). They generate ATP via oxidative phosphorylation (OXPHOS) consisting of the electron transport chain (ETC), which transfer electrons to reduce oxygen and generate water. To maintain its indispensable functions, cells employ a machinery to cope with mitochondrial metabolic dysfunction or unfolded protein accumulation within mitochondria through a transcriptional response known as the mitochondrial unfolded protein response (UPR^{mt}) to restore homeostasis (Lin and Haynes, 2016). Mitochondria and cellular energy metabolism have long been proposed to contribute to aging. In *C. elegans,* large-scale genetic screens identified longevity-promoting mutations in genes encoding ETC components as well as related to mitochondrial metabolism to the regulation of lifespan (Lee, Lee et al. 2003; Hamilton, Dong et al. 2005; Feng, Bussiere et al. 2001).

Lysosomes from the other side are cellular organelles that regulate cellular catabolism. Their substrates are wide-ranging: from damaged organelles and intracellular macromolecules to surface receptors and pathogens delivered by endocytosis (Carmona-Gutierrez et al., 2016; Futerman and van Meer, 2004). Lysosomes contain more than fifty catabolic hydrolases including lipases, proteases, nucleases that require an acidic pH maintained by the V-type ATPase for a proper catalytic activity. Lysosomes are involved in the regulation of cellular responses to nutrient availability, autophagy, stress resistance, programmed cell death, plasma membrane repair, development, and cell differentiation, among other functions (Settembre et al., 2013).

During the autophagy process, damaged organelles are engulfed in a double membrane organelle called the autophagosome, which then fuses to the lysosome for degradation of its cargo (Cesen et al., 2012). Autophagy is required and sufficient to promote longevity across species and modalities (Gelino and Hansen, 2012; Jia and Levine, 2007; Lapierre et al., 2013;

Melendez et al., 2003), and inhibition of autophagy accelerates aging (Cuervo et al., 2005; Rubinsztein et al., 2011).

Recent evidences suggested that mitochondria can coordinate with other organelles to regulate cellular homeostasis (D'Amico et al., 2017; Pellegrino et al., 2013; Quirós et al., 2016; Ramachandran et al., 2019). The mechanisms of communication include direct interaction via physical contact and indirect interaction via signaling and transcriptional pathways. In a recent study, physical contact between lysosome and mitochondria has been observed *in vitro* using Hela cells by the most advanced methods in live cell imaging (Wong, Ysselstein et al. 2018). In our lab, we previously identified a channel of lysosome-to-nucleus retrograde communication as well as a lysosome-to-mitochondria crosstalk resulting in lifespan extension (Folick et al., 2015; Ramachandran et al., 2019). Additionally, diseases caused by mutations in mitochondrial genes display lysosomal defects, and lysosome-related diseases display mitochondrial defects (Yambire et al., 2019; Fernandez-Mosquera et al., 2019).

Because the organelles defects spectrum is involved in metabolic homeostasis and aging, investigating such modes of communication remains an exciting area of research. Furthermore, mechanisms by which mitochondria communicate cell-autonomously and cell non-autonomously remain unknown. Antebi and colleagues addressed this question by identifying the nuclear transcription factor Y beta subunit (NFYB-1) as a crucial regulator of mitochondrial function in *C. elegans.* NFYB-1 is a conserved histone-like transcription factor that together with NFYA and NFYC forms a protein complex binding the CCAAT motif (Donati et al. 2008; Ceribelli et. al 2008). The authors also highlighted NFYB-1's role as a repressor of lysosomal prosaposin, a lysosomal and secreted glycoprotein involved in the hydrolysis of distinct glycosylated ceramides whose deletions cause sphingolipidoses and lysosomal storage disease (Elleder et al. 2005; Schuette et al. 2001). These results revealed a novel NFYB-1–prosaposin axis coordinating lysosome-to-mitochondrial crosstalk via lipid pools of cardiolipins and ceramides to enhance cellular mitochondrial function and longevity.

Results.

In *C. elegans,* mitochondria undergo intensive physiological and metabolic changes upon adult reproductive diapause (ARD) induced by late larval starvation, such as reduced mitochondrial

DNA levels, decreased oxygen consumption, downregulated expression of the mitochondrial reporter encoding for the complex IV subunit, and altered mitochondrial dynamics. Taking advantage of these reversible phenotypic changes upon refeeding and ARD recovery, the authors screened an RNAi library containing transcription factors, nuclear hormone receptors, chromatin regulators, phosphatases and kinases to identify regulators of mitochondrial function. Among 36 candidates that differentially mediate mitochondrial physiology, the authors identified NFYB-1, a protein that is expressed in the nucleus of various tissues. Inactivation of *nfyb-1* strongly reduced mitochondrial reporter expression and oxygen consumption and induced mitochondrial fragmentation during ARD recovery and ad libitum (AL) feeding. In the long-lived mutants harboring Rieske iron-sulfur protein defects, such as *isp-1(qm150)* and *cco-1* RNAi knockdown, the loss of *nfyb-1* suppressed this longevity phenotype, in part through impaired mitochondrial protein folding response (mitoUPR) by hindering the nuclear localization of essential mitoUPR transcriptional regulators.

Although NFYB-1 elicits transcription regulation with NFYC and NFYA in a protein complex, the role of NFYB-1 is unique in the context of altering mitochondrial function. To reveal new pathways regulated by NFYB-1, the authors performed unbiased transcriptomic and proteomic analyses. Although ER unfolded protein responses were significantly upregulated upon *nfyb-1* loss, the reduction of ER factors did not reverse the shortened lifespan upon *nfyb-1* loss in the *isp-1(qm150)* long-lived mutant. The lysosomal saposin-like protein family (SPP-8) was also identified through multi-omics analysis. Interestingly, the loss of *spp-8* fully restored mitochondrial longevity phenotype in *nfyb-1* mutant, suggesting that NFYB-1 normally promotes longevity by inhibiting SPP-8 function. SPP-8 is ortholog of human prosaposin PSAP, which plays a role in glycosphingolipid metabolism in the lysosome. Using lipidomic analysis, the authors examined the effect of *nfyb-1* mutation and *spp-8* knockdown to sphingolipid metabolism. As expected, the deficiency of SPP-8 reduced specific ceramide species and restored mitochondrial longevity upon *nfyb-1* loss. Furthermore, ceramide supplementation abolished mitochondrial longevity phenotype, which confirms the regulation of ceramide composition is important. Together, these results indicate that the NFYB-1-SPP-8 regulatory pathway impacts mitochondrial longevity through sphingolipid metabolism.

Since increased ceramide levels are often related to decreased levels of mitochondrial cardiolipins, the authors also evaluated cardiolipin composition using MS and *in vivo* staining. They found that reduced ceramides in the *spp-8* deficiency indeed correlated with increased cardiolipin levels in the *nfyb-1* mutants, likely through regulation of cardiolipin synthesis genes.

Cardiolipin supplementation also reversed a number of the mitochondrial defects in the *nfyb-1* mutant, suggesting that NFYB-1 may promote mitochondrial function by inducing cardiolipin synthesis as well as by limiting ceramide pools. .

Discussion

In this work, *Tharyan et al*. discovered that the transcription factor NFYB-1 regulates *C. elegans* mitochondrial function and morphology under a variety of physiological conditions, including ad libitum feeding, adult reproductive diapause, elevated temperature, and aging. Surprisingly, loss of NFYA or NFYC do not affect mitochondrial function, suggesting that NFYB-1 has a novel role outside of the NF-Y complex. It is not clear whether this role is unique to *C. elegans*, or whether NFYB homologues also regulate mitochondrial function in organisms as diverse as yeast, plants, and humans. If the latter is true, NFYB-1 may be an ancestral regulator of mitochondrial function.

NFYB-1 orchestrates an integrated cellular response, acting in concert with other nuclear transcription factors such as SKN-1 and DVE-1 to regulate genes whose products localize to the ER, mitochondria, and lysosomes. These products then participate in further inter-organelle crosstalk. For instance, lysosomal SPP-8 appears to mediate many of the mitochondrial phenotypes caused by loss of *nfyb-1,* perhaps by altering cellular ceramide and cardiolipin pools. This paper contributes to a growing body of literature demonstrating that communication between organelles is critical for cellular and organismal homeostasis.

In this study, the authors outlined a signaling cascade in which NFYB-1 inhibits SPP-8, which in turn modulates cellular lipid pools and mitochondrial function. The exact links between each step in the cascade offer ample room for future investigation. Loss of *nfyb-1* causes broad gene expression changes, and the mechanism by which it regulates *spp-8* has not yet been explored. The NF-Y complex is known to bind CCAAT motifs, which are absent from the *spp-8* promoter. NFYB-1 may bind to other sequence motifs when it acts independently of the NF-Y complex, and perhaps function cooperatively with other transcription factors. Alternatively, NFYB-1 may transcriptionally regulate upstream factors, which in turn regulate *spp-8.* Future studies are also needed to determine how SPP-8 affects lipid pools. Saposins typically function by making lipids more accessible to hydrolytic enzymes. Studies of the lipid substrate(s) of SPP-8 and their corresponding hydrolases will lend insight into how lipid homeostasis is controlled. Finally, the connection between lipid homeostasis and mitochondrial function is a ripe area for future

investigation. The most direct hypothesis is that lipids are transferred from the lysosome to the mitochondria, thus changing the properties of the mitochondrial membrane and therefore mitochondrial function. It will not be trivial, however, to test how membrane composition affects mitochondrial enzymes and fission/fusion dynamics.

A further outstanding question is whether NFYB-1 activity is dynamically regulated. While NFYB-1 is primarily found in the nucleus, it also exhibits some lysosomal localization. This raises the possibility that NFYB-1 localization is regulated by binding to lysosomal factors. This would provide a mechanism for NFYB-1 activity to respond to cellular stresses and environmental factors, perhaps using a mechanism analogous to mTOR. Given that NFYB-1 regulates many immune genes, infection is another possible trigger of NFYB-1 activity. A detailed examination of NFYB-1 protein interaction partners and posttranslational modifications may lend insight into what inputs, if any, trigger NFYB-1-mediated modulation of lysosomal and mitochondrial activities.

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