Mitochondria - lysosome communication mediates longevity

Preview for “Tharyan, R.G., Annibal, A., Schiffer, I. *et al.* NFYB-1 regulates mitochondrial function and longevity via lysosomal prosaposin. *Nat Metab* 2, 387–396 (2020). https://doi.org/10.1038/s42255-020-0200-2”

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INTRODUCTION

Mitochondrial function and behavior are central to the cellular vitality and organismal fitness. Mitochondria act as a key factor in diverse biological processes including energy modulation, lipid metabolism, apoptosis, and calcium balance. Abnormalities in mitochondria are also associated with various human neurodegenerative and metabolic diseases. Importantly, this dynamic organelle has been increasingly recognized as an important player in the aging process. It has been appreciated that the decline in mitochondrial function is accompanied by age and contributes to the age-related decline of organ health. Nonetheless, the modest disruption of mitochondrial function often leads to an increase in lifespan (Durieux J et al., 2007). This seeming paradox might be explained by the multidimensionality of mitochondrial function. One mechanism of the extended lifespan caused by decreased mitochondrial function might be the activation of the mitochondrial unfolded protein response (UPRmt) (Sun et al., 2016). Similarly to UPR induced by the endoplasmic reticulum, UPRmt is stress triggered by the accumulation of misfolded proteins in the mitochondria or the depletion of the mitochondrial genome. UPRmt can induce the retrograde signaling to the nucleus which ultimately upregulates the expression of mitochondrial chaperones to restore the homeostasis.

Besides interacting with the nucleus, mitochondria transduce the signaling pathway to other cellular compartments such as lysosomes, forming a dynamic network to cooperate to extend lifespan. To illuminate the major regulators in mitochondrial function and reveal the role of interaction between mitochondria and lysosome in longevity, Tharyan *et al*. utilized RNAi screen in *C. elegans* to identify nuclear

transcription factor Y, beta subunit (NFYB-1) as a crucial regulator in mitochondrial function. NFYB-1 is one of the subunits of the NF-Y transcription factor and contains a conserved histone fold motif (Ceribelli M et al., 2008). The trimeric factor NF-Y can bind to the CCAAT box which is a widespread motif of most ER stress promoters. However, it has been unclear whether NFYB-1 helps to maintain mitochondrial function according to the previous research. To further illustrate the potential NFYB-1 mediated crosstalk between mitochondria and other organelles, Tharyan *et al.* performed the secondary screen and revealed that NFYB-1 acted as a potent repressor of lysosomal saposin-like protein (SPP-8) to regulate mitochondrial function. *spp-8* encodes an ortholog of human prosaposin (PSAP) and prosaposin-like (PSAPL1) proteins. The PSAP deficiency can cause a neonatal neurovisceral lipid storage disease (Elleder, M et al., 2005), but little is known about its role in mitochondria. In this paper, the authors revealed that the NFYB-1-prosaposin axis coordinated the signaling between lysosome and mitochondria to mediate mitochondrial function and organismal health.

RESULTS

Tharyan *et al.* found that adult reproductive diapause (ARD) causes a reduction in both mitochondrial DNA (mtDNA) levels and oxygen consumption as well as a reduction in mitochondrial circularity and mitochondrial fluorescence that are examined by mitochondrial reporters, *pmyo-3::GFP*^{mt} and *pcco-1::GFP*, respectively. Additionally, they showed that ARD recovery can reverse these phenotypes. Using these mitochondrial features as a starting point, they performed an RNAi screen to identify genes that can alter *pcco-1::GFP* levels upon ARD recovery. Among ~2200 RNAi library clones that contained transcription factors, nuclear hormone receptors, chromatin regulators, phosphatases, and kinases, they identified 30 candidates that downregulate *pcco-1::GFP* level and 6 candidates that upregulate it. To narrow down their candidate numbers, the authors assessed the genes whose loss abolishes lifespan extension caused by *isp-1* mitochondrial mutants but does not affect the longevity of wild type. They identified histone-like transcription factor, NFYB-1 as a potent regulator of mitochondrial function and longevity. They also confirmed NFYB-1's nuclear localization in almost all tissues and developmental stages by endogenously tagging it with mKate2.

Authors' data revealed that in addition to reduced *pcco-1::GFP* levels upon ARD recovery, *nfyb-1* loss also perturbs oxygen consumption rate and nuclear localization of mitochondrial stress factors, DVE-1 and ATFS-1.

Next, Tharyan *et al.* performed unbiased transcriptomics and proteomics analyses using WT, *nfyb-1* mutants, *isp-1* mutants, and *isp-1; nfyb-1* double mutants to examine novel potential pathways regulated by NFYB-1. With this multi-omics study, they identified many differentially expressed transcripts and proteins that suggest roles for NFYB-1 in organellar functions and stress response as assessed by *nfyb-1* mutant results compared to both WT and *isp-1; nfyb-1* double mutant results. Then the authors focused on multi-omics results that showed an enrichment in *nfyb-1* mutants, hypothesizing that these enriched transcripts/proteins may be responsible for the suppression of *isp-1*-mediated longevity upon *nfyb-1* loss. They tested whether knockdown of upregulated proteins would restore longevity in *isp-1; nfyb-1* double mutant background. Their secondary screen on longevity revealed that knockdown of lysosomal saposin-like protein (SPP-8) restores *isp-1; nfyb-1* longevity. To assess regulators of increased SPP-8 expression in *nfyb-1* mutants, they performed an RNAi screen against transcription factors that bind to the *spp-8* promoter and identified mitochondrial regulators *dve-1* and *skn-1*. They further examined whether loss of *spp-8* rescues mitochondrial dysfunction that is caused by *nfyb-1* loss and found that knockdown of *spp-8* in *nfyb-1* mutants rescues longevity impairment, mitochondrial morphology defects, and oxygen consumption rate reduction.

Through a targeted proteomics analysis, they found that long-lived *isp-1* mutants have lower ceramide levels whereas short-lived *isp-1; nfyb-1* double mutants have higher levels of ceramide. They found that *spp-8* knockdown restores ceramide back to lower levels in double mutants and ceramide supplementation in *isp-1* mutants abolishes longevity. As data from the literature suggests an inverse association between ceramide and cardiolipin (Kim et al., 2016; Okino et al., 2003), they tested cardiolipin levels in the mutants. As expected, their data revealed that both *isp-1* and *nfyb-1* show reduction in cardiolipin and *spp-8* knockdown increases cardiolipin levels. Finally, the authors found

that cardiolipin supplementation restores mitochondrial morphology and oxygen consumption rate in *nfyb-1* mutants as well as mitochondrial longevity in *isp-1; nfyb-1* double mutants.

SIGNIFICANCE/FUTURE DIRECTIONS

Altogether, Tharyan *et al*. revealed a novel communication pathway between lysosomes and mitochondria that regulates mitochondrial function and longevity through the NFYB-1-prosaposin axis. They investigated its impact on mitochondrial physiology and longevity from several aspects by performing transcriptomics, proteomics, and lipidomics. Their multi-omics data revealed that NFYB-1 regulated an organellar network - promotes the expression of mitochondria stress response factors, inhibits ER stress response genes, and restrains lysosomal prosaposin, but overall enhances mitochondrial function and longevity.

There still remains exciting questions to study further. First, NFYB-1 is expressed in multiple tissues, including neurons, hypodermis, muscle, intestine, and germline. It would be interesting to examine whether there is any particular tissue where NFYB-1 functions to regulate longevity. Neurons could be the functional tissue, as they are cell non-autonomous regulators of UPRmt. Muscle has very active mitochondrial metabolism and therefore could also be a possible candidate site. On the other hand, metabolically most active tissue in the worm is the intestine and it is likely the site where the other partner of this regulatory axis, SPP-8, resides. Or the correct answer could be "above all" and NFYB-1/SPP-8 could be acting systemically in every tissue to regulate longevity. The second compelling question is about the organelle NFYB-1 functions to modulate mitochondrial function, because, in addition to the nucleus, NFYB-1 also localizes to the lysosomes in the intestine. Is the lysosomal localization of NFYB-1 an artifact of its overexpression or indeed it translocates to the lysosomes to regulate SPP-8 function directly? This may be examined by generating transgenic worms that express NFYB-1 without nuclear localization sequence or lysosomal localization sequence and determine whether they fail to rescue the *nfyb-1* mutant phenotypes. Along these lines,

another exciting future direction could be to investigate whether and how NFYB-1 regulates lysosome-mitochondria contact site formation and SPP-8 mediates this interaction. Finally, how does the NFYB-1/SPP-8 regulatory axis modulate ceramide and cardiolipin levels? Where are the ceramides and cardiolipins generated to impact mitochondrial activity? One likely answer is the lysosome. It could be interesting to study this question by performing immunopurification of lysosomes and/or mitochondria and compare their proteome and metabolome in wild-type worms and mitochondrial longevity mutants. These multi-omic studies will also shed light onto the poorly studied cardiolipin metabolism in worms. Overall, this study by Tharyan *et al*. discovered that NFYB-1/SPP-8 regulatory axis affects mitochondrial physiology and longevity by controlling the levels of ceramides and cardiolipins that have inhibiting and promoting effects on lifespan, respectively. In addition, their findings brought new aspects in studying how inter-organellar communication impacts organismal health and lifespan.

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