Worm

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/kwrm20

Interfacing mitochondrial biogenesis and elimination to enhance host pathogen defense and longevity

Konstantinos Palikaras[ab], Eirini Lionaki[a] & Nektarios Tavernarakis[ac]

[a] Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, Heraklion 70013, Crete, Greece

[b] Department of Biology, University of Crete, Heraklion 70013, Crete, Greece

[c] Department of Basic Sciences, Faculty of Medicine, University of Crete, Heraklion 70013, Crete, Greece

Accepted author version posted online: 29 Jul 2015.

Running head: Energy homeostasis and mitochondrial turnover

Interfacing mitochondrial biogenesis and elimination to enhance host pathogen defense and longevity

Konstantinos Palikaras[1,2], Eirini Lionaki[1] and Nektarios Tavernarakis[1,3,*]

[1]Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, [2] Department of Biology, University of Crete, [3]Department of Basic Sciences, Faculty of Medicine, University of Crete, Heraklion 70013, Crete, Greece

*Correspondence to: Nektarios Tavernarakis; Email: tavernarakis@imbb.forth.gr

Keywords: ageing, autophagy, homeostasis, mitochondria, mitophagy, stress, innate immunity

Related Article: Palikaras, K., Lionaki E., Tavernarakis N. Coordination of mitophagy and mitochondrial biogenesis during ageing in *Caenorhabditis elegans. Nature*, 521: 525-528, 2015; doi: 10.1038/nature14300.

Abstract

Mitochondria are highly dynamic and semi-autonomous organelles, essential for many fundamental cellular processes, including energy production, metabolite synthesis and calcium homeostasis, among others. Alterations in mitochondrial activity not only influence individual cell function but also, through non-cell autonomous mechanisms, whole body metabolism, healthspan and lifespan. Energy homeostasis is orchestrated by the complex interplay between mitochondrial biogenesis and mitochondria-selective autophagy (mitophagy). However, the cellular and molecular pathways that coordinate these two opposing processes remained obscure. In our recent study, we demonstrate

that DCT-1, the *Caenorhabditis elegans* homolog of the mammalian BNIP3 and BNIP3L/NIX, is a key mediator of mitophagy, and functions in the same genetic pathway with PINK-1 and PDR-1 (the nematode homologs of PINK1 and Parkin respectively) to promote longevity and prevent cell damage under stress conditions. Interestingly, accumulation of damaged mitochondria activates SKN-1 (SKiNhead-1), the nematode homologue of NRF2, which in turn initiates a compensatory retrograde signaling response that impinges on both mitochondrial biogenesis and removal. In this commentary, we discuss the implications of these new findings in the context of innate immunity and ageing. Unraveling the regulatory network that governs the crosstalk between mitochondrial biogenesis and mitophagy will enhance our understanding of the molecular mechanisms that link aberrant energy metabolism to ageing and disease.

Molecular mechanisms of mitochondrial quality control and homeostasis

Mitochondria are organelles specialized in energy production and critically influence metabolism, healthspan and lifespan of living organisms. Changes in mitochondrial number, morphology and function affect cellular and organismal homeostasis. Mitochondrial activity is determined by a combination of large gene regulatory networks, cellular processes, tissue communication, hormonal systems and environmental factors. The complexity of controlling mitochondrial function is underlined by the regulated interaction between two genomes. Mitochondria are semi-autonomous organelles that contain their own DNA[1]. Mitochondrial genes encode thirteen essential subunits of the electron transport chain multiprotein complexes. These proteins are synthesized in the mitochondrial matrix and directly inserted into the inner mitochondrial membrane. The vast majority of mitochondrial proteins, however, is encoded by nuclear genes. These proteins are synthesized in the cytoplasm and are loaded on sub-mitochondrial compartments through a highly specialized import system[2, 3]. Stoichiometric imbalance between nuclear and mitochondrial encoded proteins can lead to proteotoxic and metabolic stress[4].

Several quality control mechanisms have evolved in eukaryotes to sustain mitochondrial function and prevent cellular damage. Numerous, redundant molecular pathways preserve and restore mitochondrial activity (**Fig. 1**) [5, 6]. Mitochondria contain their own proteolytic system. These proteases are the first line of defense against mitochondrial damage and mediate the degradation of non-assembled, misfolded or unfolded proteins, ameliorating mitochondrial dysfunction (**Fig. 1A**) [7]. In addition, the mitochondrial unfolded protein response (UPR^{mt}) is activated under proteotoxic stress. Unfolded and/or non-assembled mitochondrial proteins are digested into peptides by mitochondrial proteases and transported to the cytoplasm through the HAF-1 transporter[8]. In turn, the bZip transcription factor ATFS-1 becomes activated. Recent studies show that ATFS-1, which contains both a mitochondrial translocation signal and nuclear localization sequence, shuttles between mitochondria and the nucleus in response to mitochondrial stress. Mitochondrial import efficiency is diminished upon mitochondrial dysfunction and ATFS-1 accumulates in the nucleus

driving the expression of mitochondrial chaperones and proteases, which in turn restore mitochondrial proteostasis and augment mitochondrial quality control[9-11].

Mitochondria form a dynamic network that constantly changes shape and morphology in response to energy demands. The processes of fission and fusion regulate expansion and shrinkage of the mitochondrial network, facilitating repair of mitochondrial damage through segregation or exchange of components and materials (**Fig. 1B**) [12]. Mitochondrial dynamics enhance organellar maintenance by separation and/or dilution of toxic material, lowering its impact on mitochondrial physiology.

When damage exceeds the restoration capacity of protein quality control mechanisms, dysfunctional mitochondria are removed by selective mitochondrial autophagy (mitophagy) (**Fig. 1C**). During mitophagy mitochondria are engulfed by autophagosomes and are delivered to lysosomes for degradation[13]. Besides its pivotal role in the maintenance of energy homeostasis under conditions of stress, mitophagy is also stimulated in a programmed fashion during development[14, 15]. The PINK1/Parkin pathway has been implicated in the regulation of mitophagy in mammals. The PTEN-induced mitochondrial kinase 1 (PINK1) becomes stabilized on the outer mitochondrial membrane upon mitochondrial dysfunction and recruits the cytosolic E3 ubiquitin ligase Parkin. In turn, Parkin ubiquitylates numerous outer mitochondrial membrane proteins, including mitofusin 1 and 2 (MFN1and MFN2), voltage-dependent ion channel proteins (VDACs) and components of the mitochondrial import system (TOM70, TOM40 and TOM20) among others, leading to mitochondrial fragmentation and isolation of dysfunctional mitochondria from the healthy mitochondrial population. Subsequently, damaged mitochondria are recognized, targeted and eliminated by the autophagic machinery[13, 15].

In addition to the PINK1/Parkin pathway, the outer mitochondrial membrane proteins BINP3 and BNIP3L/NIX mediate mitophagy in mammals. BNIP3 and BNIP3L/NIX function as mitophagy receptors targeting mitochondria to autophagosomes via their interaction with the autophagosomal protein microtubule-associated protein 1 light chain 3 (MAP1LC3/LC3)[16]. In our

recent study, we demonstrated that DCT-1 is the functional homolog of the mammalian BNIP3 and BNIP3L/NIX in *C. elegans*. Similar to its mammalian counterparts, DCT-1 interacts with the autophagosomal protein LGG-1, the homolog of MAP1LC3/LC3, under conditions of stress. Notably, DCT-1, PINK-1 and PDR-1 (the nematode homologs of the mammalian PINK1 and Parkin respectively) function in the same genetic pathway to mediate mitophagy, thereby preserving mitochondrial integrity and homeostasis. DCT-1 acts downstream of PINK-1 and PDR-1. DCT-1 ubiquitination is enhanced in a PINK-1-dependent manner in response to oxidative stress. Co-localization between DCT-1 and PDR-1 suggests that DCT-1 ubiquitination is likely facilitated by PDR-1[17].

Mitochondrial selective autophagy and mitochondrial biogenesis promote cellular adaptation in response to impaired mitochondrial activity. Generation of newly synthesized, and elimination of damaged organelles, are highly regulated and fine-tuned processes. The tight coordination of mitochondrial biogenesis and mitophagy is essential for the maintenance of energy homeostasis. An imbalance between these two opposing processes has detrimental consequences for cellular and organismal survival.

The interplay between mitophagy and mitochondrial biogenesis during ageing

The capacity of cells to cope with several stress conditions, including misfolded proteins, damaged organelles, pathogens invasion, temperature and oxygen fluctuations, declines with age. Age-dependent disruption of mitochondrial homeostasis, which is characterized by accumulation of dysfunctional mitochondria and excessive increase in the overall mitochondrial content, contributes to impairment of cellular function[18].

Several studies have revealed a gradual accumulation of mitochondrial mass in several cell types of diverse organisms during ageing and in various human age-related pathologies[5, 19-24]. The molecular basis of this disruption of mitochondrial mass homeostasis remained elusive. We observed a progressive accumulation of mitochondria with age in several tissues of *C. elegans*[17, 19]. Impairment of autophagy recapitulates the effect of ageing on mitochondrial mass in young animals.

Similar to general autophagy deficiency, specific inhibition of mitophagy by knocking down *dct-1, pink-1* and *pdr-1* expression results in increased mitochondrial mass. Additionally, mitophagy-deficient animals display pronounced mitochondrial dysfunction, increased sensitivity to various stressors and abrogation of lifespan prolonging interventions. Thus, induction of mitophagy mediates the elimination of dysfunctional mitochondria under stress conditions, promoting survival and longevity. Moreover, age-dependent attenuation of mitophagy contributes to accretion of mitochondria during ageing and deterioration of cellular function[17].

Accumulation of damaged mitochondria causes oxidative stress and triggers SKN-1 activation. Indeed, we found that SKN-1 becomes activated in mitophagy-deficient animals and drives the expression of several mitochondrial biogenesis genes. Surprisingly, *dct-1* is among the transcriptional targets SKN-1 (**Fig. 2**). In addition to SKN-1, DAF-16 also regulates the expression of *dct-1.* Consistently, we found that DCT-1, DAF-16 and SKN-1 function non-redundantly to regulate mitophagy and sustain mitochondrial function in response to oxidative stress[17]. SKN-1 is tethered to mitochondria through its association with the outer mitochondrial protein PGAM-5[25]. Thus, SKN-1 could function as a barometer of mitochondrial homeostasis, sensing mitochondrial damage to promote detoxification and cell survival.

In parallel, mitochondrial stress stimulates ATFS-1, which orchestrates induction of UPR^{mt}. Under physiological conditions, ATFS-1 is constitutively imported into mitochondria and degraded by mitochondrial proteases. By contrast, mitochondrial dysfunction results in nuclearization of ATFS-1, which then drives the expression of numerous genes encoding mitochondrial proteases, chaperones, and components of mitochondrial fission and import machinery among others, in a compensatory effort to restore mitochondrial activity[11]. Accumulating evidence indicates a tight crosstalk between mitophagy and UPR^{mt}. Mitochondrial proteins expressed upon ATFS-1 activation should be imported in mitochondria. However, mitochondrial import efficiency is diminished under conditions of mitochondrial dysfunction. ATFS-1 activation also promotes mitochondrial fission, which is a prerequisite for mitophagy[11]. In turn, mitophagy eliminates damaged mitochondria and enriches the

healthy mitochondrial pool. Additionally, ATFS-1 likely enhances mitophagy and mitochondrial biogenesis indirectly through the transcriptional upregulation of the *skn-1* gene[11] (**Fig. 2**). It would be interesting to further investigate how these distinct quality control pathways integrate to optimize their complementary effects on the maintenance of energy metabolism.

Interestingly, in this context, a recent study has revealed a nuclear role for the CLK-1 protein. CLK-1 is a mitochondrial enzyme participating in the biosynthesis of ubiquinone, an essential co-factor of the electron transport chain (ETC)[26, 27]. CLK-1 deficiency results in SKN-1 activation, induction of UPR^{mt} and lifespan extension[28]. Notably, mitophagy is also required for the enhanced longevity of *clk-1* mutants[17]. Nuclear CLK-1 affects longevity independently of its role in energy production, acting as biosensor of reactive oxygen species (ROS) to regulate basal levels of ROS and maintain mitochondrial homeostasis. Reduced ROS promotes CLK-1 localization to mitochondria. By contrast ROS elevation leads to nuclearization of CLK-1, which then associates with chromatin to regulate gene expression. Nuclear CLK-1 diminishes UPR^{mt} activity and prevents elevation of *skn-1* expression, upon mitochondrial stress. These findings indicate that nuclear CLK-1 prevents stimulation of UPR^{mt} under non-stress conditions[28]. Additionally, nuclear CLK-1 might inhibit mitophagy and mitochondrial biogenesis indirectly by suppressing the expression of *skn-1*.

Mitochondrial quality control and mitophagy: An intricate crosstalk during bacterial infection

As a soil-dwelling nematode, *C. elegans* encounters, grows on and defends itself against many microorganisms in its natural habitat. Animals detect the presence of pathogens not only through physical interaction, but also through sensing pathogen-mediated perturbations in essential cellular processes. Pathogens exploit an extensive arsenal of virulence factors to attack and invade host barriers. Bacterial toxins and secreted proteins, alter host mitochondrial function to promote pathogen proliferation and progression of infection[29-32]. As a consequence, mitochondria have developed protective mechanisms against pathogen invasion, including ROS generation[33], inflammasome induction[34], autophagy[31] and UPR^{mt} activation[35] among others.

In addition to pronounced mitochondrial dysfunction, several bacterial species are known to induce UPR^{mt} in nematodes, in the absence of other stressors[32, 35-37]. ATFS-1, the master regulator of UPR^{mt}, is nuclearized in response to *Pseudomonas aeruginosa* infection and drives expression of several immune-response genes, including anti-microbial peptides and secreted lysozyme, similar to those induced by impairment of mitochondria function[35]. These findings suggest a link between bacterial infection, mitochondrial activity and UPR^{mt}.

Besides UPR^{mt}, accumulating evidence indicates an essential role for autophagy in the defense against bacterial infection. Autophagy-depleted animals display increased accumulation of *Salmonella enterica* in intestinal cells, suggesting that autophagy blocks invasion and/or replication of the pathogen[38, 39]. Furthermore, autophagy impairment leads to decreased survival upon *S. enterica* and *Nematocida parisii* infection[40]. HLH-30, the homolog of the mammalian transcription factor EB (TFEB), is the master regulator of lysososomal biogenesis and autophagy, as well as a critical component of the innate immune host defense system[41, 42]. HLH-30 is activated and rapidly translocated from the cytoplasm to the nucleus upon exposure to *Staphylococcus aureus.* HLH-30 regulates expression of several host genes in response ti infection, including anti-microbial and autophagy genes, to promote tolerance and cell survival[43]. Furthermore, mitochondrial-selective autophagy has been implicated in the defense against pathogens. PINK-1- and PDR-1-depleted animals are less resistant to *P. aeruginosa*[31]. *P. aeruginosa* infection impairs mitochondrial function by chelating iron, which is pivotal for ETC activity. Iron depletion triggers mitophagy to eliminate damaged organelles and preserve energy homeostasis[44]. These findings, in their totality, indicate that induction of autophagy enhances resistance against pathogenic bacteria by simultaneously promoting two distinct, selective types of autophagy: xenophagy, that targets and eliminates entire intracellular pathogens, and mitophagy, which promotes degradation of dysfunctional mitochondria (**Fig. 3**).

Bacterial infection also triggers the activation of SKN-1 and DAF-16 transcription factors[31, 45]. SKN-1 is induced in a TIR-1- and PMK-1-depedent manner (the nematode homologs of the

mammalian Toll/IL-1 receptor and p38 MAPK kinase respectively) to promote survival upon *P. aeruginosa* and *Enterecoccus faecalis*[45]. In addition, DAF-16 is activated during *P. aeruginosa* infection to ensure host defense[31]. As mentioned above, SKN-1 and DAF-16 modulate mitophagy through transcriptional regulation of the mitophagy receptor DCT-1. Moreover, SKN-1 orchestrates the expression of several nuclear genes encoding mitochondrial related factors, thereby regulating mitochondrial biogenesis[17]. Thus, SKN-1 maintains energy homeostasis by coordination of mitophagy and the generation of new mitochondria during bacterial infection.

Concluding remarks

Our findings unravel a key regulatory mechanism that coordinates mitochondrial biogenesis and degradation of dysfunctional mitochondria through mitophagy. The mitophagy receptor DCT-1 is a nodal element of the pathway integrating extracellular and intracellular signals to promote mitophagy. Pronounced mitochondrial defects and runaway accumulation of damaged mitochondria trigger SKN-1 activation. In turn, SKN-1 orchestrates a bipartite retrograde signaling response, facilitating the coordinated expression of both mitochondrial biogenesis and mitophagy genes. Bacterial toxins and secreted proteins impair mitochondrial physiology leading to pathogen propagation. In response, quality control mechanisms become activated, including UPR^{mt}, mitophagy, HLH-30 and SKN-1 among others, to promote tolerance and resistance against pathogens, and to repair or eliminate damaged mitochondria. Mitochondrial stress promotes ATFS-1 nuclearization, which in turn induces expression of several genes to maintain mitochondrial homeostasis. Interestingly, ATFS-1 also regulates *skn-1* expression establishing an intimate link between UPR^{mt}, mitophagy and mitochondrial biogenesis. Thus, SKN-1 serves a central rheostat function for mitochondrial homeostasis, sensing mitochondrial damage to promote healthspan, stress resistance and survival in response to diverse intracellular and environmental stimuli, including nutrient availability, growth factors and hormones, pathogens, toxins, temperature and oxygen fluctuations, among others. Unraveling the regulatory network that couples mitochondrial

biogenesis and mitochondrial quality control mechanisms during ageing or upon bacterial infections will provide new insights into therapeutic interventions promoting organismal longevity and resistance to pathogens.

Disclosure of Potential Conflict of Interest

No potential conflicts of interest were disclosed.

Funding

Work in the authors' laboratory is funded by grants from the European Research Council (ERC), the European Commission Framework Programs, and the General Secretariat for Research and Technology of the Greek Ministry of Education.

References

1. Nunnari J, Suomalainen A. Mitochondria: in sickness and in health. Cell 2012; 148:1145-59; PMID: 22424226; http://dx.doi.org/10.1016/j.cell.2012.02.035
2. Opalinska M, Meisinger C. Metabolic control via the mitochondrial protein import machinery. Current opinion in cell biology 2015; 33:42-8; PMID: 25497717; http://dx.doi.org/10.1016/j.ceb.2014.11.001
3. Schmidt O, Pfanner N, Meisinger C. Mitochondrial protein import: from proteomics to functional mechanisms. Nature reviews Molecular cell biology 2010; 11:655-67; PMID: 20729931; http://dx.doi.org/10.1038/nrm2959k
4 Andreux PA, Houtkooper RH, Auwerx J. Pharmacological approaches to restore mitochondrial function. Nature reviews Drug discovery 2013; 12:465-83; PMID:23666487; http://dx.doi.org/10.1038/nrd4023
5. Palikaras K, Tavernarakis N. Mitochondrial homeostasis: the interplay between mitophagy and mitochondrial biogenesis. Experimental gerontology 2014; 56:182-8; PMID:24486129; http://dx.doi.org/10.1016/j.exger.2014.01.021
6. Scheibye-Knudsen M, Fang EF, Croteau DL, Wilson DM, 3rd, Bohr VA. Protecting the mitochondrial powerhouse. Trends in cell biology 2015; 25:158-70; PMID:25499735; http://dx.doi.org/10.1016/j.tcb.2014.11.002
7. Roy M, Reddy PH, Iijima M, Sesaki H. Mitochondrial division and fusion in metabolism. Current opinion in cell biology 2015; 33:111-8; PMID:25703628; http://dx.doi.org/10.1016/j.ceb.2015.02.001
8. Quiros PM, Langer T, Lopez-Otin C. New roles for mitochondrial proteases in health, ageing and disease. Nature reviews Molecular cell biology 2015; 16:345-59; PMID:25970558; http://dx.doi.org/10.1038/nrm3984

9. Haynes CM, Yang Y, Blais SP, Neubert TA, Ron D. The matrix peptide exporter HAF-1 signals a mitochondrial UPR by activating the transcription factor ZC376.7 in C. elegans. Molecular cell 2010; 37:529-40; PMID:20188671; http://dx.doi.org/10.1016/j.molcel.2010.01.015

10. Haynes CM, Petrova K, Benedetti C, Yang Y, Ron D. ClpP mediates activation of a mitochondrial unfolded protein response in C. elegans. Developmental cell 2007; 13:467-80; PMID:17925224; http://dx.doi.org/10.1016/j.devcel.2007.07.016

11. Benedetti C, Haynes CM, Yang Y, Harding HP, Ron D. Ubiquitin-like protein 5 positively regulates chaperone gene expression in the mitochondrial unfolded protein response. Genetics 2006; 174:229-39; PMID:16816413; http://dx.doi.org/10.1534/genetics.106.061580

12. Nargund AM, Pellegrino MW, Fiorese CJ, Baker BM, Haynes CM. Mitochondrial import efficiency of ATFS-1 regulates mitochondrial UPR activation. Science 2012; 337:587-90; PMID:22700657; http://dx.doi.org/10.1126/science.1223560

13. Pickrell AM, Youle RJ. The roles of PINK1, parkin, and mitochondrial fidelity in Parkinson's disease. Neuron 2015; 85:257-73; PMID:25611507; http://dx.doi.org/10.1016/j.neuron.2014.12.007

14. Novak I, Dikic I. Autophagy receptors in developmental clearance of mitochondria. Autophagy 2011; 7:301-3; PMID:21206218; http://dx.doi.org/10.4161/auto.7.3.14509

15. Ashrafi G, Schwarz TL. The pathways of mitophagy for quality control and clearance of mitochondria. Cell death and differentiation 2013; 20:31-42; PMID:22743996; http://dx.doi.org/10.1038/cdd.2012.81

16. Liu L, Sakakibara K, Chen Q, Okamoto K. Receptor-mediated mitophagy in yeast and mammalian systems. Cell research 2014; 24:787-95; PMID:24903109; http://dx.doi.org/10.1038/cr.2014.75

17. Palikaras K, Lionaki E, Tavernarakis N. Coordination of mitophagy and mitochondrial biogenesis during ageing in C. elegans. Nature 2015; 521:525-8; PMID:25896323; http://dx.doi.org/10.1038/nature14300

18. Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell 2013; 153:1194-217; PMID:23746838; http://dx.doi.org/10.1016/j.cell.2013.05.039

19. Artal-Sanz M, Tavernarakis N. Prohibitin couples diapause signalling to mitochondrial metabolism during ageing in C. elegans. Nature 2009; 461:793-7; PMID:19812672; http://dx.doi.org/10.1038/nature08466

20. Kaeberlein M. Lessons on longevity from budding yeast. Nature 2010; 464:513-9; PMID:20336133; http://dx.doi.org/10.1038/nature08981

21. Malpass K. Neurodegenerative disease: defective mitochondrial dynamics in the hot seat-a therapeutic target common to many neurological disorders? Nature reviews Neurology 2013; 9:417; PMID:23877644; http://dx.doi.org/10.1038/nrneurol.2013.138

22. Preston CC, Oberlin AS, Holmuhamedov EL, Gupta A, Sagar S, Syed RH, et al. Aging-induced alterations in gene transcripts and functional activity of mitochondrial oxidative phosphorylation complexes in the heart. Mechanisms of ageing and development 2008; 129:304-12; PMID:18400259; http://dx.doi.org/10.1016/j.mad.2008.02.010

23. Vafai SB, Mootha VK. Mitochondrial disorders as windows into an ancient organelle. Nature 2012; 491:374-83; PMID:23151580; http://dx.doi.org/10.1038/nature11707

24. Wallace DC. Diseases of the mitochondrial DNA. Annual review of biochemistry 1992; 61:1175-212; PMID:1497308; http://dx.doi.org/10.1146/annurev.bi.61.070192.005523

25. Paek J, Lo JY, Narasimhan SD, Nguyen TN, Glover-Cutter K, Robida-Stubbs S, et al. Mitochondrial SKN-1/Nrf mediates a conserved starvation response. Cell metabolism 2012; 16:526-37; PMID:23040073; http://dx.doi.org/10.1016/j.cmet.2012.09.007

26. Felkai S, Ewbank JJ, Lemieux J, Labbe JC, Brown GG, Hekimi S. CLK-1 controls respiration, behavior and aging in the nematode Caenorhabditis elegans. The EMBO journal 1999; 18:1783-92; PMID:10202142; http://dx.doi.org/10.1093/emboj/18.7.1783

27. Wong A, Boutis P, Hekimi S. Mutations in the clk-1 gene of Caenorhabditis elegans affect developmental and behavioral timing. Genetics 1995; 139:1247-59; PMID:7768437

28. Monaghan RM, Barnes RG, Fisher K, Andreou T, Rooney N, Poulin GB, et al. A nuclear role for the respiratory enzyme CLK-1 in regulating mitochondrial stress responses and longevity. Nature cell biology 2015; 17:782-92; PMID:25961505; http://dx.doi.org/10.1038/ncb3170

29. Pellegrino MW, Haynes CM. Mitophagy and the mitochondrial unfolded protein response in neurodegeneration and bacterial infection. BMC biology 2015; 13:22; PMID:25857750; http://dx.doi.org/10.1186/s12915-015-0129-1

30. Bos LD, Sterk PJ, Schultz MJ. Volatile metabolites of pathogens: a systematic review. PLoS pathogens 2013; 9:e1003311; PMID:23675295; http://dx.doi.org/10.1371/journal.ppat.1003311

31. Kirienko NV, Ausubel FM, Ruvkun G. Mitophagy confers resistance to siderophore-mediated killing by Pseudomonas aeruginosa. Proceedings of the National Academy of

Sciences of the United States of America 2015; 112:1821-6; PMID:25624506; http://dx.doi.org/10.1073/pnas.1424954112

32. Liu Y, Samuel BS, Breen PC, Ruvkun G. Caenorhabditis elegans pathways that surveil and defend mitochondria. Nature 2014; 508:406-10; PMID:24695221; http://dx.doi.org/10.1038/nature13204

33. Arsenijevic D, Onuma H, Pecqueur C, Raimbault S, Manning BS, Miroux B, et al. Disruption of the uncoupling protein-2 gene in mice reveals a role in immunity and reactive oxygen species production. Nature genetics 2000; 26:435-9; PMID:10.1038/82565; http://dx.doi.org/10.1038/82565

34. Zhou R, Yazdi AS, Menu P, Tschopp J. A role for mitochondria in NLRP3 inflammasome activation. Nature 2011; 469:221-5; PMID:21124315; http://dx.doi.org/10.1038/nature09663

35. Pellegrino MW, Nargund AM, Kirienko NV, Gillis R, Fiorese CJ, Haynes CM. Mitochondrial UPR-regulated innate immunity provides resistance to pathogen infection. Nature 2014; 516:414-7; PMID:22700657; http://dx.doi.org/10.1126/science.1223560

36. Cohen LB, Troemel ER. Microbial pathogenesis and host defense in the nematode C. elegans. Current opinion in microbiology 2015; 23:94-101; PMID:25461579; http://dx.doi.org/10.1016/j.mib.2014.11.009

37. Dunbar TL, Yan Z, Balla KM, Smelkinson MG, Troemel ER. C. elegans detects pathogen-induced translational inhibition to activate immune signaling. Cell host & microbe 2012; 11:375-86; PMID:22520465; http://dx.doi.org/10.1016/j.mib.2014.11.009

38. Benjamin JL, Sumpter R, Jr., Levine B, Hooper LV. Intestinal epithelial autophagy is essential for host defense against invasive bacteria. Cell host & microbe 2013; 13:723-34; PMID:23768496; http://dx.doi.org/10.1016/j.chom.2013.05.004

39. Jia K, Thomas C, Akbar M, Sun Q, Adams-Huet B, Gilpin C, et al. Autophagy genes protect against Salmonella typhimurium infection and mediate insulin signaling-regulated pathogen resistance. Proceedings of the National Academy of Sciences of the United States of America 2009; 106:14564-9; PMID:19667176; http://dx.doi.org/10.1073/pnas.0813319106

40. Curt A, Zhang J, Minnerly J, Jia K. Intestinal autophagy activity is essential for host defense against Salmonella typhimurium infection in Caenorhabditis elegans. Developmental and comparative immunology 2014; 45:214-8; PMID:24674884; http://dx.doi.org/10.1016/j.dci.2014.03.009

41. Settembre C, Di Malta C, Polito VA, Garcia Arencibia M, Vetrini F, Erdin S, et al. TFEB links autophagy to lysosomal biogenesis. Science 2011; 332:1429-33; PMID:21617040; http://dx.doi.org/10.1126/science.1204592

42. Tiller GR, Garsin DA. Of worms and men: HLH-30 and TFEB regulate tolerance to infection. Immunity 2014; 40:857-8; PMID:24950206; http://dx.doi.org/10.1016/j.immuni.2014.06.002

43. Visvikis O, Ihuegbu N, Labed SA, Luhachack LG, Alves AM, Wollenberg AC, et al. Innate host defense requires TFEB-mediated transcription of cytoprotective and antimicrobial genes. Immunity 2014; 40:896-909; PMID:24882217; http://dx.doi.org/10.1016/j.immuni.2014.05.002

44. Allen GF, Toth R, James J, Ganley IG. Loss of iron triggers PINK1/Parkin-independent mitophagy. EMBO reports 2013; 14:1127-35; PMID:24176932; http://dx.doi.org/10.1038/embor.2013.168

45. Papp D, Csermely P, Soti C. A role for SKN-1/Nrf in pathogen resistance and immunosenescence in Caenorhabditis elegans. PLoS pathogens 2012; 8:e1002673; PMID:22577361; http://dx.doi.org/10.1371/journal.ppat.1002673

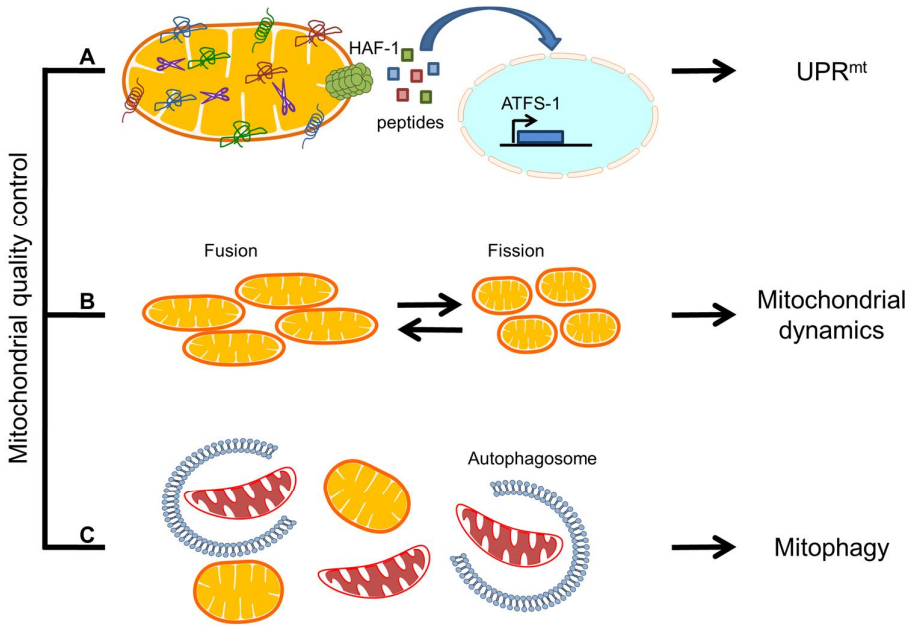

Figure 1
Palikaras, Lionaki & Tavernarakis

Figure 1. Mitochondrial quality control mechanisms. (**A**) Mitochondrial unfolded protein response (UPR^{mt}) is activated under proteotoxic and metabolic stress. Misfolded and non-functional mitochondrial proteins are digested into peptides by mitochondrial proteases and are transferred to the cytoplasm through the HAF-1 transporter. In turn, ATFS-1 is activated and translocates to the nucleus to regulate transcription of nuclear genes encoding mitochondrial proteins, thus restoring and enhancing proteostasis and mitochondrial activity. (**B**) Mitochondrial dynamics facilitate quality control by segregating or exchanging damaged proteins and mutated mitochondrial DNA (mtDNA). (**C**) Severe damage triggers mitochondria-selective autophagy. Entire defective organelles are recognized, engulfed by autophagosomes and delivered to lysosomes for destruction.

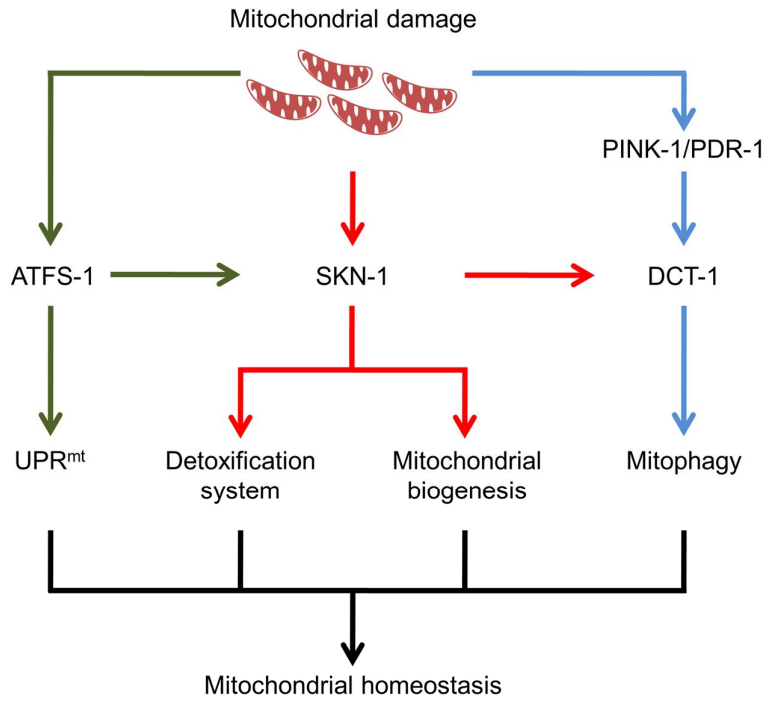

Figure 2
Palikaras, Lionaki & Tavernarakis

Figure 2. Coordination of UPR^{mt}, mitophagy and mitochondrial biogenesis during ageing. Proteotoxic and metabolic stress impair mitochondrial homeostasis and induce ATFS-1 nuclearization. In turn, ATFS-1 orchestrates the mitochondrial unfolded protein response (UPR^{mt}), which restores mitochondrial function. Excessive mitochondrial damage causes oxidative stress and initiates a bipartite retrograde response, mediated by SKN-1, to preserve mitochondrial homeostasis. SKN-1 coordinates expression of both mitochondrial biogenesis and mitophagy genes. Mitophagy is stimulated to eliminate entire damaged mitochondria. DCT-1, PINK-1 and PDR-1 act in the same pathway to mediate mitophagy. SKN-1 transcriptionally regulates expression of the DCT-1 mitophagy receptor. Notably, the *skn-1* gene is among ATFS-1 transcriptional targets during activation of UPR^{mt}, thus, establishing a complex interplay between UPR^{mt}, mitophagy and mitochondrial biogenesis.

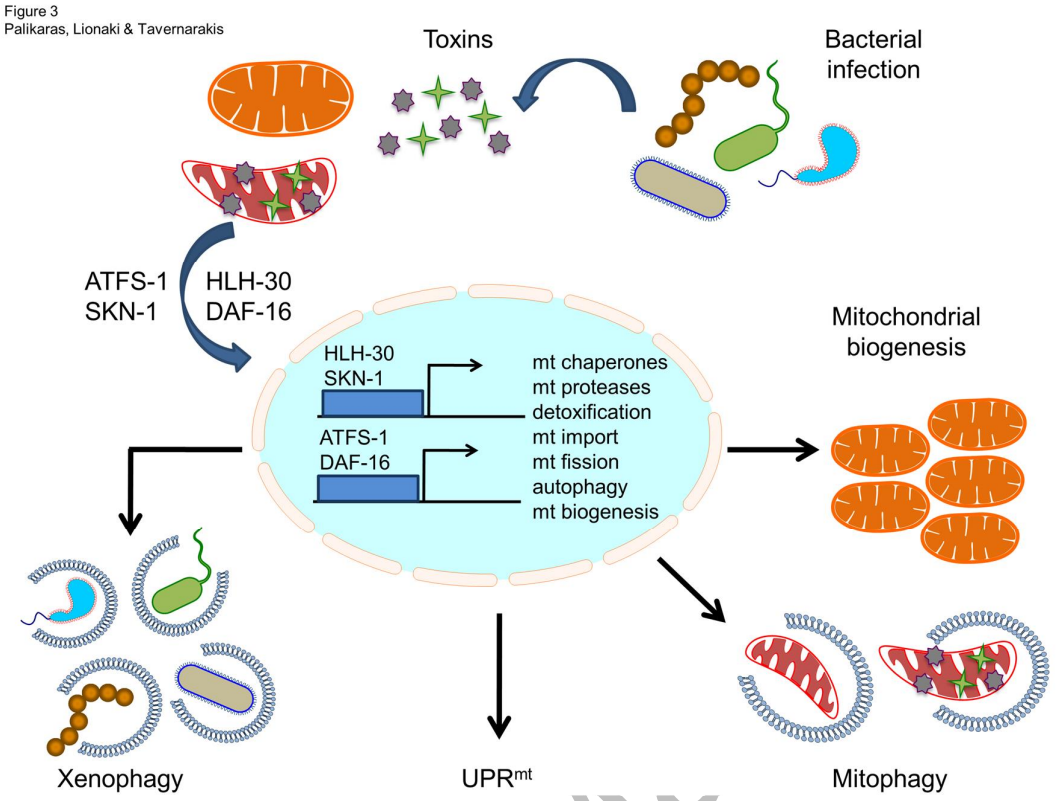

Figure 3. An intricate crosstalk between mitochondrial quality control pathways during bacterial infection. Bacterial toxins and secreted proteins impair mitochondrial function to enhance pathogen propagation. Mitochondrial damaged triggers activation of the ATFS-1, SKN-1, DAF-16 and HLH-30 transcription factors to augment mitochondrial quality control mechanisms (UPR^{mt}, detoxification system, mitophagy and mitochondrial biogenesis) and pathogen elimination via xenophagy. This sophisticated crosstalk between mitochondrial quality control pathways upholds energy homeostasis and organismal survival during bacterial infection.