Mitochondrial quality control in aging and lifespan control of the fungal aging model \textit{Podospora anserina}

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Abstract

Aging of biological systems is a fundamental process controlled by a complex network of molecular pathways. In the filamentous fungus \textit{Podospora anserina}, a model in which organismal aging can conveniently be analysed, mitochondria play a central role. A wide range of relevant pathways were identified that contribute to the maintenance of a population of functional mitochondria. These pathways act in a hierarchical manner, but all the pathways are limited in capacity. At the end of the life cycle, when the various surveillance pathways are overwhelmed and damage has passed certain thresholds, programmed cell death brings the life of individual \textit{P. anserina} to an end.

Introduction

The filamentous fungus \textit{Podospora anserina} has been extensively studied as a model organism to unravel the molecular pathways involved in the control of biological aging. Soon after the first description of the 'senescence syndrome' in the early 1950s [1] elucidating the degeneration of \textit{P. anserina} cultures after a strain-specific period of growth, classical genetic experiments suggested that genetic traits, which are now known to be located in mitochondria, are involved in the control of the lifespan of this fungus [2]. In fact, in the late 1970s, \textit{P. anserina} was the first system in which molecular pathways linked to age-dependent reorganization of the DNA located in mitochondria, mtDNA (mitochondrial DNA), were reported to occur during aging [3]. The analysis of such age-related mtDNA instabilities was the focus of the first decade of research after their initial description. Strains of \textit{P. anserina} in which mtDNA reorganization is delayed for different reasons were found to be long-lived. Some of these mutants even appear to be immortal since, instead of growing for only approximately 3–5 weeks, they are viable for years. The research on \textit{P. anserina} has inspired studies on other fungi such as \textit{Neurospora intermedia}, in which impairment of mitochondrial functions as a result of gross mtDNA reorganization was found to play a general role during aging. This group of studies has been summarized in a number of reviews (see [4–6]). Subsequently, a number of specific molecular pathways, which emphasize the fundamental role of mitochondria in aging, have been reported. More recent review articles summarize this group of studies and address the relevance for biological aging in general [7–13]. The focus of the present review is to integrate the individual pathways into a network of hierarchical pathways (Figure 1) that appear to follow a common goal: keeping mitochondria functional for as long as possible.

Mitochondria in aging

Mitochondria are not only the 'power plants' of eukaryotic cells via the generation of most of the ATP needed for life of organisms but are also fundamental for the generation of iron–sulfur clusters, amino acid synthesis, lipid and copper metabolism, autophagy and apoptosis. One of the hypotheses that inspired aging research for almost 30 years is the MRFT (mitochondrial free radical theory of aging) [14], which states that ROS (reactive oxygen species) generated in mitochondria during oxidative phosphorylation lead to the age-related accumulation of oxidative damage and functional impairments of cells and organs (Figure 1). Ultimately this kind of damage leads to physiological degeneration and organismal death. Numerous investigations support a role of ROS in aging, but also data have been gathered that appear to be controversial with regard to the refined version of the original theory put forward by Harman [14].

Respiration, ROS and ROS scavenging

In \textit{P. anserina}, a number of mutants respire by an alternative respiration mechanism instead of the standard COX (cytochrome c oxidase)-dependent pathway that is also used in mammals. In strains in which there are deletions of mtDNA or the nuclear DNA coding for components of COX, or in which COX is not properly assembled, e.g. due to impairment of the supply of copper as a cofactor,
Figure 1 | A network model integrating individual molecular pathways with relevance to aging of *P. anserina*

Mitochondria generate ATP that is essential for driving all the energy-consuming processes of the organism. They also generate, as ‘by-products’, ROS. According to the MRFT, these aggressive compounds are able to damage basically all molecules in a cell and thus lead to the impairment of pathways in which these molecules are involved (red symbols with a minus sign). The accumulation of damage induces batteries of surveillance systems indicated in the different boxes (e.g. ‘DNA repair’ or ‘mitochondrial dynamics’). Finally, as the last response, a PCD machinery is induced bringing the life of the impaired organism to an end. In addition, ROS are known to specifically regulate gene expression. Such a response can induce/repress different components of pathways also involved in the maintenance of a population and functional mitochondria. Autophagy/mitophagy is likely to be a part of the surveillance system but as such has not been experimentally investigated yet. Proteins indicated in bold have been investigated for their role in *P. anserina*. Others, not in bold, are encoded in the *P. anserina* genome. Reprinted from [12] with permission. © 2010 Wiley-Blackwell.

an AOX (alternative, iron-dependent terminal oxidase) is induced via retrograde signalling. The AOX accepts electrons from ubiquinol, leading to a ‘bypass’ of complex III of the respiratory chain. Complex III of the mitochondrial electron-transport chain is involved in proton pumping across the inner mitochondrial membrane, contributing to the generation of the membrane potential that is the force for ATP formation of complex V. In addition, complex III does generate superoxide anion radicals that are released both to the matrix as well as to the inter-membrane space of mitochondria. Strains bypassing complex III and respiring via the alternative pathways are characterized by lower ROS and ATP generation. These strains are generally impaired in growth and fertility but are long lived, indicating the contribution of ROS to the normal aging process [15–17]. Apart from the respiratory chain, there are other sources of mitochondrial ROS generation. Increasing the enzymatic activities of the O-methyltransferase PaMTH1, and thereby interfering with this particular type of ROS generation, was also found to lead to lifespan extension [18,19]. Conversely, deletion of the gene encoding PaMTH1 results in accelerated aging [20].

A general increase in the ROS scavenging capacity of *P. anserina* via overexpression of genes involved in carotenoid biosynthesis was found to increase the lifespan of transgenic strains [21]. However, interfering in other specific components of enzymatic pathways with the potential to scavenge particular ROS was recently found to lead to results that, at first glance, seem to contradict the predictions of the refined MRFT [22]. Specifically we found that deletion of the gene *PaSod3* coding for the mitochondrial manganese SOD (superoxide dismutase; PaSOD3) did not lead to the expected acceleration in aging. Moreover, strains overexpressing *PaSod3* that were demonstrated to have increased levels of this SOD were characterized by a reduced lifespan. This latter phenotype is correlated with increased levels of the general stress protein PaHSP60, a clearly reduced abundance of mitochondrial peroxiredoxin (PaPRX) that converts hydrogen peroxide into water, and reduced amounts of a soluble protease, PaCLPP, in the mitochondrial matrix [22]. While the relevance of results of the deletion of the mitochondrial matrix SOD is still unclear, those from strains overexpressing *PaSod3* are highly informative. They explain that an increase in abundance of one component of the ROS scavenging system is not sufficient to efficiently intervene in the ROS system in a way that is positive for the organism. It becomes clear that individual components of these systems have to be well balanced in order not to lead to impairments due to the accumulation of reactive intermediates that can cause molecular damage. Contradictions with the MFRT also
obtained in other systems may be solved by careful, more holistic analyses of the consequences of defined experimental manipulations in a given system.

**Remodelling of damage**

During the lifetime of any biological system, molecular damage of biomolecules occurs. Since molecular systems that take care of and destroy potential damaging agents are limited in their capacity, molecular damage does accumulate. In such a situation a variety of additional surveillance systems can be activated. As in other systems, *P. anserina* encodes different components of the enzymatic systems able to repair damage in DNA [23,24]. Also, repair of some protein modifications is possible. In the cases where repair is not possible, which accounts for most types of protein damage, damaged proteins become degraded by different proteolytic systems. Outside the mitochondrion, the proteasome system and systems recently identified to be active in yeast [25] may also be part of a maintenance system in *P. anserina*. A role for a soluble AAA (ATPase associated with various cellular activities) protease termed PaLON has recently been demonstrated in *P. anserina* [25]. Overexpression of the gene coding for this protein leads to a reduced oxidation of proteins. In particular, mitochondrial aconitase, an enzyme of the tricarboxylic acid cycle, was found to be less carbonylated in *PaLon* overexpressors. The corresponding strains were more resistant against inducers of apoptosis and display an increased lifespan [26]. It appears that mitochondrial fission leading to the separation of damaged and impaired parts of filamentous mitochondria is a way to keep mitochondrial activity high. Such mitochondria might be degraded as a whole by mitophagy, a type of specialized autophagy (a cellular ‘self-eating’ process). In the genome of *P. anserina* a number of potential genes coding for homologues involved in this kind of autophagy in yeast are found.

**Cellular surveillance systems: PCD (programmed cell death)**

The final response to accumulated damage is the induction of PCD. In a previous study, we observed that in mitochondria from senescent wild-type strains of *P. anserina*, PaCypD, a mitochondrial PPlase (peptidylprolyl cis–trans isomerase), increases in abundance [27]. In other systems this protein is known to bind to mitochondrial membrane proteins, leading to the opening of the mPTP (mitochondrial permeability transition pore). The pore allows the entrance of low-molecular-mass solutes up to 1.5 kDa in size into mitochondria and leads to the dissipation of the mitochondrial membrane potential, an unfolding of the inner mitochondrial membrane, disruption of the outer mitochondrial membrane, the release of apoptogens such as cytochrome *c* and to execution of cell death. While the structure of the mPTP remains to be elucidated, mitochondrial permeability transition is well demonstrated to occur in different situations (e.g. in human disease). We have recently investigated the role of PaCYPD in *P. anserina* by genetic manipulation of the abundance of the protein and by inhibiting it with the inhibitor cyclosporin A. We found that deletion of *PaCypD* does not have an effect on lifespan and other phenotypic characteristics [28]. This is probably due to redundant pathways controlling PCD in *P. anserina*. However, a remarkably strong effect was observed in *PaCypD* overexpressors. These strains exhibited an accelerated aging phenotype with cytochrome *c* release from mitochondria, nuclear DNA condensation, accelerated fission of mitochondria, reduced female fertility and vegetative growth, increased sensitivity to exogenous oxidative stress and the induction of apoptosis. In addition, the mitochondrial ultrastructure showed an accelerated conversion from mitochondria with lamellar cristae into those with a reticulate structure as they are characteristic of mitochondria from senescent cultures. The lifespan of *PaCypD* overexpressors was reduced by approximately 50–61% when compared with the wild-type. Significantly, treatment with cyclosporin A rescued the strain to wild-type characteristics [28]. In addition, a mitochondrial contribution to the induction of PCD in *P. anserina* was the induction of an increase in lifespan via the deletion of genes coding for four potential members of the protein family of AIF (apoptosis-inducing factor)-like proteins. Lifespan increase was most pronounced in those strains lacking the two mitochondrially located PaAIF-like proteins PaAIF2 and
PaAMID2 respectively [29]. The question of the detailed molecular mechanisms leading to the execution of death remains to be evaluated. However, at least two metacaspases are part of this system in the P. anserina. Deletion of both the genes encoding them leads to a clear lifespan increase. In the double mutant this increase is much less pronounced, suggesting a vital function for both of the two metacaspases. In the single deletion strain this function is complemented by the metacaspase that remains present in the strain. In the double mutant this function is absent [30]. The presence of caspase-like proteins as they are discussed to occur in yeasts is currently unclear.

As in the unicellular ascomycete S. cerevisiae, PCD pathways bring the life of impaired individuals of P. anserina to an end. From the evolutionary point of view, such a programmed pathway is not easy to understand. It has been suggested that PCD in yeast is an altruistic process that leads to the death of affected organisms for the sake of non-impaired ones that are competing for the same resources [31,32]. Such an explanation may also apply to P. anserina.

**ROS in signalling and gene regulation**

While the capacity of ROS as damaging agents has extensively been investigated in aging research, another role of ROS has been less explored. This is the role of ROS in signalling and in the control of gene expression. In P. anserina such a function of ROS has been demonstrated to control developmental processes such as fruiting body development and spore germination [33]. With respect to aging, this potential role, as it is suggested to be active in P. anserina (Figure 1), remains to be studied in detail.

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**References**


