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To die or not to die – How mitochondrial processes affect lifespan of *Podospora anserina*

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ABSTRACT

The filamentous ascomycete *Podospora anserina* is a well-established model system to study organismic aging. Its senescence syndrome has been investigated for more than fifty years and turned out to have a strong mitochondrial etiology. Several different mitochondrial pathways were demonstrated to affect aging and lifespan. Here, we present an update of the literature focusing on the cooperative interplay between different processes.

1. Introduction

Basic research often requires the availability of model systems which allow the efficient experimental investigation of specific questions. In aging research, different models are used, from different cell models up to whole organisms such as *Drosophila melanogaster*, *Caenorhabditis elegans* and mice. Certainly, research with models closely related to the human species provides results which can be transferred to humans more easily. However, some characteristics make "simpler" model systems far more attractive. Especially in aging research, a short-lived model allows the convenient identification of lifespan-extending pathways. Such a model organism is the filamentous ascomycete *Podospora anserina*. Its wild type "s" [1] is characterized by a short life-span of about 3–4 weeks, which can be simply measured through the time period an individual can grow on solid medium. Already in the 1980s the reason for this short lifespan was uncovered: An intron within the mitochondrial DNA (mtDNA) encoded cytochrome *c* oxidase I gene (*PaCoxI*) leads to mtDNA instabilities and accelerates the aging process. More specifically, it was shown that the intron gives rise to the formation of a covalently closed circular DNA, termed plasmid-like DNA (plDNA), which thereafter reintegrates at specific sites into the mtDNA (Fig. 1). Subsequent recombination between repetitive integrated plDNA sequences leads to gross mtDNA rearrangements and loss of larger parts of the mtDNA including sequences coding for essential mitochondrial proteins [2,4–13]. This rather unique feature makes *P. anserina* an ideal candidate to investigate mitochondria-related processes that affect lifespan, since processes interfering with mitochondrial functions are expected to directly impact the onset of senescence. These studies are facilitated by the availability of convenient methods to isolate large amounts of age-matched mitochondria for physiological and biochemical analyses.

In fact, during the last decades, several mitochondrial processes have been shown to affect the lifespan of *P. anserina* (Table 1). Surprisingly, in some cases the outcome of these studies was rather unexpected and could only be explained by more complex interactions between different processes. Some of them cooperate synergistically and extend lifespan while the interaction of others accelerates aging. Overall, now a more holistic view about the molecular control of *P. anserina* aging is emerging. In this review, we will highlight recent findings on the interplay of mitochondrial processes and how these interactions affect lifespan in *P. anserina*. For a more comprehensive treatise and discussion of *P. anserina* aging and the relevance of this work for aging in general, the reader is referred to a recent review and earlier reviews cited herein [14].

2. Pro-death signaling: detrimental interaction of different mitochondrial processes

Autophagy describes a conserved process of "self-eating" which is required to maintain cellular homeostasis [56,57]. While non-selective or general autophagy is mainly thought to recycle nutrients during starvation, selective autophagy acts as quality control pathway. It is

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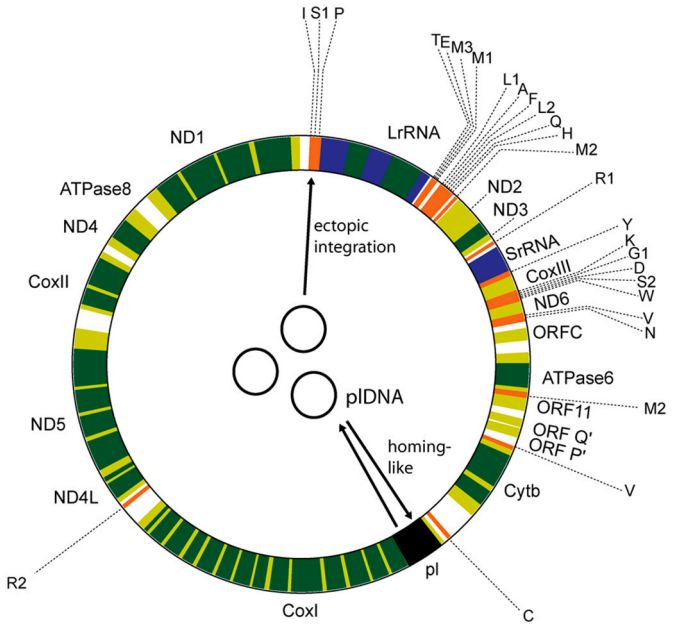

Fig. 1. In *P. anserina* during aging massive reorganization of the mtDNA occurs. The mtDNA of *P. anserina* wild type "s" consists of about 94 kbp. Regions coding for proteins are depicted in light green. ND1, ND2, ND3, ND4, ND4L, ND5 encode subunits of complex II. Cytb is the gene coding for a subunit of complex III, while CoxI, CoxII, and CoxIII encode the respective subunits of complex IV. ATPase6 and ATPase8 are the genes for the mitochondria-encoded subunits of complex V. Additional open reading frames with hitherto unknown function are ORFC, ORFP', ORFQ', and ORF11. The mitochondrial tRNA genes are shown in red (single letter code), and genes encoding the large and small mitochondrial ribosomal RNA subunits (LrRNA and SrRNA) are depicted in blue. With the exception of the pl-intron (pl) of the CoxI gene (marked in black), all introns are indicated in dark green. By a yet unknown mechanism the pl-intron forms stable covalently closed DNA circles, the so-called "plasmid-like" DNA (plDNA). The amount of plDNA dramatically increases during wild-type aging and forms the basis for gross mtDNA rearrangements. Single plDNA molecules are able to re-integrate into the mtDNA, specifically at two positions: Either at the CoxI exon/first intron site in a process termed "homing-like" integration or ectopically between $tRNA^{I}$ and $tRNA^{S}$ (I, S1, encoding the $tRNA^{isoleucin}$ and $tRNA^{serine}$) [2,3]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

dedicated to remove superfluous but also damaged cellular components, such as dysfunctional mitochondria by mitophagy (reviewed in [58]). However, in certain settings, mitophagy turned out to be rather detrimental and results in lifespan reduction. The first *P. anserina* mutant, in which such a role was observed, strongly overexpresses the gene coding for PaCYPD, a mitochondrial peptidyl prolyl-*cis*, *trans*-isomerase, implicated in the regulation of the mitochondrial permeability transition pore (mPTP). Strong constitutive overexpression of *PaCypD* lowers mitochondrial membrane potential suggesting that opening of the mPTP is induced leading to the dissipation of the membrane potential. Lifespan is strongly decreased in the corresponding mutant [52] and goes along with an increase in general autophagy but to a much higher extent mitophagy [43]. Accordingly, deletion of *PaCypD* significantly reduces mitophagy and general autophagy. A pro-survival role of autophagy, which was demonstrated for the wild type [59], can be excluded in the *PaCypD* overexpressor because concomitant deletion of *PaAtg1* encoding a core component of the autophagy machinery, does not result in additional lifespan reduction. Thus, it can be concluded that the observed autophagy/mitophagy induction is not beneficial for the *PaCypD* overexpressor. In another mutant, an even detrimental role of excessive mitophagy is observed. Deletion of the gene encoding the mitochondrial superoxide dismutase PaSOD3 impairs mitochondrial superoxide scavenging. As expected, this mutant is highly sensitive against paraquat [60], a generator of superoxide at the mitochondrial respiratory chain [61,62]. The mutant's lifespan is strongly reduced on paraquat containing medium. This reduction depends on a functional autophagy machinery [60]. It seems that the over-activation of mitophagy leads to a dramatic shortage of functional mitochondria resulting in lifespan reduction.

Not only impaired mitochondrial superoxide scavenging but also the function of mitochondrial F_1F_o-ATP-synthase (complex V) is linked to excessive mitophagy induction. Dimeric complex V regulates mitochondrial ultrastructure and ablation of the assembly factor PaATPE, which is required for dimer formation, leads to premature death in *P. anserina* [21]. Excessive PaCYPD-dependent induction of mitophagy was found to be responsible for this lifespan reduction [22].

These different examples demonstrate that impairments in different mitochondrial processes such as the regulation of mPTP, superoxide scavenging, and complex V function, are linked to a detrimental mitophagy induction. Excessive mitophagy induction thus seems to be a crucial negative feedback mechanism leading to lifespan reduction upon mitochondrial impairments.

3. Pro-survival signaling: lifespan extension through positive feedback mechanisms

3.1. Mitohormesis: mild oxidative stress results in lifespan extension

At least eleven distinct sites in mitochondria were demonstrated to generate superoxide or hydrogen peroxide in isolated mitochondria (reviewed in [63]). Although both molecules are ROS, their potential impact on the cellular function is quite different. Superoxide hardly permeates membranes, it is short-lived, and thus mostly affects molecules in the close vicinity and has low direct signaling potential. Matrix-localized superoxide dismutase continuously converts superoxide into hydrogen peroxide, which readily passes membranes and thus is able to transmit signals to the cytosol or other organelles. In the presence of copper or iron ions hydrogen peroxide induces the formation of hydroxyl radicals, which are highly reactive and immediately causes molecular damage. Strong oxidative stress is undoubtedly detrimental and accelerates the aging process by damaging various cellular compounds including proteins, lipids and DNA. Also mild oxidative stress was long time considered to reduce lifespan. However, within the last about 15 years, the role of mild oxidative stress has been reconsidered. The term "mitohormesis" describes the beneficial alterations induced upon sublethal mitochondrial stress [64]. Nowadays, the concept of mitohormesis is well accepted and describes the induction of an adaptive response to ROS produced in mitochondria. This retrograde response results in a health-promoting long-term reduction of oxidative stress (reviewed in [65]) and thus is able to lead to lifespan extension. The mitohormetic response in *C. elegans* is completely lost upon treatment with antioxidants [66]. In humans antioxidants prevent health-promoting effects of physical exercise, which also increases mitochondrial ROS production. Obviously, the beneficial effects of mild oxidative stress strictly depend on ROS levels. Such a mitohormetic response was also observed in *P. anserina*. It was demonstrated that paraquat increases wild-type's lifespan at low concentrations up to 2.5 fold [43,60,67]. This lifespan extension is at least partly dependent on the autophagy machinery [60] and requires PaCYPD, suggesting that formation of the mitochondrial permeability transition pore (mPTP) is involved in signal transmission from mitochondria to the cytosol. Mild mitochondrial oxidative stress through paraquat treatment induces mitophagy [60], resulting in an overall increased capacity to handle mitochondrial damage. Not only paraquat exerts such a beneficial effect, but also curcumin, a natural polyphenol from the rhizome of turmeric, *Curcuma longa*. In *P. anserina*, curcumin increases lifespan in an autophagy- and superoxide dismutase-dependent manner [68]. Neither a mutant ablated for the core autophagy protein PaATG1 nor for all superoxide dismutases responds to curcumin treatment, suggesting that a retrograde response, similar to

that induced by paraquat, is responsible for the curcumin-dependent lifespan extension [68].

Such a retrograde response is not only induced by drug treatment but also during aging. Recently, a complexome study of *P. anserina* mitochondria uncovered an up-regulated recruitment of proteasome components and endoplasmic reticulum proteins to mitochondria during aging [69]. It appears that intimate mitochondria/ER contact and CDC48 mediated proteasomal protein surveillance act as salvage

Table 1
Mitochondrial processes affecting *P. anserina* lifespan.

Process/ gene	Protein function/description	Kind of mutation	Impact on lifespan	Reference
Energy metabolism				
Nuo19.3	19.3 kDa subunit of complex I	Deletion	Increase	[15]
PaAnt1	Adenine nucleotide translocator	*PaAnt1*^{M106P}, *PaAnt1*^{A121P}	Increase or decrease, outcome depends on *PaRmp1* allele	[16]
		PaAnt1^{S296M}	Decrease	
PaAox	Alternative terminal oxidase	Overexpression	Decrease in complex IV and cyc1 mutants	[17,18]
		Deletion	Lethal in complex III or IV-mutants	[19]
PaAtp9	Subunit of complex V, two paralog genes (*Atp9-5* and *Atp9-7*) exist	Deletion of either of the two paralogs	Exclusive use of *Atp9-7* increases lifespan while exclusive use of *Atg9-5* decreases lifespan	[20]
PaAtpe	Subunit of complex V	Deletion	Decrease	[21,22]
PaAtpg	Subunit of complex V	Deletion	Decrease	[21]
PaCox1	Subunit 1 of complex IV	Deletion of first intron and few adjacent nucleotides of upstream exon	Increase	[10]
PaCox5	Subunit 5 of complex IV	Deletion	Increase	[15,19,23,24]
PaCox17	Chaperone delivering copper to complex IV	Deletion	Increase	[25]
PaCyc1	Cytochrome *c1*	Mutation	Increase	[18,19]
PaNdi1	Internal alternative NADH oxidase	Overexpression	Decrease in complex I-mutant	[15]
PaOxa1	Respiratory complex assembly	Thermosensitive allele *oxa1*^{ts}	Increase or decrease, outcome depends on *PaRmp1* allele	[26]
PaRcf1	Supercomplex assembly	Deletion	Decrease	[27]
		Overexpression	Increase	
PaRse2	Transcription factor required for *PaAox* expression	Deletion	Decrease, lethal in complex III/IV mutants	[28]
		Mutation leading to increased *PaAox* expression	Decrease in complex III/IV mutants	
PaRse3	Transcription factor required for *PaAox* expression	Deletion	Decrease, lethal in complex III/IV mutants	[28]
		Mutation leading to increased *PaAox* expression	Decrease in complex III/IV mutants	
Mitochondrial proteostasis				
PaClpP	Mitochondrial matrix protease	Deletion	Increase	[29–31]
PaIap	Mitochondrial inner membrane protease	Deletion	Increase	[29,32,33]
PaLon1	Mitochondrial matrix protease	Overexpression	Increase	[34]
		Deletion	Decrease	[35]
Protein import				
PaTim54	Component of mitochondrial inner membrane transport complex	Mutation which impairs it expression	Increase, level depends on *PaRmp1* allele	[36]
PaTom70	Component of mitochondrial outer membrane transport complex	Mutation altering the last 97 amino acids	Increase	[37]
Reactive oxygen species (ROS) scavenging				
PaMth1	Mitochondrial methyltransferase	Overexpression	Increase	[38]
		Deletion	Decrease	[39]
PaNdk1	Mitochondrial NAD(H) kinase	Deletion	Increase	[40]
PaSod3	Mitochondrial MnSOD	Overexpression	Decrease	[41–43]
mtDNA integrity				
mtHMG1	mtDNA binding?	Deletion	Decrease	[44]
pAl2-1	Mitochondrial plasmid	Presence	Decrease of calorie-reduction-mediated longevity	[45,46]
		Integration in mtDNA	Increase	[46]
Others				
Grisea	Transcription factor, regulation of copper homeostasis	Loss-of-function mutation	Increase	[47–49]
Pa_1_10620	Component of mitochondrial ribosome?	Overexpression	Increase	[50]
PaAif2	Mitochondrial AIF-like oxidoreductase	Deletion	Increase	[51]
PaAmid2	Mitochondrial AIF-like oxidoreductase	Deletion	Increase	[51]
PaCrd1	Cardiolipin synthase	Deletion	Decrease	[33]
PaCypD	Mitochondrial peptidyl prolyl-*cis*, *trans*-isomerase	Overexpression	Decrease	[43,52]
PaDnm1	Mitochondrial fission factor	Deletion	Increase	[53]
		Overexpression	Decrease	[54]
PaMdm10	Component of ERMES	Missense mutation	Decrease in *mat-* (*PaRmp1-1*)	[37]
PaMt1	Cytosolic metallothionein (copper chaperone)	Targeting to mitochondria	Increase	[55]

pathways helping mitochondria to counteract the age-dependent accumulation of damage.

Also in a *PaSod3* deletion mutant retrograde signaling is observed. Here, the compensatory induction of a mitohormetic response leading to mitophagy induction explains the mild phenotype of the mutant under standard growth conditions [60]. These data demonstrate that the analysis of mutants impaired in pathways considered to be required for a long healthspan, allows the identification of compensatory pathways.

3.2. Induction of alternative respiration

An outstanding function of mitochondria is the generation of ATP through oxidative phosphorylation at the respiratory chain (Fig. 2). Standard respiration requires the interaction of three large protein complexes: complex I (NADH:ubiquinone oxidoreductase), complex III (ubiquinol:cytochrome *c* oxidoreductase), and complex IV (cytochrome *c* oxidase). Electron transport through these complexes leads to the translocation of protons across the inner mitochondrial membrane resulting in the formation of a protonmotive force which drives ATP synthesis at complex V (F_1F_o-ATP-synthase). At complex I and complex III single electron transfer to molecular oxygen is possible leading to the formation of the superoxide anion, a ROS. In addition to the three core complexes, branching points of the respiratory chain exist in filamentous fungi which allow them to cope with varying environmental conditions such as nutrient and co-factor availability, development or oxidative stress (reviewed in [70]), and also were shown to play a pivotal role in aging of *P. anserina*. By-passing complex I is possible either via the use of complex II (succinate dehydrogenase), which feeds electrons from succinate into the respiratory chain, or of alternative NADH:ubiquinone oxidoreductases that either transfer electrons from matrix-located NADH (internal NADH dehydrogenases) or from the intermembrane space (external NADH dehydrogenases) to ubiquinol. Finally, instead of complex IV (and complex III) an alternative quinol oxidase, the salicylhydroxamate (SHAM)-sensitive alternative oxidase AOX, which is described in plants, algae, some protists, and in *P. anserina* as in most other fungi (reviewed in [71]) can be used (Fig. 2).

In *P. anserina* a number of studies demonstrate the induction of *PaAox* transcription, respectively AOX-dependent respiration associated with lifespan extension. *Aox* induction results from mutational changes in the mtDNA [49], impairment of complex IV assembly [23,27], lack of the complex IV essential co-factor copper [25,48,49,55,72,73], mutation in the adenine nucleotide translocase gene *PaAnt1* [16], deletion of the gene encoding the mitochondrial NAD(H) kinase PaNDK1 [40], down-regulation of PaTIM54, a homolog of the yeast mitochondrial inner membrane import machinery component Tim54p [36], mutation of the cytochrome *c* gene [18], impaired respiratory complex assembly [26], and oxidative stress [42,67]. In all these mutants, lifespan is affected (see Table 1). Since AOX-dependent respiration by-passes complex III and IV, it results in a lower mitochondrial membrane potential, and less superoxide formation since complex III-dependent ROS formation is circumvented. Both consequences alleviate or prevent oxidative stress not only directly by omitting complex III but also indirectly by decreasing the reduction state of coenzyme Q which provides the reduction equivalents for AOX. Although the AOX protein is barely detectable in young *P. anserina* wild type under standard conditions, oxygen consumption measurements demonstrate a partly SHAM-sensitive AOX-dependent respiration even at young age [69,74]. During aging, the membrane potential dissipates. Addition of SHAM leads to a further reduction of this residual membrane potential [75] supporting earlier findings that during aging respiration becomes more SHAM-sensitive [76]. *PaAox* transcript can be readily detected in middle-aged cultures and its level increases during aging [77]. Recently the age-dependent induction of AOX was demonstrated [69]. This suggests, that AOX (at least in low amounts) is constitutively present, and thus AOX induction is a highly effective measure to rapidly adapt to variable environmental and physiological conditions.

AOX induction is not only linked to lifespan extension but also to reduced mycelial pigmentation, growth retardation, and female sterility. Especially the latter two most probably result from the lowered ATP production during AOX-dependent respiration. The organism seems to counter-act these ATP limitations by the transcriptional induction of glycolytic enzymes [24]. Interestingly, deletion of the gene encoding PaMED13, a component of the evolutionary conserved Mediator complex, improves growth rate and pigmentation of the complex IV-deficient mutant *ΔPaCox5*, but still results in lifespan extension [24]. Mediator regulates gene expression by integrating different input signals and transducing them to the RNA polymerase II machinery (reviewed in [78]). In *ΔPaMed13/ΔPaCox5* the transcription of genes encoding glycolytic enzymes, such as hexokinase, fructose-biphosphate aldolase, triose phosphate isomerase, phosphoglycerate kinase, enolase, and pyruvate kinase, is even higher than in *ΔPaCox5* and thus glycolytic ATP production might be enhanced without detrimental effects on lifespan. Taken together, these data suggest that not AOX induction per se is lifespan-extending but rather the (mild) reduction of the mitochondrial membrane potential and electron flow through the respiratory chain accompanied by a decreased reduction state of coenzyme Q. Further up-regulation of AOX level and thereby most probably also the mitochondrial ATP production restores wild-type fertility and pigmentation, but

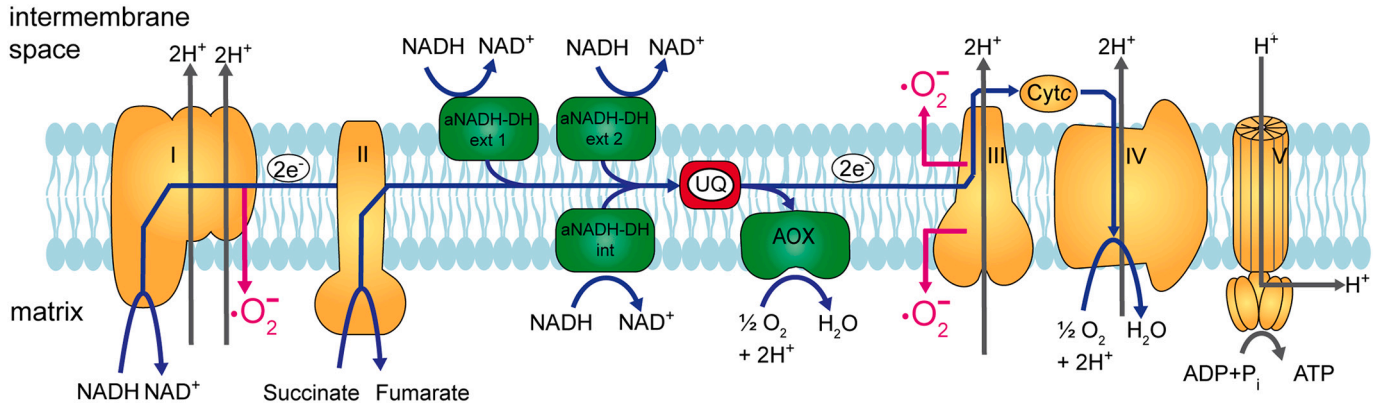

Fig. 2. The respiratory chain of *P. anserina*. The electrons from internal NADH enter the respiratory chain either at complex I or at the internal NADH dehydrogenase (aNADH-DH int), while electrons from external NADH are transferred to ubiquinone (UQ) via two different alternative external NADH dehydrogenases (aNADH-DH ext. 1 and 2). Alternatively, electrons from succinate are delivered to ubiquinone (UQ) via complex II. The electrons from reduced ubiquinone are transferred to complex III and subsequently via cytochrome *c* (Cytc) to complex IV, or alternatively, to the alternative terminal oxidase AOX. At complex I, III, and IV protons are pumped across the inner mitochondrial membrane resulting in a protonmotive force which is used by complex V to produce ATP. At complex I and III single electron transfer to molecular oxygen results in the formation of superoxide anion ($\bullet O_2^-$), a free radical.

also reduces lifespan to wild-type level [17], supporting this idea.

The individual respiratory complexes I, III, and IV are assembled together into supercomplexes in so-called respirasomes ([79], see recent review by [80]). The functional relevance of their formation is still not completely elucidated. Supercomplexes have been suggested to allow efficient substrate transfer between the individual complexes ("substrate channeling", [79]). A recent study questions this role. To test for substrate channeling, AOX was incorporated in mammalian heart mitochondria to create a competing pathway for quinol oxidation [81]. The authors demonstrated that the quinol generated in the supercomplexes by complex I is more rapidly reoxidized by the AOX outside the supercomplexes than by complex III within supercomplexes. Quinone/quinol freely diffuse in and out of the supercomplexes and therefore, it seems that substrate channeling does not occur and is not required for respiration. However, one commonly accepted role of supercomplexes at least in mammals is the assembly and/or stabilization of the largest respiratory complex, complex I. Consequently, in mammals complex III and IV are required for complex I stability/assembly [82–85]. On the one hand, supercomplexes might provide a scaffold that helps to assemble complex I [86]. On the other hand, inactivation of complex III and IV results in over-reduction of coenzyme Q, and stimulates reverse electron transport (RET) that generates superoxide [87]. Its formation damages complex I proteins inducing their degradation. Unlike in mammals, in *P. anserina* complex III [18], or complex III together with IV are not required for stabilization/assembly of complex I [19], and the authors speculate that instead AOX is involved in complex I stabilization. This idea is supported by the observation that *PaAox* expression is induced in a complex I-deficient mutant (mentioned in [15]), possibly as a compensatory response. Interestingly, in mouse cell lines heterologous expression of a fungal *Aox* gene stabilizes complex I in the absence of complex III and IV [87]. It is thus rather plausible that in fungi (and plants) AOX plays naturally an important role in complex I stabilization and may thus also be induced upon complex I impairment. Indeed in maize plants, complex I deficiency also results in AOX induction [88]. However, until now, no evidence exists for a direct interaction of AOX with complex I. Obviously, further studies on a potential interplay between complex I stability and AOX are required to fully understand the extent of compensatory capacity of AOX induction.

Beside AOX induction, another form of alternative respiration linked to longevity was demonstrated to occur upon complex I deficiency [15]. In this case, the alternative internal NADH dehydrogenase PaNDI1 is required for viability of a mutant impaired in complex I assembly to bypass complex I at the expense of strongly reduced male and female fertility. Interestingly, similar to the senescence-restoring effect by overexpressing *PaAox* in complex III or IV mutants, overexpression of *PaNdi1* also restores the short wild-type lifespan. This again strongly argues for a beneficial role of lowered membrane potential and decreased coenzyme Q reduction state while preserving the electron flow through the respiratory chain. In accordance with the data described above, by-passing complex I and II by an oleic acid diet recently also was shown to increase *P. anserina* lifespan, here without negatively affecting growth rate [89]. Moreover, even at the level of complex V composition lifespan can be modulated. *P. anserina* encodes two different c-subunit isoforms, ATP9-5 and ATP9-7, which antagonistically affect longevity [20] (Table 1).

Overall, these data demonstrate that the plasticity of the composition of the respiratory chain provides efficient compensatory mechanisms which are able to delay the aging process.

3.3. Impairments in mitochondrial proteostasis

The mitochondrial proteome consists of about 1000 to 1500 different proteins. Most of them are imported from the cytosol. Their processing, import, and also folding has to be tightly controlled. To ensure proper mitochondrial function, the degradation and replacement of dysfunctional proteins is necessary. Moreover, as part of a rewiring program to adapt to altered metabolic conditions, the removal of superfluous proteins is essential. A diverse group of proteases evolved that forms the so-called mitodegradome (reviewed in [90]). Some of these proteases control proteins just in one mitochondrial compartment, e.g. the matrix, while others control proteins in several compartments, such as the outer mitochondrial membrane (OMM), the intermembrane space (IMS), and the inner mitochondrial membrane (IMM) (recently reviewed in [91]). An example for the latter one is an i-AAA protease located in the inner mitochondrial membrane (PaIAP in *P. anserina*, YME1 in yeast, YME1L in humans). This protease controls protein import, lipid metabolism, mitochondrial dynamics and the level of different IMM, IMS, and OMM proteins (reviewed in [91]). It was thus unexpected and surprising to observe a pronounced lifespan extension in the *PaIap* deletion mutant of *P. anserina*. This effect is seen at 27 °C growth temperature. At 37 °C, the mutant turned out to be short-lived [32], indicating that PaIAP is required for heat-stress adaptation. A recent study links the altered lipid metabolism of this mutant to the observed role in lifespan control [33]. It was found that PaIAP regulates the level of the cardiolipin synthase PaCRD1 and other enzymes in phospholipid (PL) metabolism to allow adaptation to different conditions (e.g. temperature changes). Its ablation results in a stimulation of cardiolipin synthesis and a pronounced reorganization of the mitochondrial PL profile. At standard conditions, these alterations lead to lifespan extension. Thus, ablation of a component of the mitochondrial protein quality control system beneficially affects PL metabolism. These data imply a compensatory role of mitochondrial PL composition rewiring upon proteostasis impairment.

Proteostasis is also impaired in mutants lacking another mitochondrial protease, the matrix-located caseinolytic protease CLPP. While the phenotypic consequences of CLPP ablation are quite different, its role in degrading subunits of the N-module of complex I [92] is conserved across eukaryotes (recently reviewed in [93]). Similar to what is seen with PaIAP, a *P. anserina* mutant ablated for PaCLPP shows a pronounced lifespan extension [29]. Like in mammals and plants, complex I subunits as well as components of the pyruvate dehydrogenase complex and the tricarboxylic acid cycle are presumable substrates of PaCLPP [94]. Accordingly, ablation of PaCLPP affects the TCA cycle, glucose and amino acid metabolism and nucleotide levels [31]. PaCLPP therefore obviously plays a central role in controlling energy metabolism. The lifespan extension of the deletion mutant is thus rather unexpected. Subsequent investigations revealed that induction of autophagy is not only able to compensate the deficits in energy metabolism of the mutant but leads to the observed lifespan extension [30]. Surprisingly, concomitant deletion of *PaSnf1*, the gene encoding the catalytic α-subunit of AMP-activated protein kinase (AMPK), a master regulator of autophagy, does not shorten lifespan but results in dramatic lifespan extension on glucose-containing medium [31]. Such a synergistic interaction indicates that AMPK signaling limits the lifespan of the *PaClpP* deletion mutant. AMPK is not only involved in autophagy induction but also in the stimulation of various catabolic processes (reviewed in [95]) and was shown in yeast to be required for the metabolic switch from glycolysis to respiration [96]. It seems that in the *PaClpP* deletion mutant – on glucose-containing media – such a switch is unfavorable, perhaps by overwhelming mitochondrial metabolism. Interestingly, the level of this synergistic interaction depends on the presence of fully functional PaRMP1 [31]. This protein is homolog to the yeast SLS1 protein, which coordinates transcription of mtDNA encoded genes and the subsequent translation [97]. In accordance, PaRMP1 was linked to respiratory complex assembly [26], but unfortunately, a concrete function has not yet been elucidated. Nevertheless, the data obtained with the *ΔPaClpP/ΔPaSnf1* mutant suggest that translation of mitochondria-encoded proteins is relevant for the longevity phenotype. Indeed, this idea is supported by findings in mice. Here, CLPP was shown to regulate mitoribosome maturation [98]. Ablation of CLPP delays maturation and in a mouse mutant with dysfunctional mitochondrial translation, *ClpP* deletion results in a strong lifespan extension [99]. Taken together, these studies suggest that downscaling of

mitochondrial metabolism by preventing AMPK signaling and impairing mitochondrial translation is highly beneficial upon PaCLPP ablation.

As indicated by the above introduced examples, impairments in mitochondrial proteostasis do not necessarily accelerate aging, but might rather stimulate compensatory pathways.

4. Conclusions

Each of the above described processes is obviously important for the maintenance of mitochondrial function. However, one should not underestimate the ability of other – non-redundant – processes to respond to disturbances in mitochondrial function. In a changing environment with different temperatures, limited nutrient availabilities or fluctuating oxidative stress load, different pathways gain differential importance and therefore allow a high plasticity to respond to changes. Model organisms such as *P. anserina* have invaluable advantages compared to complexer organisms to study such aspects, because they often show low redundancy of pathways. It is thus much less complicated to efficiently interfere in these pathways. Moreover, intervention in processes, which in animal models are lethal, e.g. because of being involved in embryonic development, are often feasible to study in models like *P. anserina*. Especially the latter feature allows to uncover compensatory interactions upon mitochondrial impairment not only on a cellular basis but also in fully developed organisms. Translation of this knowledge to humans is possible in cases in which evolutionary conserved processes are studied. Such studies can provide valuable tools to overcome mitochondrial dysfunction. One example for this is the elaborate study of alternative respiration in fungi. The hereby identified alternative respiratory complexes allow to study the distinct consequences of complex I deficiency in mammals. For example, heterologous expression of the gene encoding the yeast alternative internal NADH dehydrogenase NDI was found to increase survival of a complex I-deficient mouse mutant [100]. While NDI prevents neuro-inflammation, it does not prevent ataxia, demonstrating that other processes than impaired NAD^+ regeneration contribute to the development of motor dysfunction in this mouse model. Also another study provided valuable information on the processes affected upon respiratory impairments. Heterologous expression of fungal *Aox* restores electron transport in mtDNA-less mouse cells [101] and helped to uncover the role of coenzyme Q over-reduction in complex I destabilization in human cells [87]. Since mitochondrial dysfunction in humans is associated with aging and a number of age-associated diseases (for recent reviews see: [102–104]), information about compensatory or counteractive mechanisms should facilitate the development of suitable treatments to improve the healthy period, the healthspan, in the life of organisms including our own species.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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