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Tobias A. Weber, Andreas S. Reichert

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Review

Impaired quality control of mitochondria: aging from a new perspective

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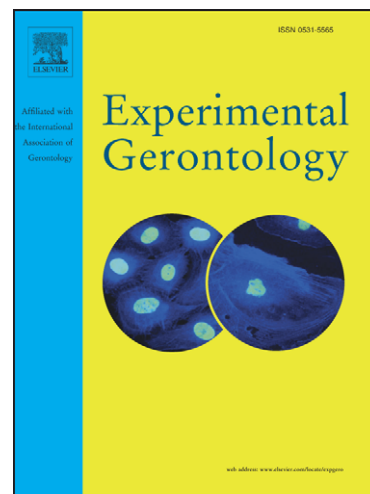
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Review

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6 Impaired quality control of mitochondria: aging from a new perspective
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10 **Tobias A. Weber and Andreas S. Reichert***
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12 CEF Makromolekulare Komplexe, Mitochondriale Biologie, Fachbereich Medizin,
13 Goethe-Universität Frankfurt am Main, Theodor-Stern-Kai 7, D-60590 Frankfurt/Main,
14
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18 Germany
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21 *Corresponding author
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24 e-mail: reichert@zbc.kgu.de
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Abstract

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3 Mitochondria fulfill a number of essential cellular functions and play a key role in the
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5 aging process. Reactive oxygen species (ROS) are predominantly generated in this
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7 organelle but next to inducing oxidative damage they act as signaling molecules.
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10 Autophagy is regulated by signaling ROS and is known to affect aging as well as
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12 neurodegenerative diseases. Many cellular components that influence autophagy are
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14 linked to longevity such as members of the sirtuin protein family. Recent studies
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16 further link mitochondrial dynamics to the removal of dysfunctional mitochondria by
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18 mitophagy, thereby representing a novel mechanism for the quality control of
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20 mitochondria. Here we summarize the current views on how mitochondrial function is
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22 linked to aging and we propose that quality control of mitochondria has a crucial role
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25 in counteracting the aging process.
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34 Keywords: aging, mitochondrial dysfunction, fusion, fission, autophagy, mitophagy,
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36 sirtuins, longevity, ROS
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Biological aging is commonly defined as the successive accumulation of adverse effects in an organism with increasing age which leads to functional impairment of individual cells, tissues, as well as the whole organism, finally resulting in the death of the organism. Several theories aiming to explain the rather complex process of aging have been put forward in the last decades (Fleming et al., 1992; Masoro, 2000). Here we will discuss the prominent role of mitochondria in the aging process. In particular, recent findings on the roles of mitochondrial dynamics and selective autophagy of mitochondria (mitophagy) in maintaining mitochondrial functionality will be highlighted. Mitochondria play central roles in numerous diseases and biological processes and their functions go beyond being merely the power plants of cells (Schatz, 2007). With increasing lifetime mitochondrial function has been reported to be impaired in humans (Rooyackers et al., 1996; Short et al., 2005). Whether dysfunctional mitochondria are the cause of cellular impairment or a consequence thereof is a matter of intense discussions. Resolving this issue certainly is of great importance and some advances in this direction are discussed here.

Reactive oxygen species and their possible implications on aging

A good example in this regard is the proposed causative role of reactive oxygen species (ROS) for inducing mitochondrial dysfunction and at the same time of mitochondrial dysfunction leading to increased ROS formation. This vicious cycle is the basis of the 'free radical theory of aging' proposed by Harman (Harman, 1956, 1972). ROS are often considered to be the major source of cellular damage (Davies, 1995; Raha and Robinson, 2000; Sohal and Weindruch, 1996). They are predominantly generated in mitochondria, lead to oxidative damage (Sesti et al., 2009), and are proposed to have gross implications on aging (Fleming et al., 1992).

1 ROS can be neutralized by the addition of antioxidants or by the expression of ROS
2 scavenging enzymes. In fact, overexpression of catalase, which converts H_2O_2 to
3 H_2O and O_2 , leads to an increased lifespan in mice (Schriner et al., 2005). It is
4 unclear which effect is responsible for this observation as higher ROS production is
5 not strictly correlated to accelerated aging. Despite their destructive potential ROS
6 are essential for cellular functions since they also act as signaling molecules (Veal et
7 al., 2007) and by that are involved in a number of pro-survival pathways (Brigelius-
8 Flohe, 2009); e.g. ROS are important in autophagy (Scherz-Shouval et al., 2007).
9 ROS are subdivided into two groups: “signaling ROS” (sROS) and “excessive ROS”
10 (eROS). Increased ROS production due to an increased metabolic rate does not
11 necessarily lead to increased cellular damage, e.g. rat muscle cells adapt to ROS in
12 response to exercise by upregulating ROS scavenging pathways (Gomez-Cabrera et
13 al., 2008). Also an increased rate of mitochondrial biogenesis in chronically
14 contracting muscle was observed leading to an expansion of the mitochondrial
15 reticulum in muscle (Hood, 2001). The benefits of physical activity on health and
16 longevity were reported earlier (Warburton et al., 2006) but are not restricted to
17 normal levels of physical activity as even individuals with an exceptionally high
18 physical activity such as elite endurance and elite mixed-sports athletes with a highly
19 aerobic metabolism show increased longevity and lower mortality compared to
20 normally active individuals (Teramoto and Bungum, 2009). During aerobic
21 metabolism the majority of chemical energy in form of adenosine triphosphate (ATP)
22 is obtained via the citric acid cycle and oxidative phosphorylation. In contrast,
23 anaerobic metabolism mediates ATP production mainly via glycolysis and lactic acid
24 fermentation. Interestingly, during calorie restriction (CR) in yeast the relative
25 proportion of ATP generated via the respiratory pathway is increased at the expense
26 of fermentation (Lin et al., 2002). As sports associated with aerobic metabolism are
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beneficial for health and longevity it is tempting to speculate that performing aerobic sports, besides other positive effects, results in an overall increased resistance to ROS and/or to a net reduction of potentially damaging eROS. Thus, increased generation of ROS would not necessarily promote aging as long as compensatory mechanisms are present. Possibly, a surplus of natural resistance against ROS, or against cellular damage in general, is build up in response to physical exercise. In particular, under normal, non-exercising conditions ROS levels would be minimized more efficiently compared to cells which have not undergone an adaption by physical training. However, the exact contribution of ROS and ROS scavenging pathways on longevity after physical training are difficult to assess since also other factors come into play; e.g. calorie intake/restriction and increased insulin sensitivity (Fontana et al., 2009; Lanza et al., 2008).

Role of insulin sensitivity and calorie restriction in aging

Insulin sensitivity describes the level of insulin that is necessary to keep a constant blood glucose level maintained by insulin-susceptible cells. Lower insulin sensitivity has been associated with accelerated aging (DeFronzo, 1981). In contrast, higher insulin sensitivity has been reported after calorie restriction (CR) and extensive endurance training (Barzilai et al., 1998; Dhahbi et al., 2001) which are both associated with extended longevity (Barger et al., 2003; Teramoto and Bungum, 2009). However, as insulin and insulin-like peptides show contrary effects on longevity (Cohen and Dillin, 2008) it is unclear how longevity and a high insulin-sensitivity are mechanistically linked.

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Calorie restriction (CR) is clearly associated with delayed aging in a number of organisms from yeast to mice (Barger et al., 2003). Some physiological parameters commonly associated with CR in mammals are reduced fat mass, reduced blood glucose levels, reduced insulin levels, improved glucose tolerance, decreased blood pressure, and reduced fat and cholesterol levels. In general, CR increases the amount of ATP generated via respiration, stimulates mitochondrial biogenesis, and leads to reduced levels of ROS (Nisoli et al. 2005; Guarente 2008). Still, the underlying mechanisms of CR on aging are controversially debated (Morley et al., 2009). CR does not promote longevity in all species and is not proven so far to do so in humans (Shanley and Kirkwood, 2006). Studies with monkeys often lacked sufficient number of individuals and the amount of food intake might have been inappropriate as the amount of calories fed to the CR group were comparable to the amount available in the wild; thus, not representing a true CR (Banks et al., 2003; Morley et al., 2009). Several studies confirmed the crucial role of sirtuins, NAD⁺-dependent deacetylases, in the CR-dependent deceleration of aging. Also the extent or way of CR appears to be crucial as e.g. when using a moderate protocol of CR the yeast sirtuin Sir2 was reported to be required for the CR-dependent increase of the number of cell divisions a mother cell can undergo (termed replicative lifespan) in yeast (Kaeberlein et al., 1999). However, this dependency on Sir2 was not observed using a more strict protocol applying a higher degree of CR (Kaeberlein et al., 2005).

The sirtuins – a protein family linked to longevity

The sirtuin protein family in mammals consists of seven members, SIRT1 to SIRT7. Sirtuins are linked to histone deacetylation and chromatin remodeling (Imai et al., 2000; Denu, 2003). Human longevity has been associated with mutations in the

1 enhancer-regions of the *SIRT3* gene (Bellizzi et al., 2005). Upon cellular stress
2 SIRT3 was reported to be processed in the nucleus and subsequently translocated
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4 into mitochondria (Scher et al., 2007). It was shown to deacetylate acetyl-CoA
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6 synthetase 2 within the mitochondrial matrix (Hallows et al., 2006; Schwer et al.,
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8 2006) thereby activating this metabolic enzyme. SIRT1 suppresses stress-induced
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10 apoptosis which was proposed to explain the observed link to longevity (Brunet et al.,
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12 2004; Langley et al., 2002; Luo et al., 2001; Vaziri et al., 2001). SIRT1 was
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14 suggested to be involved in the lifespan-extending mechanism exerted by CR (Cohen
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16 et al., 2004) as well as by extensive endurance training (Lanza et al., 2008). While
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18 the amount of ROS production in both cases is apparently unequal, in both situations
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20 a high level of mitochondrial NAD⁺ is present. In the case of CR high mitochondrial
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22 NAD⁺ levels and the presence of mitochondrial SIRT3 and SIRT4 have been
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24 suggested to lead to improved cell survival (Yang et al., 2007; Guarente 2008). This
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26 points to a putative role of sirtuins as regulatory proteins that are, being NAD⁺-
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28 dependent deacetylases, regulated by altered NAD⁺/NADH ratios and thus respond
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30 to an altered metabolic state of the cell. However, the molecular mechanisms that
31
32 would explain the role of sirtuins, in particular of mitochondrial sirtuins, in the aging
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34 process are still open. One future challenge will be the identification and
35
36 characterization of other relevant substrates and how they are regulated by sirtuins.
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38 One interesting aspect of SIRT1 is its role in autophagy. Lack of SIRT1 inhibits
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40 autophagy *in vivo* and this protein physically interacts with several components of the
41
42 autophagy machinery (Lee et al., 2008). Recently SIRT1 induced autophagy was
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44 suggested to be required for the lifespan-prolonging effect of CR (Morselli et al.,
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46 2010). This prompted us to discuss in more detail what is known about the role of
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48 autophagy in determining longevity?
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Autophagy and aging

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3 Autophagy is a process where intracellular components such as damaged or
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5 superfluous organelles or aggregated proteins are engulfed by autophagosomes and
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7 degraded within lysosomes (Xie and Klionsky, 2007). A recent study linked
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9 autophagy to longevity as age-related impairment of autophagy in skeletal muscle of
10
11 rats that lived under mild CR was slowed down (Wohlgemuth et al., 2009). Another
12
13 study reported that autophagy induced by spermidine increases lifespan in various
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15 organisms (Eisenberg et al., 2009). Autophagy therefore seems to have positive
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17 effects on longevity. Conversely, decreased autophagy shows an opposite effect as
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19 *Drosophila* mutants with a decreased capacity to perform autophagy have been
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21 shown to be short-lived and hyper-sensitive to metabolic stress exhibiting a
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23 neurodegenerative phenotype (Juhasz et al., 2007). Mice tend to develop
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25 neurodegenerative diseases when autophagy is suppressed or lost (Hara et al.,
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27 2006; Komatsu et al., 2006). Furthermore mice treated with rapamycin, a known
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29 inductor of autophagy (Diaz-Troya et al., 2008), have an increased lifespan (Harrison
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31 et al., 2009). Moreover, autophagy was shown to decrease with increasing life time
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33 (Cuervo et al., 2005). In summary, several lines of evidence place autophagy as a
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35 central regulatory mechanism in the aging process (Vellai et al., 2009).
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45 As discussed above, SIRT1 affects autophagy as well as aging. Interestingly, the
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47 tumor-suppressor protein p53, also known as the “guardian of the genome” (Efeyan
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49 and Serrano, 2007) appears to be regulated by SIRT1 (Langley et al., 2002). The
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51 main biological role of p53 is tumor-suppression by cell cycle arrest and apoptosis,
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53 approximately 70% of all human cancers are linked to malfunction of p53 (Finlan and
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55 Hupp, 2007). Mice lacking p53 develop normally, but have a highly increased rate of
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57 tumorigenesis (Donehower et al., 1992). Besides its function as a tumor suppressor
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1 protein p53 has been reported to be involved in the regulation of autophagy (Crighton
2 et al., 2006; Tasdemir et al., 2008). Tasdemir *et al.* (2008) showed that cytoplasmic
3 p53 inhibits autophagy in a transcription-independent manner. Thus, depletion of p53
4 might lead to an increased autophagy resulting in a lifespan extension. Indeed, in *C.*
5 *elegans* loss of the p53 orthologue cep-1 leads to an increased lifespan
6 (Tavernarakis et al., 2008). But even when the loss of p53 in humans would have
7 similar effects on the lifespan by increasing autophagy, the positive effects on
8 lifespan would presumably be overwhelmed by the negative effects in humans. Due
9 to a significantly increased tumorigenesis rather a dramatically shortened lifespan
10 would be observed. However increased autophagy could represent an anti-aging, or
11 survival, mechanism for tumor cells as they often lack functional p53.
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31 **Novel implications of P63 on aging**

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34 The p53-protein family consists of three members: p53, p63 and p73, which share
35 structural and to some extent functional similarity (Levrero et al., 2000; Yang et al.,
36 2002). P73 was reported to be involved in autophagy (Crighton et al., 2007;
37 Rosenbluth and Pietsenpol, 2009) whereas for p63 such a link was not clearly
38 established so far. However, p63 was shown to play a role in aging as a conditional
39 knockout of all p63 isoforms induced cellular senescence (Keyes et al., 2005).
40
41 Furthermore, a p63-isoform lacking of the N-terminal transactivation domain,
42 $\Delta\text{Np63}\alpha$, was linked to aging (Sommer et al., 2006). Overexpression of $\Delta\text{Np63}\alpha$ in
43 basal skin cells led to a premature aging phenotype together with a significantly
44 shortened lifespan in mice. Interestingly, senescence in lung epithelial cells caused
45 by overexpression of $\Delta\text{Np63}\alpha$ was rescued by SIRT1 demonstrating that SIRT1 is
46 involved in $\Delta\text{Np63}\alpha$ -mediated premature aging. The molecular function of p63 has
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1 not been clarified yet but it will certainly be interesting to reveal its role in autophagy
2 and the aging process.
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8 **Mitophagy and its role in mitochondrial quality control**

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11 The selective removal of cellular components including organelles and misfolded
12 protein aggregates occurs via selective, i.e. cargo-specific, autophagy (Xie and
13 Klionsky, 2007). In the case of mitochondria this process is termed mitophagy but the
14 molecular mechanisms for cargo recognition and transport into autophagolysosomes
15 are only known to a limited extent. In particular, how dysfunctional mitochondria are
16 distinguished from functional ones on a molecular level is not understood. Mitophagy
17 was reported to be increased in mammalian and yeast cells harbouring dysfunctional
18 mitochondria (Kim et al., 2007; Lemasters, 2005; Nowikovsky et al., 2007; Priault et
19 al., 2005). It is also induced by nutrient starvation and depends on the general
20 autophagy machinery. In yeast, Uth1, a mitochondrial outer membrane protein, as
21 well as Aup1, a mitochondrial phosphatase, were reported to be essential for
22 mitophagy (Kanki and Klionsky, 2008; Kissova et al., 2004; Tal et al., 2007).
23
24 Recently, systematic screens for components involved in this process revealed
25 several additional components required for this organelle-specific type of autophagy
26 including Atg11, Atg20, Atg24, Atg32, Atg33 (Kanki and Klionsky, 2008, 2009; Kanki
27 et al., 2009; Okamoto et al., 2009). Atg32 is of particular interest as it was reported to
28 act as a mitophagy receptor. It is anchored to the outer membrane of mitochondria
29 and is involved in the local recruitment of ATG8, a component essential for
30 autophagosome formation. In mammalian cells, the following components have been
31 reported to be involved in the degradation of mitochondria: NIX (Sandoval et al.,
32 2008; Schweers et al., 2007), BNIP3 (Zhang et al., 2008), PARKIN (Narendra et al.,
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2008) and PINK1 (Dagda et al., 2009; Geisler et al., 2010; Narendra et al., 2010).
NIX and BNIP3 are BH3 proteins required for mitochondrial clearance during
erythrocyte formation and under hypoxia, respectively. PARKIN, an E3-like ubiquitin-
ligase, and PINK1, a mitochondrial kinase, are associated with Parkinson's disease.
NIX was recently identified as mitophagy receptor in mammalian cells (Novak et al.
2010). The roles of PINK1, PARKIN, and mitochondrial dynamics in mitophagy are
discussed below. In summary, the first components involved in this fundamental
process have been identified but the molecular mechanisms of how either of these
proteins mediates mitochondrial degradation and how these processes are linked to
aging is still open.

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A recent study linked the mitochondrial protein Cisd2 to autophagy and aging. Cisd2
is a member of the gene family containing the CDGSH iron sulfur domain; its cellular
function is unclear. Cisd2 knockout mice show phenotypes of premature aging which
appears to be a consequence of mitochondrial dysfunction accompanied by
increased mitophagy and 'autophagic cell death' (Chen et al., 2009). It is still under
debate whether so called 'autophagic cell death' is merely accompanied or actually
executed by autophagy (Kroemer and Levine, 2008). Here, the authors at least
showed that cell death in Cisd2^{-/-} cells is not caused by activation of apoptosis or
starvation and that there was no significant difference in ROS production compared
to Cisd2^{+/+} cells. Furthermore, murine cells lacking the essential autophagy
component Atg5 show accumulation of damaged mitochondria and altered
mitochondrial morphology (Twig et al., 2008). Thus, on the one hand mitochondria
have to remain functional as otherwise cell death, possibly by autophagic pathways,
is induced. But on the other hand, autophagy has to remain functional in order to
prevent accumulation of cellular debris and dysfunctional mitochondria which also
impairs cell survival. Accumulation of dysfunctional mitochondria and an impaired

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removal thereof is very likely to result in decreased cell viability and in the long run senescence. Thus, quality control of mitochondria is of major importance occurring at different levels. Mitophagy acts at an intracellular level as entire organelles are degraded. In addition, mitochondrial chaperones and proteases prevent the accumulation of misfolded and aggregated proteins within mitochondria. In particular, three classes of ATP-dependent proteases located in mitochondria are important for the quality control of mitochondrial proteins: the AAA-proteases (ATPase associated with a number of cellular activities), the Lon and the Clp proteases (Koppen and Langer, 2007). For example, overexpression of the Lon protease in *Podospora anserina*, an established fungal aging model, led to an extended lifespan while not impairing respiration, growth or fertility (Luce and Osiewacz, 2009). Impairment of mitochondrial proteases leads to neurodegenerative diseases and is linked to aging as well (for review see Germain, 2008; Tatsuta and Langer, 2008). In summary, several lines of evidence suggest that quality of mitochondria is a crucial process in maintaining cellular homeostasis, ensuring longevity, and preventing the occurrence of neurodegenerative disorders.

Mitochondrial dynamics allows distinguishing functional from dysfunctional mitochondria

How are dysfunctional mitochondria recognized and distinguished from functional mitochondria? A hint to answer this question came from recent studies deciphering the molecular mechanisms that link mitochondrial dynamics to the functionality of mitochondria in yeast and mammalian cells. Mitochondrial morphology is highly

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variable and is known to be altered in many pathological situations. Normally mitochondria form a large network of interconnected tubules which is maintained by a balance of fission and fusion events of mitochondria (Bereiter-Hahn and Voth, 1994; Nunnari et al., 1997). Initially, mitochondrial fission was reported to be a prerequisite for mitochondrially mediated apoptosis (Frank et al., 2001; Karbowski et al., 2002; Lee et al., 2004). Moreover, Bax and Bak, two proapoptotic proteins of the Bcl-2 family, were reported to promote mitochondrial fission (Autret and Martin, 2009). In contrast, apoptosis was apparently not blocked in a tissue-specific knock-out mouse lacking the fission factor DRP1, dynamin-related GTPase 1 (Ishihara et al., 2009). Also another study reported that apoptosis was not efficiently inhibited upon downregulation of the mitochondrial fission machinery (Parone et al., 2006). Thus, the strict requirement for mitochondrial fission in apoptosis is a matter of debate and awaits further clarification (Suen et al., 2008).

Very little is known on the effect of mitochondrial dynamics on aging. However, a considerable lifespan prolonging effect was reported upon deletion of the homolog of DRP1, *Dnm1*, in *Saccharomyces cerevisiae* and in *Podospora anserina*, an established aging model organism (Scheckhuber et al., 2007). It is important to note that in the reported study longevity was not accompanied by impaired fitness of *P. anserina* and thus can be regarded as an example of healthy aging. In addition, fragmentation of mitochondria was observed to occur progressively with age in *Podospora anserina* which was inhibited by deletion of *Dnm1* (Scheckhuber et al., 2007). The lifespan extension (244 days for the *Dnm1* deletion mutant versus 22 days for wild type strain of *Podospora anserina*) was proposed to be caused by an increased resistance to apoptosis which could be linked to the impairment of mitochondrial fission. Even though the connection between fission and apoptosis needs to be elucidated further this study strongly points to a link between

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mitochondrial dynamics, apoptosis, and aging. A comparable positive effect of impaired fission on lifespan does not appear to occur in mammals as deletion of DRP1 has been shown to abolish embryonic development and synapse formation in mice (Ishihara et al., 2009; Wakabayashi et al., 2009). In general, alterations in mitochondrial dynamics were so far rather linked to various disorders in humans; e.g. three human neuropathies have been associated with mutations in genes encoding proteins that are required for fission or fusion of mitochondria. The corresponding genes encoding GDA1, Mitofusin2, and OPA1 are affected in Charcot-Marie-Tooth neuropathy type 4a, type 2a, and autosomal dominant optic atrophy type I, respectively (Alexander et al., 2000; Delettre et al., 2000; Niemann et al., 2005; Zuchner et al., 2004). LETM1 is deleted in patients suffering from Wolf-Hirschhorn syndrome, and its yeast orthologue, Mdm38, was shown to be essential for wild type mitochondrial morphology (Dimmer et al., 2002), mitochondrial ion homeostasis (Jiang et al., 2009; Nowikovsky et al., 2004), and import/assembly of OXPHOS complexes (Frazier et al., 2006). Moreover, deletion of Mdm38 was accompanied with increased mitophagy (Nowikovsky et al., 2007). Interestingly, the *Cisd2* gene discussed above is mutated in a second type of Wolf-Hirschhorn syndrome, namely WHS2 (Amr et al., 2007). We recently showed that after dissipation of the mitochondrial membrane potential as well as in several *in vivo* model systems of mitochondrial dysfunction proteolytic processing of the fusion factor OPA1 is induced and that this is a key step in inducing fragmentation of dysfunctional mitochondria (Duvezin-Caubet et al., 2006). This is in line with other studies showing that dissipation of the mitochondrial membrane potential and inducing apoptosis also led to proteolytic processing of OPA1 (Ishihara et al., 2006; Olichon et al., 2007). OPA1 processing and mitochondrial fragmentation was observed in mouse embryonic fibroblasts derived from a knock-in mouse expressing an error-prone mitochondrial

1 DNA Polymerase γ (Trifunovic et al., 2004), the so-called 'mutator mouse' (Duvezin-
2 Caubet et al., 2006). Several proteases responsible for OPA1 processing were
3
4 proposed: the mitochondrial rhomboid protease PARL (Cipolat et al., 2006; Pellegrini
5 and Scorrano, 2007), the i-AAA protease Yme1L (Griparic et al., 2007; Song et al.,
6 2007), and the m-AAA protease (Duvezin-Caubet et al., 2007; Ishihara et al., 2006).
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8 Depletion of prohibitin, a mitochondrial protein complex known to regulate protein
9 degradation by m-AAA proteases (Steglich et al., 1999), leads to a severe growth
10 defect and altered mitochondrial cristae morphology (Merkwirth et al., 2008).
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12 Interestingly, these phenotypes are rescued by a non-cleaved large OPA1 isoform
13 further supporting a role of the m-AAA protease in OPA1 processing. Until recently it
14 was unclear which protease is responsible for the stress-induced processing of OPA1
15 as opposed to OPA1 processing during biogenesis. Two recent studies provide
16 evidence that the ATP-independent protease OMA1 is involved in stress-induced
17 processing of OPA1 (Ehse et al., 2009; Head et al., 2009). This is of particular
18 importance since OPA1 cleavage is a key process linking mitochondrial morphology
19 to the functionality of mitochondria (Duvezin-Caubet et al., 2006) which explains how
20 distinguishing functional from dysfunctional mitochondria occurs mechanistically. We
21 hypothesized earlier that a spatial separation of dysfunctional mitochondria could act
22 as a mechanism to prevent further damage, exerted e.g. by continued production of
23 ROS, and could represent a prerequisite for removal of damaged mitochondria from
24 the cell (Duvezin-Caubet et al., 2006; Herlan et al., 2004). The role of mitochondrial
25 degradation in ensuring functional mitochondria was discussed also by others
26 (Kirkwood, 2000; Skulachev et al., 2004) but the molecular mechanisms linking the
27 functionality of mitochondria with their morphology were only determined recently
28 (Duvezin-Caubet et al., 2006; Herlan et al., 2004; Ishihara et al., 2006). For further
29 details we refer to another review of our group (Schäfer and Reichert, 2009).
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1 A recent report showed that fission of mitochondria under normal growth conditions
2 results in the generation of depolarized mitochondria promoting the engulfment of
3 mitochondria by autophagosomes (Twig et al., 2008). Further, expression of a
4 dominant-negative variant of the fission factor DRP1 as well as overexpression of the
5 fusion factor OPA1 led to an impairment of mitophagy. This shows that mitochondrial
6 fission is required for efficient mitophagy in mammalian cells. Moreover, mitochondria
7 with a low membrane potential are more readily degraded than mitochondria with a
8 high membrane potential. It is still open whether removal of dysfunctional
9 mitochondria as observed under several pathological conditions or after oxidative
10 stress is mechanistically equivalent to mitophagy under normal growth conditions or
11 after starvation.
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31 **Neurodegenerative diseases and their connection to mitochondrial dynamics**

32 A number of studies confirm the pivotal role of mitochondrial dynamics in
33 neurodegenerative diseases such as Parkinson's and Alzheimer's disease. This may
34 not be surprising as the factors determining neurodegenerative diseases strongly
35 overlap with those implicated in aging and both processes often correlate with each
36 other. Two genes associated with Parkinson's disease, PINK1 and PARKIN, play a
37 not yet fully understood role in mitochondrial fusion/fission processes (Deng et al.,
38 2008; Exner et al., 2007; Poole et al., 2008; Yang et al., 2008; Gispert et al., 2009;
39 Lutz et al., 2009). Interestingly, cumulating evidence show that PARKIN and PINK1
40 promote the removal of mitochondria by autophagy further supporting the idea that
41 mitochondrial dynamics and mitophagy are linked processes and that this link may be
42 crucial in Parkinson's disease (Cherra et al., 2009; Michiorri et al., 2010; Narendra et
43 al., 2008, 2010). It is interesting to note that overexpression of PARKIN does promote
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mitophagy only when in addition the membrane potential is dissipated suggesting
that additional events triggered by mitochondrial dysfunction are required for
mitophagy. Loss of PINK1 or PARKIN induces DRP1-dependent mitochondrial
fragmentation (Exner et al., 2007; Lutz et al., 2009). It could well be that in this
situation mitochondrial morphology is indirectly affected as presumably damaged
mitochondria accumulate in the absence of PINK1 and PARKIN.

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The fission factor DRP1 is required for embryonic development in mice (Ishihara et
al., 2009; Wakabayashi et al., 2009) and also appears to be involved in Alzheimer's
disease (Wang et al., 2009). Increased levels of A β , a neurotoxic fragment derived
from the amyloid precursor protein APP found in Alzheimer disease patients, led to
S-nitrosylation of DRP1 which in turn promoted mitochondrial fragmentation (Cho et
al., 2009). In addition, S-nitrosylated DRP1 was increased in brains from Alzheimer
disease patients compared to control patients. Downregulation of DRP1 has various
effects on mitochondria such as disturbed lipid composition, a lowered respiration
(Benard et al., 2007), and decreased mitochondrial DNA (mtDNA) levels (Parone et
al., 2008). Also downregulation of OPA1 was reported to impair oxidative
phosphorylation (Chen et al., 2005) and mutations in OPA1 have been associated
with increased occurrence of mtDNA deletions in humans (Stewart et al., 2008). In
summary, from all these reports it becomes clear that mitochondrial dynamics and
mitophagy are linked and that these processes play an important role also in the
pathogenesis of neurodegenerative disorders.

The mitochondrial genome and aging

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In the 1950s a successive accumulation of mutational hits in the genome was proposed to explain aging (Szilard, 1959). Later this theory was modified and mutations in mtDNA were added to the somatic mutation theory (Ames, 1989; Harman, 1972; Linnane et al., 1989). In mtDNA the mutation rate is considerably higher than in nuclear DNA. Several reasons for this have been put forward including higher ROS levels, reduced DNA repair mechanisms, altered chromatin packaging due to the lack of histones, and a high degree and asymmetry of mtDNA replication (Neiman and Taylor, 2009; Richter et al., 1988). Therefore, mutational damage accumulates more rapidly in mtDNA compared to nuclear DNA leading to dysfunctional proteins and defects in the respiratory chain (Cottrell et al., 2001; Fayet et al., 2002; Muller-Hocker, 1989). The reorganization of mtDNA during aging was first described in *Podospora anserina* (for detailed review see Osiewacz, 2002; Osiewacz & Scheckhuber, 2006) which suggested early that accumulation of mtDNA alterations could be a good marker for cellular senescence. Indeed, certain pathogenic mtDNA deletions were found in adult but not in fetal human tissue (Cortopassi and Arnheim, 1990). Another study also reported the accumulation of mtDNA mutations in the brain of older individuals versus younger individuals (Corral-Debrinski et al., 1992). Similar results were found for monkeys and mice (Khaidakov et al., 2003; Schwarze et al., 1995). More recently premature aging phenotypes were reported in mice expressing a mitochondrial DNA polymerase γ lacking a proof-reading activity (Kujoth et al., 2005; Trifunovic et al., 2004). In both reports the reported lifespan of the 'mutator' mice was significantly lower compared to the wild type. Kujoth et al. (2005) reported that the observed mtDNA mutations do not contribute to increased ROS production in mitochondria and suggest that tissue dysfunction is rather associated with increased apoptosis. Since these reports have been published a debate has been and is still going on about the interpretation of

1 these findings (Khrapko et al., 2006; Khrapko and Vijg, 2007, 2009). One major issue
2 debated is the magnitude of mtDNA mutations and deletions and their relative
3 contribution to aging in mice; e.g. the level of mtDNA alterations in the mutator mice
4 was reported to be several orders of magnitude higher when compared to aged wild
5 type mice (Vermulst et al., 2007). The authors of the latter study suggested that
6 neither mutations nor deletions in mtDNA do limit the lifespan of mice. However, high
7 enough mutation levels seem to be sufficient to induce a premature aging phenotype.
8 This certainly does not exclude that other mechanisms are eventually equally
9 important. One other important mechanism may indeed be reduction of mitophagy
10 with age. Such a scenario is well possible as autophagic flux was shown to be
11 reduced with age in mice (Morimoto et al., 2007).
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31 **Conclusions on the link between aging and mitochondrial quality control**

32 Several possible causes of aging associated with mitochondria have been
33 addressed. Due to the fact that many processes are interconnected, it is crucial to
34 distinguish cause and effect under these circumstances. ROS might lead to damaged
35 DNA or proteins and this might lead to accumulation of dysfunctional mitochondria
36 thus increasing autophagy. Each described process seems to have its essential
37 components but those are often also involved in other important biological processes
38 like in the case of p53 or SIRT1. One feature connecting the different pathways is
39 energy. And as energy is one of the things mitochondria are clearly connected to, it is
40 not really surprising that mitochondria are important for aging. Also the Sir2-family
41 members are linked to the energy metabolism of the cell as they depend on the
42 cellular NAD⁺/NADH ratio. Recent studies allow us now to propose an extended view
43 on the role of mitochondria and aging. When mitochondria are functional, fusion and
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1 fission of mitochondria occur in a constant and balanced manner. As soon as
2 individual parts of the mitochondrial network become dysfunctional (e.g. by oxidative
3 damage), damaged mitochondria become spatially isolated and re-fusion with the
4 intact network is blocked (Fig. 1). Thus, dysfunctional mitochondria are distinguished
5 on a morphological basis from the rest. Spatial separation as well as possibly
6 selective degradation of dysfunctional mitochondria helps to minimize accumulation
7 of cellular debris and subsequent damage. This type of intracellular quality control
8 mechanism is of particular importance for post-mitotic tissues such as neurons and
9 muscle cells. Still future studies will have to investigate whether impaired quality
10 control of mitochondria indeed limits lifespan in eukaryotes.
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Figure legends

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3 Figure 1 A: Mitochondrial dynamics and removal of dysfunctional mitochondria in
4 non-aged cells. Functional mitochondria can undergo fusion and fission cycles (green
5 pathway) while dysfunctional mitochondria, i.e. damaged by excessive ROS (eROS),
6 are not able to fuse anymore (red crossed arrow). This leads to a spacial separation
7 and degradation of damaged mitochondria by mitophagy (red pathway). A selection
8 of important factors for individual steps are indicated: OPA1, Mitofusin1/2, DRP1,
9 signalling ROS (sROS), SIRT1, PINK1, PARKIN, NIX and BNIP3.
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21 B: Impaired quality control of mitochondria as hypothesized to occur in an aged cell.
22 Inhibition or block of the pathway depicted in panel A can occur at different steps;
23 e.g. by impaired fission, impaired autophagosome formation surrounding damaged
24 mitochondria, or by impaired degradation in lysosomes. Block of any of these steps
25 (black crossed arrows) would lead to the progressive accumulation of dysfunctional
26 mitochondrial and finally in senescence.
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Figure 1A

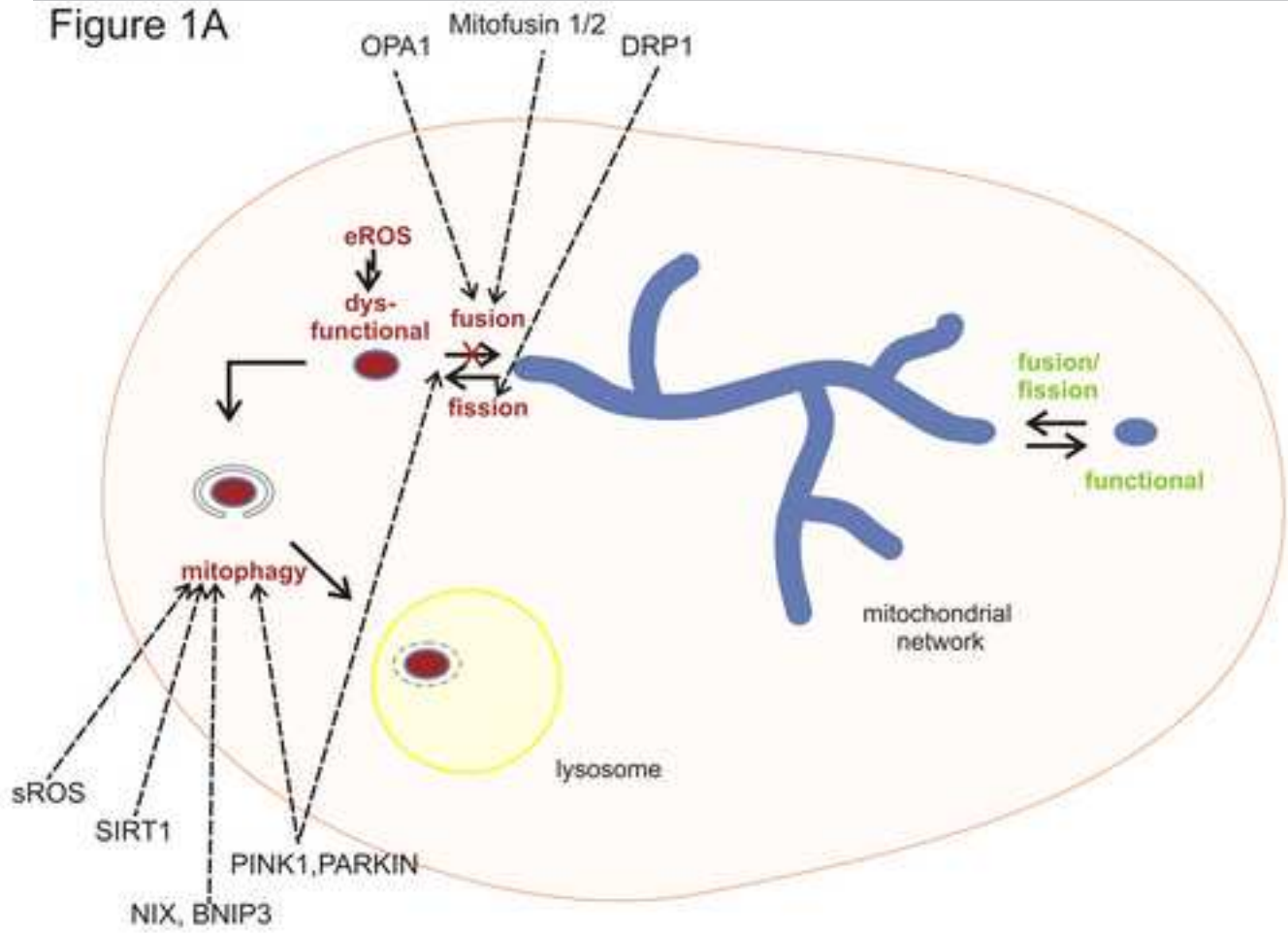


Figure 1B

