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Age-related decline in mitochondrial bioenergetics: Does supercomplex destabilization determine lower oxidative capacity and higher superoxide production?

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Abstract

Mitochondrial decay plays a central role in the aging process. Although certainly multifactorial in nature, defective operation of the electron transport chain (ETC) constitutes a key mechanism involved in the age-associated loss of mitochondrial energy metabolism. Primarily, mitochondrial dysfunction affects the aging animal by limiting bioenergetic reserve capacity and/or increasing oxidative stress via enhanced electron leakage from the ETC. Even though the important aging characteristics of mitochondrial decay are known, the molecular events underlying inefficient electron flux that ultimately leads to higher superoxide appearance and impaired respiration are not completely understood. This review focuses on the potential role(s) that age-associated destabilization of the macromolecular organization of the ETC (i.e. supercomplexes) may be important for development of the mitochondrial aging phenotype, particularly in post-mitotic tissues.

Keywords

aging; mitochondrial dysfunction; electron transport supercomplexes.

1. Introduction

Alterations of the components of the mitochondrial energy transduction system represent an important part of the etiology of mitochondrial decay and cellular dysfunction in aging tissues [1]. In particular, accumulation of age-related changes to both the structure and function of the electron transport chain (ETC) [2-5] and alterations of the lipid milieu of the inner mitochondrial membrane (IMM) have been noted [6-11]. Moreover, these changes to

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the IMM may contribute to decreased aerobic metabolism, increased generation of reactive oxygen species (ROS) and oxidative damage (for reviews see [12-15]), and higher appearance of proapoptotic factors in the cytosol [16-18]. Nevertheless, the relationship between IMM structural alterations with age and their precise consequence to mitochondrial decay is only poorly understood. This is particularly true for the protein complexes of the ETC, whose overall structural organization is currently being revised from that of individual electron transport complexes in the IMM to large “supercomplexes” composed of differing stoichiometries [19-21]. Assembly and stability of supercomplexes appears to be highly important for regulation of mitochondrial bioenergetics as even slight losses in their formation, as shown in Barth syndrome, correlate with severe cellular dysfunction [22]. Furthermore, supercomplex destabilization is the main underlying factor involved in the loss of mitochondrial bioenergetics in a canine model of acute heart failure [23, 24]. Thus, there is growing evidence that supercomplex destabilization plays a critical role in the progression of pathophysiologies where mitochondrial dysfunction has been detected. By analogy, a rationale exists that disintegration of supercomplexes is one of the characteristic features of age-related mitochondrial decay in post-mitotic tissues [25-27]. The focus of this paper is thus two-fold, namely, to review the critical factors associated with mitochondrial decay and how alterations to supercomplex ultrastructure may be an important underlying facet leading to the mitochondrial aging phenotype.

2. Mitochondrial dysfunction in the aging process

2.1. General aspects regarding the etiology of mitochondrial decay in aging

Mitochondrial decay plays a central role in the aging process [1]. Although the age-associated loss of mitochondrial function is undoubtedly multifactorial, several lines of evidence indicate that certain molecular and cellular alterations are significantly involved in the progression of changes that ultimately lead to impaired mitochondrial energy metabolism. Age-related oxidative damage and deletions of mitochondrial DNA (mtDNA) that correlate with lower respiratory activity have been reported in liver [9] and cardiac muscle [28] of rats, liver [29] and skeletal muscle [30] of primates, and skeletal [31] and cardiac muscle [28, 32] of humans. Moreover, mtDNA deletions accumulate during the progression of atrial fibrillation in human aging [32]. On the other hand, impaired autophagy that regulates mitochondrial homeostasis also appears to contribute to the age-related accumulation of respiratory defects in tissues [33-35]. In this regard, Terman et al. showed that inhibition of autophagy in neonatal rat cardiomyocytes leads to morphological alterations of mitochondria and changes in membrane potential (ψ_m), similar to that of senescent cells [36]. This group further concluded that abnormal mitochondria accumulate in post-mitotic tissues as a consequence of a defective autophagy process [36, 37].

It has been hypothesized that the aforementioned characteristics of mitochondrial decay are symptomatic of a vicious downward spiral where defective ETC complexes contribute to enhanced ROS generation by mitochondria, which in turn increases mtDNA damage and mutations, which eventually reciprocally affects structure of the ETC components (for recent reviews see [12, 13]). Ultimately, higher rates of ROS appearance increase oxidative modification of membrane lipids and proteins to the point where accumulation of molecular

defects overcomes the capacity to maintain mitochondrial homeostasis through autophagy [13, 33, 36].

2.2. Consequences of mitochondrial decay on cellular function

An immediate consequence of the age-related impairment of mitochondrial function would be decreased aerobic energy transduction. A consistent picture has emerged from examination of mitochondrial bioenergetics in intact cells from young and old animals of many species. Here, mitochondrial membrane potential (ψ_m) and cellular energy status progressively decline with age in both freshly isolated rat hepatocytes [9] and in human skin fibroblasts taken from very young (fetal material) versus very old (103 years) human donors [38]. This work is now buttressed by reports connecting mitochondrial decay to organ decline *in vivo*. Using ^{31}P magnetic resonance spectroscopy and optical approaches to simultaneously monitor ATP synthesis and O_2 uptake, Marcinek et al. showed that working skeletal muscle of aged C57Bl/6 mice experienced a 50% decline in the mitochondrial P/O ratio, an indicator of the efficiency of ATP synthesis coupled to respiration, and a consequent loss in energy charge versus young animals [39]. This is in agreement with Kostler et al. who observed that high-energy phosphates in the human heart declined significantly *in vivo* with age, suggesting energy reserve capacity in this organ is highly attenuated [40]. Furthermore, using positron emission tomography for analyzing cardiac function in humans, Kates et al. found that aging significantly decreases fatty acid oxidation rates, with respect to myocardial oxygen consumption rates [41].

In addition, aging reportedly diminishes State 3 respiration rates during oxidation of NADH-associated substrates in rat mitochondria isolated from hippocampus and brain cortex [42, 43], skeletal muscle [44], liver [45], kidney [46], and heart [3, 4, 17, 44], as well as in human skeletal muscle mitochondria [47]. Nevertheless, it is less clear whether aging modifies respiratory control (RCR) and P/O ratios when using *in vitro* models. Experiments on isolated rat heart mitochondria showed that even though State 3 respiration declines with age, the P/O ratio and RCR remain unaffected [4, 44, 48]. In line with these findings, O'Toole et al. reported a lack of age-related changes in the P/O and RCR in rat kidney mitochondria [46]. In contrast, other reports reveal that RCR values decline with age in rat liver mitochondria [45] and also in mitochondria from rat hippocampus [43]. To summarize, aging decreases mitochondrial ADP-stimulated respiration and cellular aerobic metabolism, but the degree of this decline is controversial. *In vitro* studies may not precisely reflect the magnitude that age-related mitochondrial decay impairs cellular energy metabolism *in vivo*.

Analysis of mitochondrial calcium handling, a key aspect of overall mitochondrial function, shows that aging induces higher vulnerability to calcium overload and propensity to permeability transition in mitochondria in brains and livers from B6D2F1 mice [18] and in interfibrillar mitochondria from Fischer 344 [17] and Fischer 344 $\times$ Brown Norway rats [16]. Furthermore, Hofer et al. showed that the age-related loss of calcium-accumulating capacity in mitochondria from Fischer 344 $\times$ Brown Norway rats correlated with higher concentrations of cytosolic cytochrome *c* and resultant higher caspase 3 activity [16]. In further support for these observations, it was recently observed that human cardiomyocytes

display higher expression of proapoptotic proteins (e.g. Bax) with age, which correlate with increased cytosolic levels of cytochrome *c* and caspase 9 [49].

Lastly, a strong relationship has emerged between mitochondrial decay and oxidative damage to mitochondrial and extramitochondrial cellular components (for reviews see [12-15]). Consistent with this view are studies, including our own, showing higher levels of mtDNA deletions and oxidative damage (see Section 2.1.), oxidative DNA damage [49, 50], lipid peroxidation [50-53], and lipid adduction to proteins [54-56] with age. Cardiolipin, a mitochondrial specific phospholipid, may be particularly prone to oxidative damage and loss (see Section 3.2. and also [57]). This is because of the highly unsaturated nature of its acyl side-chains and its proximity to ROS emanating from the ETC [58-61]. Additionally, most amino acid residues can be oxidized by ROS, which lead to formation of disulfide bonds and carbonyl derivatives [49, 62-66]. Proteins in proximity to decaying mitochondria may be particularly susceptible to oxidative damage and dysfunction. Thus, mitochondrial-driven oxidative damage may not only influence progression of mitochondrial decay but also adversely affect cell survival and overall organ function.

Even though the aforementioned characteristics of mitochondria in aged tissues show varying degrees of alterations, the available evidence nevertheless supports the view that that electron flux efficiency through the ETC declines while oxidant leak is enhanced with age. Both of these age-associated deficits emanate from an altered IMM. The following section will provide evidence for the functional consequences of mitochondrial decay, especially with respect to ETC function.

3. Age-associated changes of the mitochondrial energy transduction system

3.1. Catalytic and structural alterations of the respiratory chain complexes in aging

Dysfunction of the components of the ETC strongly correlates to the age-related mitochondrial decline in bioenergetic reserve observed in different organs [2-5]. In both rodents and humans, complex I catalytic activity declines with age in liver [5, 67], brain [43, 68], and heart [5, 49, 69, 70]. In addition, several alterations of the complex III holo-protein result in its lower activity, particularly in post-mitotic tissues of aging rodents [2, 4, 69, 71] and primates [72]. These results agree with other studies showing that defects of complex IV correlate with lower mitochondrial oxidative capacity in post-mitotic tissues of elder humans [73, 74], primates [72, 75], and rodents [3, 43, 76-79].

Age-associated alterations of the ETC components promote inefficient electron transport (i.e. higher electron leakage) and increased ROS appearance in mitochondria [70, 71, 80, 81]. In turn, higher ROS generation contributes to ETC dysfunction by initiating oxidative modifications of ETC proteins [55, 69, 76] and mtDNA [30, 82] (see also Section 2.2.). However, the mechanism(s) of superoxide ($O_2^{\bullet-}$) generation under physiological circumstances are not completely understood, and the role that the ETC plays in increased $O_2^{\bullet-}$ with age is not yet clear. In this regard, experimental observations by Moghaddas et al. indicate that oxidative modification of the Q_0 binding site in complex III is the main factor involved in higher $O_2^{\bullet-}$ generation in the aged heart [71]. On the other hand, complex I may

also be an important source of $O_2^{\bullet-}$ generation that could contribute to enhanced $O_2^{\bullet-}$ with age [70, 81, 83, 84].

Regardless of the specific site of production, increased ROS from an impaired ETC correlates with a decline in mitochondrial antioxidant status with age. The mono-thiol antioxidant, glutathione (GSH), is particularly diminished in mitochondria from aged tissue. We showed that aging leads to deficits in both mitochondrial GSH levels and its redox ratio (GSH/GSSG) in the brain and heart of rats [85]. Moreover, we observed both an age-related loss of GSH and ascorbate in rat hepatocytes [86] and isolated rat heart mitochondria [76]. The aging rat heart also displayed significantly lower ascorbate concentrations [50]. Thus, mitochondria from aged tissues of a variety of species display increased ROS output, lower antioxidant defenses, and greater oxidative damage.

3.2. Age-related changes in the lipid composition of the inner membrane

Aging leads to alterations of the lipid composition of the IMM that also contribute to impaired bioenergetic capacity of organs and tissues. An age-related decline in coenzyme Q, a key isoprene-derivative that mediates electron transfer between several IMM protein complexes, has been observed in plasma [87] and also in cardiac muscle from human subjects [88, 89]. In addition, tissue levels of coenzyme Q appear to decline in heart, kidney, and skeletal muscle from aged rats [90]. This loss appears to be more pronounced when coenzyme Q levels are measured in isolated mitochondria versus whole tissues [10, 11]. Additionally, multiple studies suggest that aging leads to lower cardiolipin levels and/or its acyl side-chain composition. Cardiolipin is a phospholipid that is almost exclusively located to mitochondria and acts as an important cofactor of several IMM proteins [58, 59]. Studies using brain [68], liver [91], and cardiac mitochondria [6-8, 92-94], and our own work with isolated rat hepatocytes [86, 95] indicate that cardiolipin significantly declines with age. However, the extent of cardiolipin loss and/or whether such declines are functionally consequential is controversial. Hoppel and colleagues provided evidence that interfibrillar mitochondria of the aging rat heart have no changes in cardiolipin levels or composition, in contrast to earlier reports [96]. The reason for the discrepancy between this work and the aforementioned studies is not clear, although a partial answer may lie in the different methods used to extract lipids from mitochondrial fractions (e.g. one-phase organic systems versus two-phase mixtures) and the various techniques employed for separation and analysis of cardiolipin.

Despite the controversial degree of general cardiolipin loss, there is a growing consensus that significant remodeling of cardiolipin acyl side-chains occurs with age. Helmy et al. observed an age-related increase in the ratio of monolyso-cardiolipin to mature cardiolipin in the guinea pig kidney [97], an indicator of defective incorporation of acyl chain units into cardiolipin during the remodeling cycle [98]. In addition, tetralinoleoyl-cardiolipin ([18:2]₄-cardiolipin), the predominant species in the heart [61, 99], appears to be the most adversely affected on an age basis [100]. We also recently observed that aging leads to a significant loss of (18:2)₄-cardiolipin in rat heart interfibrillar mitochondria, although total cardiolipin levels were unaffected (unpublished results). These results partially agree with the observations made by Hoppel and coworkers [96]. Alteration of acyl side-chains, as evident

in aging tissues, would be expected to adversely affect ETC electron movement because of protein conformational changes. Thus, cardiolipin molecular composition is markedly affected with age, possibly due to defects in remodeling of mature cardiolipin.

Although aging does not significantly affect the levels of major phospholipids (e.g. phosphatidylethanolamine) [6-8, 91], the overall IMM composition and its fluidity are significantly changed with age. Cholesterol accumulates in the IMM, alters membrane fluidity, and may promote greater H⁺ leakage [7, 91]. In a similar vein, using LC-MS/MS analysis, we recently demonstrated that aged cardiac mitochondria accumulate ceramide, a pro-apoptotic sphingolipid, which results in inhibition of complex IV activity [79]. Furthermore, three ceramide isoforms in the IMM (e.g. those with 16, 18, and 24:1 acyl chains) caused the increase in overall ceramide levels [79]. Thus, extensive age-related alterations of the lipid milieu of the IMM occur, which adversely affect membrane structural organization and contribute to limiting the biological function of the ETC complexes.

4. Supercomplex destabilization as a new underlying factor of mitochondrial decay in aging

4.1. Supercomplex organization of the electron transport chain

The ETC comprises four large protein complexes, which along with the F₁F₀-ATP synthase (complex V) and mobile electron carriers (e.g. cytochrome *c* and coenzyme Q), constitute the machinery for converting metabolic energy transiently stored as reduced coenzymes into ATP. Until recently, the prevailing view was that the components of the ETC were distinct entities where rapid, random collisions allowed electron transfer between complexes [101-104]. However, with the advent of Blue Native-PAGE (BN-PAGE) technology [19-21], there is a growing awareness that the individual components of the ETC may actually exist as large macromolecular assemblies, or so-called supercomplexes. Evidence accumulated from functional and structural studies now supports the existence of these supramolecular assemblies, which includes oxygen consumption characteristics [105], metabolic flux control analysis [106-111], and three-dimensional structures of the I₁III₂IV₁¹ supercomplex from bovine heart mitochondria at relatively good resolution (~ 20 Å) [112, 113].

Although there was initial skepticism whether supercomplexes were artifacts from the use of a non-ionic detergent (e.g. digitonin) in their isolation, further extensive characterization of supercomplexes from mitochondria of different sources (Table 1) appears to have mitigated most concerns regarding their biological existence. This characterization includes identification of striking variations in stoichiometries of components comprising supercomplexes in tissues from the same species [114] or from cells obtained from different human tissues (Table 1). Moreover, metabolic flux control analysis measuring the kinetic behavior of the mitochondrial ETC and the ubiquinone pool further provide supporting evidence for supercomplex organization of the ETC [106-108, 110, 111]. Briefly, complexes I and III appear to be kinetically linked [106, 107, 111], while there is no association of

¹Supercomplex assemblies are presented as the Roman numeral which represents the particular electron transport complex, along with a subscript indicating its stoichiometry relative to other electron transport chain components.

complex II with any other complex. This latter result is in good agreement with BN-PAGE analysis using cardiac mitochondria [19, 25, 114, 115], which indicates that supercomplexes are comprised of varying stoichiometries of complexes I, III, and IV. Finally, cryo-electron microscopic and tomographic studies independently show the existence of $I_1III_2IV_1$ supercomplexes [112, 116]. These studies show that the distance between binding sites of coenzyme Q at complexes I and III is only ~13 nm [112, 116] and that the distance between binding sites of cytochrome *c* at complexes III and IV is ~10 nm [112, 116]. Such distances are much shorter than the minimum effective lengths determined for diffusion of coenzyme Q (37.9 nm) and cytochrome *c* (24.8 nm) during electron transfer by a random collision mechanism [102]. This suggests that supercomplexes offer a catalytic advantage of faster and more efficient electron transfer by limiting the overall distance between redox cofactors.

Finally, and in relation to the role that supercomplex destabilization plays in mitochondrial aging (see Section 4.2.), supercomplexes may be necessary for general stability of the ETC. Moreno-Lastres et al. demonstrated that complexes III and IV are required for full assembly of complex I in mitochondria from human osteosarcoma cybrids [117]. Other studies show that removing complex III results in loss of the I_1III_2 supercomplex in human mitochondria from skeletal muscle [118], skin fibroblasts [119], and osteosarcoma cybrid cells [120]. Therefore, it is reasonable to theorize that not only does supercomplex organization mediate respiratory activity, but these macromolecular assemblies also regulate stability of individual components of the ETC.

In summary, there is growing evidence that the ETC is actually assembled as a solid-state macromolecular assembly where defective supercomplex organization results in pathologies as varied as heart failure [23, 24], Barth syndrome [22], and Leigh syndrome [118, 119]. Both the theoretical and experimental evidence suggesting that supercomplex destabilization is part of mitochondrial aging will now be discussed.

4.2. Destabilization of supercomplexes and its implications on mitochondrial decay in aging

4.2.1. Evidence of supercomplex destabilization in aged tissues—While only a limited number of studies have examined how aging affects supercomplexes, the information gleaned so far strongly suggests that the size and complexity of these assemblies diminish on an age basis. To our knowledge, we were the first to show supercomplex deterioration in cardiac mitochondria from old rats [25]. Primarily, supercomplexes comprising the highest molecular weight assemblies declined to the greatest extent with age, although most of the electron transport supercomplexes showed some degree of loss [25]. However, it is notable that supercomplex disintegration was not from age-associated decrements in a particular ETC component [25]. In support of our work in the heart, Frenzel et al. also reported supercomplex destabilization in cortical tissue of aging rat brains [26]. This study also noted that $I_1III_2IV_N$ supercomplexes are particularly adversely affected [26]. However in contrast to these studies, Lombardi et al. noted an age-associated increase in $I_1III_2IV_N$ supercomplexes in rat skeletal muscle [27], which was hypothesized to be a compensation for the significant loss of the smaller I_1III_2 supercomplex. Finally, a recent report showed no age-associated alterations in supercomplex organization at all in rat kidney

mitochondria [46], even though loss of State 3 respiration was detected [46]. Taken together, there appears to be varying degrees of age-dependent supercomplex disorganization where brain and heart mitochondria are the most adversely affected, and other tissues (e.g. skeletal muscle and kidney) have lesser or no supercomplex decrements. The reason(s) for this variability are not presently clear but could stem from tissue-specific factors related to supercomplex stabilization and/or levels of individual ETC components available for supercomplex formation. However, it is quite conceivable that part of the seeming variability for supercomplex destabilization may stem from the small number of studies performed to date and differences in analytical protocols and quantitative analysis used. Nevertheless, the identification of severe age-associated alterations in supercomplex levels, especially in post-mitotic tissue, warrants further analysis of both the causes and the consequences to mitochondrial function (see also Section 4.2.2).

4.2.2. Major factors that alter supercomplex stability in aging—The precise mechanism(s) involved in supercomplex destabilization have yet to be elucidated. However, genetic studies using yeast [121-123] as well as cryo-electron microscopy [112] are beginning to elucidate a role for lipid-protein interactions as a partial mechanism for this deterioration. For example, experiments on yeast mitochondria indicate that cardiolipin lowers electrostatic repulsion at the interface between complexes III and IV [122]. Moreover, the ETC complexes assembled as the $I_1III_2IV_1$ supercomplex are not in close contact with each other (2-5 nm apart) within the IMM [112], which indicates that lipid-protein interactions are key to maintaining supercomplex stability. In addition, there is a strong correlation between the levels of $I_1III_2IV_N$ supercomplexes and mitochondria from tissues displaying relatively high content (~70-80%) of cardiolipins with (18:2)₄-acyl side-chains (Table 1). This association fits with observations that “symmetrical” cardiolipins containing similar acyl chains promote protein-protein interactions and ETC function [61, 99, 124-126]. Indeed, the aforementioned study by Frenzel et al. suggested that the age-related destabilization of brain cortical supercomplexes was mediated by altered cardiolipin acyl chain content [26]. Thus, it is tempting to hypothesize that the oxidative damage of unsaturated cardiolipin side-chains that has been observed in the heart and brain from aged animals [68, 70] leads to supercomplex deterioration by altering binding of cardiolipin to ETC proteins. In support of this hypothesis, Diaz et al. recently reported that increased ROS generation destabilizes supercomplexes in mouse lung fibroblasts [127]. In this study, lower supercomplex levels correlated with decreased stability of complex I in an antimycin A-supported model of ROS generation. Nevertheless, it is not clear whether oxidatively damaged membrane lipids also contributed to supercomplex destabilization under the same conditions [127]. These studies provide a framework for further work on the role that IMM lipid composition (see section 3.2) plays in both supercomplex formation and destabilization with age.

In addition to lipid involvement, disintegration of supercomplexes may also stem from alterations to proteins of the IMM. In particular, complex IV is a key player in supercomplex formation or, alternatively, supercomplex destabilization with age. In fact, we and others showed that complex IV activity declines in aged rat heart interfibrillar mitochondria [3, 76, 79], which correlates with extensive oxidative modification of this complex [76]. Moreover,

alterations of several complex IV subunits are associated with defective assembly of I₁III₂IV_N supercomplexes in mitochondria from human cells [118, 120, 128, 129], rodents [105, 130], and *C. elegans* [131]. For example, phosphorylation of complex IV results in the loss of respirasomes in a canine model of acute heart failure [23]. Therefore, oxidative or posttranslational modifications of complex IV may regulate supercomplex stability in cardiac mitochondria [23]. Finally, studies from multiple laboratories have supplied exciting new information showing that specific protein factors are involved in supercomplex assembly [132-134]. Rcf1 (Respiratory Supercomplex Factor 1) associates with complex IV and is required for supercomplex stabilization in yeast mitochondria. Moreover, Chen et al. showed that defective organization of I₁III₂IV_N supercomplexes results from siRNA-mediated knock down of the *rcf1* mammalian homologue, *HIG2A* (hypoxia inducible gene 1, family member 2A), in C2C12 myoblasts [132]. Thus, *HIG2A* or other as yet unidentified proteins may also regulate supercomplex stability, particularly in post-mitotic tissues (e.g. the heart and brain) that show the most severe supercomplex disintegration with age.

In summary, both lipid factor(s) and proteins associated with the IMM may be involved in the assembly and maintenance of mitochondrial supercomplexes (Fig. 1). While little evidence currently exists for a precise role of any one factor in the loss of supercomplexes with age, nevertheless, it is noteworthy that both cardiolipin structural alterations and protein oxidation markedly increases on an age basis, particularly in mitochondria where supercomplexes deteriorate most appreciably. Identification of the specific biological molecules involved in supercomplex assembly will be necessary to further identify both tissue specific variability of supercomplex assemblies and their age-associated decline.

4.2.3. Supercomplex destabilization and impaired mitochondrial bioenergetics in aging—Since their elucidation by Schägger and colleagues [19, 135, 136], it has been hypothesized that the biological reason for the existence of supercomplexes is to efficiently pass electrons through the ETC to O₂. Conversely, age-associated decrements in supercomplex assembly may theoretically result in inefficient ETC electron flux, adversely affecting energy reserve capacity. Experimental support for a role of supercomplex assembly in efficient electron transport comes from studies showing stability of the I₁III₂ supercomplex correlates with higher NADH-cytochrome *c* oxidoreductase activity [107, 108, 110]. Moreover, pioneering work by Zhang et al. [121] showed that supercomplex destabilization in a yeast strain lacking the cardiolipin synthase gene (i.e. *crd1* mutants) was associated with abnormal growth on nonfermentable substrates [121]. In addition, Greenberg et al. showed that *crd1* yeast displayed defective mitochondrial respiration and oxidative phosphorylation characteristics but only under extreme conditions, such as elevated temperature or osmotic shock [137-139]. Thus, it appears that supercomplexes are not required for maintaining basal respiratory activity but for supporting bioenergetic reserve capacity. Taken together, these observations provide support that supercomplex destabilization diminishes State 3 respiration and also limits bioenergetic reserve capacity *in vivo*. This phenotype parallels characteristics of mitochondrial decay and at least in part explains the metabolic limitations of aging in aerobically active tissues (see Section 2.2.). Significantly more work will be needed in order to fully elucidate the true implications of supercomplex destabilization on the loss of mitochondrial bioenergetics.

4.2.4. Supercomplex destabilization and ROS generation in aging—An obvious advantage for the ETC integrated as a supercomplex would be for efficient electron flux from reduced coenzymes to molecular oxygen [19]. In this regard, Panov et al. hypothesized that supercomplexes prevent superoxide formation even during maximal electron flux by maintaining all the electron carriers (i.e. Fe-S clusters and ubiquinone in complexes I and III) involved in $O_2^{\bullet-}$ generation in a permanently oxidized state [140]. Thus, supercomplexes would represent an evolutionary adaptation to prevent excessive ROS formation [140]. Alternatively, lack of supercomplexes has been implicated in high basal mitochondrial $O_2^{\bullet-}$ production in human neutrophils [141]. One study showed that mitochondria from mononuclear leukocytes and human leukemia cells (HL-60) contain $I_1III_2IV_N$ supercomplexes, but these particular macromolecular assemblies disappear when HL-60 cells differentiate into neutrophils [141]. While the respiratory supercomplexes were not necessary to maintain membrane potential (ψ_m), supercomplex loss in differentiated cells resulted in significantly higher rates of $O_2^{\bullet-}$ generation [141]. Furthermore, Lenaz et al. showed that mouse fibroblasts expressing the activated form of the *k-ras* oncogene lacked supercomplexes, which correlated with a higher rate of ROS appearance with respect to wild type fibroblasts [108]. Finally, new insights as to the role of supercomplexes in preventing ROS formation have been provided by the recent discovery of the Rcf1 subunit in yeast mitochondria [132, 133]. In these studies, *rcf1* yeast displayed significantly higher rates of ROS appearance and increased oxidative damage versus wild type cells [132, 133].

In summary, supercomplex disassembly in aged post-mitotic tissues correlates with a wealth of information showing higher superoxide leak from the ETC with age. Taken together with the aforementioned studies on supercomplexes and their influence on $O_2^{\bullet-}$ generation, it is enticing to speculate a cause-and-effect relationship between higher ROS and supercomplex deterioration. Figure 1 shows a schematic representation of this hypothesis. Nevertheless, there is still a dearth of experimental support for a functional role of supercomplexes in preventing higher rates of ROS appearance in post-mitotic tissues.

5. Conclusions and perspectives

Structural and functional alterations of the IMM components are associated with mitochondrial decay that adversely affects aerobic energy metabolism in aging tissues. Loss of catalytic function of the ETC complexes is recognized as an underlying factor of the age-related decrease in bioenergetic reserve capacity. Herein, we have presented theoretical and current experimental evidence that disintegration of ETC supercomplex ultrastructure may lead to many aspects of the mitochondrial aging phenotype, especially in post-mitotic tissues like the heart and brain. As discussed, supercomplex deterioration may be multifactorial where both IMM lipid and proteins may be involved. The precise elucidation of the factor(s) that integrate ETC components as supercomplexes should also shed light on age-related decline in supercomplex assembly and, by analogy, provide molecular target(s) for therapeutic intervention to maintain ETC function. Moreover, equal efforts should be directed toward defining the consequences of supercomplex disassembly with respect to known characteristics of mitochondrial decay. It should be noted that even small losses of supercomplex levels correlate with severe consequences to organ function (*cf* Barth Syndrome). However, studies with yeast suggest that basal bioenergetics is not affected with

supercomplex disassembly. It is likely that supercomplex deterioration would mainly limit electron transport efficiency, leading to enhanced ROS/oxidative damage, as well as limitations in energy reserve capacity. Reciprocally, increased ROS generation would cause supercomplex destabilization by oxidatively altering the structural organization of lipids (e.g. cardiolipin) and proteins involved in the assembly of mitochondrial supercomplexes. Thus, one could envision that aging leads to a vicious downward spiral of oxidative damage to IMM constituents, supercomplex disintegration and increasing oxidative damage, which is followed by more severe supercomplex destruction. This scenario could be extended to age-accelerating syndromes where chronic oxidative stress is evident. For example, it would be interesting to determine whether mitochondria from diabetic subjects also display loss of supercomplexes similar to normal aging. Further studies are needed to elucidate whether defective supercomplex organization limits energy supply and aerobic metabolism in the old animal.

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Highlights

- The inner mitochondrial membrane decays with age, affecting energy metabolism.
- Supercomplex destabilization is discussed as a novel aspect of mitochondrial aging.
- Discovery of supercomplex loss opens new possibilities to decline in bioenergetics.

Figure 1.

Supercomplex destabilization and mitochondrial decay in aging. Age-related alterations of the IMM components are presented as important factors affecting stability of supercomplex organization. Potential consequences of supercomplex destabilization on mitochondrial function include higher generation of $O_2^{\bullet-}$, which may affect supercomplexes by increasing oxidative damage of IMM lipids and proteins, and diminished bioenergetic reserve capacity. RCF1a and RCF1b: human homologues of the yeast respiratory supercomplex factor (Rcf1). HIG2A: hypoxia inducible gene 1, domain family member 2A.

Table 1

Tissue distribution of cardiolipins and identified mitochondrial electron transport supercomplexes.

Source of mitochondria	Symmetric cardiolipin ^a (% of total)	Refs.	Supercomplexes		Refs.
			I ₁ III ₂ ^b	I ₁ III ₂ IV _N ^c	
Heart					
Bovine	(18:2) ₄ (70-73)	[142]	+	+	[19, 136]
Rat	(18:2) ₄ (77)	[99, 124]	+	+	[25, 115]
Dog	(18:2) ₄ (77-79)	[23, 99]	+	+	[24]
Liver					
Rat	(18:2) ₄ (57)	[142, 143]	+	—	[114]
Mouse	n.d.		+(V _I) ^d	+(II _I)	[105]
Skeletal muscle					
Rat	(18:2) ₄ (73)	[99]	+	+	[27, 114]
Human	(18:2) ₄ (79-81)	[124, 144]	+	+	[118]
Cell line					
C2C12	n.d.		+(V _I)	+(II _I)	[132]
HeLa	n.d.		+	+	[129]
HEK-293	n.d.		+	+	[128]
HL-60	n.d.		—	+	[141]
PBMC (human)	n.d.		—	+	[141]
Lymphoblasts (human)	(18:1) ₄ (32)	[61, 145]	+	+	[22]
Skin fibroblasts					
Mouse	n.d.		+	+	[130]
Human	(18:2) ₄ (20-30)	[146, 147]	—	+	[149]
Lung fibroblasts (mouse)	(18:1) ₄ (n.d.)	[148]	—	+	[148]
Osteosarcomacybrids (human)	n.d.		—	+	[120]
TPC-1 (human)	n.d.		+	—	[107]
Other sources					
Brain cortex (rat)	n.d.		+	+	[26]
Kidney (rat)	(18:2) ₄ (50)	[124]	+	+	[46, 114]
Human placenta	(18:2) ₄ (20)	[124]	+	+	[150]
<i>S. cerevisiae</i>	(18:1) ₄ (31)	[61]	III ₂ IV ₁ ^e	III ₂ IV ₂	[19, 122]
<i>N. crassa</i>	(18:2) ₄ (29)	[143]	+	+	[151]
<i>C. elegans</i>	n.d.		+	+	[131]
<i>P. anserina</i>	n.d.		+	+	[152, 153]
<i>P. denitrificans</i>	n.d.		—	I ₁ III ₄ IV ₄	[135]
Plants					
Potato	n.d.		+	+	[154-156]
Spinach green leaves	n.d.		+	+	[157]

Source of mitochondria	Symmetric cardiolipin ^a (% of total)	Refs.	Supercomplexes		Refs.
			I ₁ III ₂ ^b	I ₁ III ₂ IV _N ^c	
<i>A. maculatum</i>	n.d.		+	+	[158]
Arabidopsis	n.d.		+	—	[159]
Bamboo	n.d.		+ (V ₁)	—	[160]
Maize	n.d.		+		[161]

n.d. = not determined

^a Molecular species are presented as the acyl chains (parenthesis) and their corresponding stoichiometries (subscript).

^b I₁III₂ denotes a supercomplex comprising a single copy of complex I and dimeric complex III.

^c I₁III₂IV_N denotes supercomplexes comprising a single copy of complex I, dimeric complex III, and variable (N=1 - 4) copies of complex IV.

^d Roman numeral in parenthesis denotes additional OXPHOS complexes also reported as part of supercomplexes.

^e Alternative mitochondrial supercomplexes, different than I₁III₂ and/or I₁III₂IV_N-type assemblies.