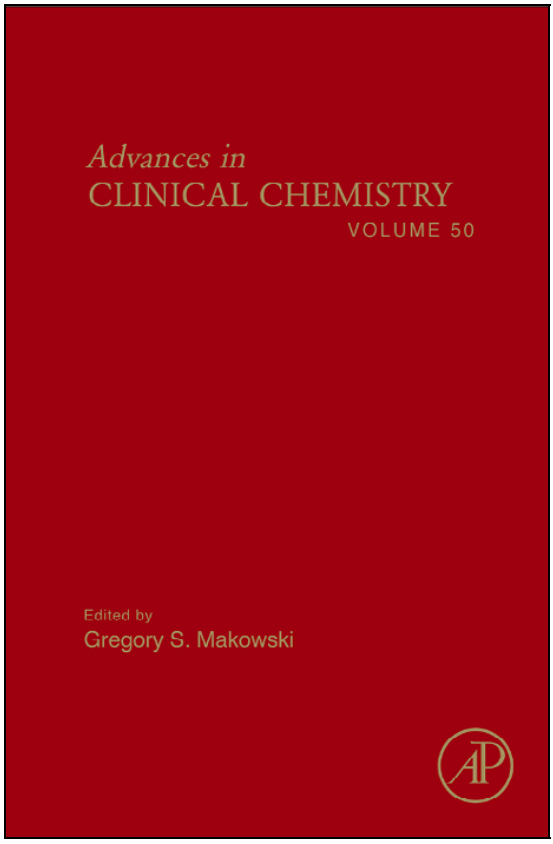

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MITOCHONDRIAL DYSFUNCTION, PROTEOTOXICITY, AND AGING: CAUSES OR EFFECTS, AND THE POSSIBLE IMPACT OF NAD$^+$-CONTROLLED PROTEIN GLYCATION

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1. Abstract

Aging is frequently characterized by the accumulation of altered proteins and dysfunctional mitochondria. This review discusses possible causes of these effects, their interdependence and the impact of energy metabolism on proteostasis, especially formation and elimination of altered proteins. It is suggested NAD^+ to some degree regulates formation of aberrant proteins and generation of oxygen free-radicals and reactive oxygen species (ROS),

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because when NAD^+ is limiting, glycolytic triose phosphates spontaneously decompose into methylglyoxal (MG), a highly deleterious glycating agent and ROS inducer. That NAD^+ has stimulatory effects on stress protein expression and autophagy, while mitochondria regenerate NAD^+ from NADH, further integrates energy metabolism into proteostasis. It is suggested that, as altered proteins can deleteriously interact with mitochondria, changes in synthesis, or elimination, of cytosolic error-proteins will affect mitochondrial activity. It is also suggested that functional mitochondria are essentially antiaging agents, while their dysfunction or inactivity accelerate ROS formation and aging. These proposals may also help explain the oxygen paradox that while ROS may be causal to aging, increased mitochondrial activity (i.e., oxygen utilization) suppresses aging and much associated pathology. Increased synthesis of glutathione, humanin, and mitochondrial chaperone proteins are other additional consequences of increased mitogenesis and which would help ensure proteostasis.

2. Introduction

Evidence from range of model systems suggests that aging is frequently associated with mitochondrial dysfunction [1–8] and almost universally accompanied by the accumulation of altered or abnormal proteins [9–11]. Among the agents that are thought to promote both mitochondrial dysfunction and formation of altered proteins are oxygen free-radicals and related reactive oxygen species (collectively termed ROS). As mitochondria are intimately associated with oxygen, it has frequently been assumed that these organelles are the primary source of the ROS which provoke altered protein formation, mitochondrial dysfunction, and the onset of aging in general. In this short review I will discuss the interrelationship between the origins and proteotoxic actions of altered proteins and age-related mitochondrial dysfunction, especially as a number of recent papers have questioned the assumption that mitochondria generate the ROS which induce protein damage. I will not, however, attempt to provide an in-depth account of age-related ROS generation and mitochondrial dysfunction.

3. Aging and Mitochondrial Dysfunction

Over the past two or three decades much evidence has accumulated showing that aging and cell senescence are frequently accompanied by mitochondrial dysfunction [1–8], and it has furthermore been clearly

demonstrated that specific mutations in mitochondrial genes can cause many pathologies [12–18], some of which accompany aging. These associations have led to the proposal that mitochondria play causal roles in the aging process, and the acceptance of the oxygen free-radical theory of aging has further reinforced this view, especially as oxygen and mitochondria are intimately related. It can be equally well argued, however, that mitochondria normally function as antiaging agents because dietary restriction and aerobic exercise, which suppress or delay aging and/or related pathologies, increase mitochondrial activity and/or mitogenesis [19–26]; it is loss of certain mitochondrial activities that provokes aging onset [3, 7, 8, 27, 28]. These and other observations have therefore led some to question many of the assumptions underlying the proposed causal roles of ROS and mitochondria in aging and associated pathologies [29–35]. Additionally, it should also be noted that aging also occurs in cells which lack any mitochondria, for example, those at the center of the eye lens and erythrocytes.

4. Proteotoxicity and Aging

There is much evidence that aging is strongly associated with accumulation of altered proteins (see Refs. [9–11] and references therein). It has been suggested that these altered proteins exert toxic effects (proteotoxicity) that compromise cell function and play causal roles in some age-related pathologies and aging generally [9, 36–40]. Indeed, the term ''protein-folding diseases'' may be applied to a number of age-related neurodegenerative conditions such as Alzheimer's disease, prion diseases, Parkinson's disease, and Huntington's disease, which frequently result in the generation of aggregates of altered proteins [36–38]. Suppression of the toxicity of these aggregates may be facilitated by the actions of chaperone proteins [40–49] in combination with proteolytic activities of proteasomes [50–59], autophagic lysosomes [60–70], and the endoplasmic reticulum [71–74]; toxicity is enhanced when these mechanisms fail [75, 76].

5. Origin of Altered Proteins

Altered proteins are formed both biosynthetically and postsynthetically [77]. The error frequency during gene expression is not inconsequential; mRNA translation is the most error-prone step. It has been estimated that approximately one codon in about 3000 is mistranslated [78], thereby generating a relatively high proportion of aberrant polypeptide chains (up to one-third of collagen chains may be erroneous due to mRNA mistranslation). In addition,

protein misfolding can significantly add to the proportion error-proteins generated during protein synthesis [79]. Deficiency in mice of a cytoplasmic stress protein recognition protein or CHIP (carboxyl terminus Hsp70-interacting protein), a ubiquitin ligase and cochaperone important for maintenance of polypeptide quality, was found to accelerate anatomical, physiological, and biochemical symptoms of aging, including increased lipid oxidative damage and proteasome dysfunction [80]. These observations, together with many findings involving compromised proteasomal/lysosomal activities [54–80], suggest that failure in protein quality control (proteostasis) may be causal to aging because of the accumulation of altered polypeptides, that is, proteotoxicity.

One early explanation of aging was the so-called error-catastrophe theory which posited that an increase in translational errors may cause aging [81], due to the eventual feedback of errors into DNA replicative enzymes, thereby increasing DNA replication errors and altered (mutant) protein formation. Although the major prediction of this theory, an age-related increase in translational error frequency, has never been realized, it should be pointed out that the theory has not been tested with respect to proteins synthesized by mitochondrial ribosomes [82]. However, it has been shown that decreasing the error frequency of mitochondrial ribosomal protein synthesis increased yeast cell longevity [83], while increasing the error frequency of cytoplasmic ribosomes in cultured human fibroblasts by paromomycin provoked the onset of cell senescence [84]. Interestingly, the phenomenon of ototoxicity of certain antibiotics has been ascribed to their miscoding effects on mitochondrial ribosomes within cells of the auditory system [13]. Whether a spontaneous increase in mitochondrial ribosomal mistranslation frequency could contribute to age-related deafness is completely speculative however.

Failure to control of protein quality within mitochondria may contribute to aging [85]. The mitochondrial enzyme aconitase is particularly prone to oxidation during aging [86], while the Lon protease may facilitate the degradation of altered mitochondrial polypeptides including aconitase [87, 88]. Furthermore, whereas mildly oxidized aconitase is very readily degraded by Lon, severely oxidized aconitase aggregates and thereby becomes relatively resistant to proteolysis by the mitochondrial protease. It was also concluded that the proteolytic activity of Lon may become compromised with age, despite increased expression of the protein in aged rat heart [89].

A recent study has reinforced the idea that maintaining the efficacy of intramitochondrial protein homeostasis may be important in aging. Luce and Osiewacz [90] found that overexpression of the mitochondrial protease Lon in the mitochondria of *Podospora anserina* extended life span and increased stress-resistance of the organism. It was further suggested that the increased Lon activity was also responsible for the decreased level of endogenous oxidative stress, presumably by ensuring the rapid elimination of

aberrant polypeptides. Indeed, Ngo and Davies [91] have recently shown that of Lon is a human stress protein as its synthesis responds to low levels stress conditions. Another mitochondrial protease, ClpP, is also thought to be involved in mitochondrial protein quality control [92]. Additionally, accumulation of unfolded proteins within mitochondria has been shown to activate the mitochondrial unfolded protein response, an effect which appears to be controlled separately from the stress-induced upregulation of ClpP and Lon proteases [93]. Conversely, it has been shown that decreased levels of mitochondrial proteases such as Lon, ClpP, and paraplegin can compromise organelle function and organism health span [93–98], while in rat brain Lon has been shown to be inactivated by peroxynitrite prior to effects on electron transport activity [99]. Other mitochondrial proteases include i-AAA- and m-AAA-proteases, ATP-dependent enzymes that reside in the intermembrane space and the matrix, respectively. These multimeric proteases are thought to contribute to the maintenance of protein quality but also seem to possess important autocatalytic protein-processing functions [100], deficiency of which may contribute to neurological disorders.

6. Altered Proteins and ROS

The conventional view of the free-radical theory of aging seems to assume that increased ROS generation modifies intracellular proteins to such an extent that the chaperone and proteolytic activities are unable to cope with the increased load of oxidatively damaged polypeptides. While there is much evidence that ROS do damage proteins [101, 102], it is also known that some altered proteins can induce ROS generation. For example, it has long been known that the amyloid peptide which is associated with Alzheimer's disease can induce ROS formation [103], while fragments of other proteins, for example, prion protein [104] and the DNA binding protein TDP-43 [105], also induce ROS formation, perhaps to ensure their proteolytic elimination. Indeed, it has been shown that ROS are necessary for protein degradation to proceed in muscle myotubes [106]. Furthermore, extracellular collagen fragments provoke oxidative stress, including increased oxidation of intracellular proteins, in cultured fibroblasts [107]. Similarly, ROS are generated following the reaction of protein-AGEs with their receptors (RAGEs). This occurs via stimulation of cytoplasmic NADPH oxidase which generates superoxide anions [108–112] and/or via effects within mitochondria [112]. It is thought that the free-radicals activate protein kinase C-β (PKC-β) to phosphorylate $p66^{shc}$ protein which permits the latter to enter mitochondria where it oxidizes cytochrome *c*, promotes formation of hydrogen peroxide and opening of the mitochondrial permeability transition pore (PTP), thereby disrupting proper mitochondrial

function [113, 114]. It is noteworthy that the β-amyloid peptide (which is associated with Alzheimer's disease) also reacts with AGE receptors (RAGEs) and induces ROS formation [115]. In addition, however, there is evidence that the β-amyloid peptide can bind directly to mitochondria and promote an increase in ROS formation [116, 117].

Changes in cytoplasmic protein biosynthetic and proteolytic activities can impact upon mitochondrial function. For example, proteasomal dysfunction (decreased proteolysis of altered proteins) can provoke ROS generation [118], although the precise mechanistic route remains obscure: either the inhibited proteasomes are directly responsible for the increase in ROS generation, or the presence of the altered proteins provokes ROS formation (discussed above) [103–107] and induce mitochondrial dysfunction [116, 117]. Other studies have revealed that impaired synthesis of the 20S proteasome can lead to increased mitochondrial DNA mutation and increased ROS levels [119].

Interestingly, it has also been found that reduced cytoplasmic protein synthesis suppresses age-related mitochondrial dysfunction [120], and that decreased protein synthesis has been shown to increase longevity in yeast [121] and *Caenorhabditis elegans* [122–124], while methionine restriction (but not other amino acids) suppressed mitochondrial oxidative dysfunction in rats and mice [125]. It is possible that all these examples decrease the generation of error-proteins simply as a result of the lowered overall bulk protein synthesis per cell, thereby decreasing the error-protein load that the chaperones and proteolytic apparatus must deal with [77, 126, 127]. Additionally and/or alternatively, decreased protein synthesis may induce beneficial alterations in gene expression [128] as it has also been demonstrated that a yeast longevity gene is upregulated when error-protein synthesis is increased [129], possibly reflecting error-protein-induced expression of stress-proteins (proteases and chaperones).

7. Aging and Autophagy of Mitochondria

Evidence is accumulating to support the idea that aging is associated with compromised autophagic elimination of dysfunctional mitochondria [60–65, 130, 131]. It is possible therefore that the age-related accumulation of dysfunctional mitochondria could simply be a consequence of age-related autophagic insufficiency; the problem then becomes what causes the age-related autophagic dysfunction [132–134]. Alternatively, excessive generation of dysfunctional mitochondria may overload the autophagic system, in which case we have to consider possible origins of mitochondrial damage and/or changes to the ameliorative homeostatic processes which fail to completely prevent/repair the damage (see below).

Autophagy is the activity by which proteins and organelles are delivered to lysosomes for destruction. There are three types of autophagy: microautophagy, macroautophagy, and chaperone-mediated autophagy (CMA). Microautophagy involves the direct entry of a protein into the lysosome via invagination of the lysosomal membrane. It is not necessary here to discuss microautophagy as it does not seem to participate in the mitochondrial turnover. CMA has been clearly shown to decrease with age [86, 87]. CMA describes the selective degradation of specific cytosolic proteins containing the amino acid sequence or signal motif KFERQ which is recognized by a specific chaperone protein (Hsc70) which translocates the target protein to the lysosome by interaction with the Lamp2a (lysosome-associated membrane protein 2a) receptor. Lamp2a abundance has been shown to decrease with age in rodent liver, possibly due to its intrinsic instability [66]. Additionally, Hsc70 has been found to be a target for glycation in senescent human fibroblasts [135], a modification which could also compromise the protein's role in autophagy. It is uncertain whether CMA contributes directly to mitochondrial turnover. However, it is possible that improved CMA, as well as upregulation of macroautophagic elimination of altered proteins, might assist dysfunctional mitochondria autophagy.

Macroautophagy involves the formation of autophagosomes containing large portions of cytoplasm or whole organelles bounded by a double membrane which then fuse with lysosomes. This process, sometimes simply termed autophagy, involves nonselective intake of cytoplasmic material for degradation into reusable building blocks. Macroautophagy responds to metabolic cues such as starvation, controlled by the target of rapamycin (TOR) regulatory complex. TOR activity is inhibited by rapamycin. The increased incidence of general protein breakdown may help to explain the beneficial effects of dietary restriction and rapamycin on longevity by suppressing the accumulation of altered proteins and dysfunctional organelles. It is observed, however, that macroautophagy activity declines with age, possibly due to the accumulation of undegradable lipofuscin (age pigment) within the autophagosomes. Lipofuscin is formed spontaneously when partially degraded material (peptides or lipid) becomes cross-linked either by the action of deleterious aldehydes or redox metal ions such as iron: mitochondria are a rich source of iron. It appears that should cross-linking occur within the autophagosomes, the resultant product (lipofuscin) cannot be catabolized further and accumulates; such accumulations are detected especially in long-lived nonmitotic cells (neurones, cardiac myocytes, and retinal pigment epithelial cells). It is thought that the slow accretion of lipofuscin eventually compromises autophagic function. It is noteworthy that long-lived mutant nematodes accumulate lipofuscin at a slower rate than the shorter lived wild-type animals, and that when life span is extended by caloric

restriction, lipofuscin accumulation is further suppressed [133, 134]. Upregulation of autophagy has been found to extend life span in yeast [70], the nematode *C. elegans* [62, 65], and *Drosophila* [63]; observations consistent with the proposal that compromised autophagy plays an important role in the aging process [60, 61, 64]. Furthermore, autophagy may be neuroprotective by removing proteotoxic altered proteins associated with neurodegenerative conditions, while defects in certain autophagic gate proteins (Atgs) limit clearance of inclusion bodies in neurones [136, 137]. However, overexpression of Atg5 can stimulate autophagy but induce cell death [138], indicating that upregulation of autophagy can also contribute to apoptosis [138]. It is likely that both excessive and insufficient autophagy contribute age-related cellular dysfunction resulting in either cell death or cell senescence, respectively. Interestingly, it has been reported that aging in progeroid mice is accompanied by increased autophagic activity [139]. This unexpected finding suggests that regulation of autophagy must be quite finely controlled, and that its overactivity can compromise organism longevity. Indeed, the claim that basal autophagy decreases with age has been challenged [140, 141].

A major factor in the selective autophagic elimination of dysfunctional mitochondria, termed mitophagy, is alteration to the mitochondrial membrane transition pore (MTP) [131]. Opening of the pore causes the mitochondria to be permeable to molecules of molecular mass under 1500 Da and thereby promotes apoptosis. It is interesting that a common metabolic aldehyde methylglyoxal (MG), also termed a glycotoxin and thought to be largely responsible for diabetic complications [142], can react with (i.e., damage) arginine residues present in the MTP and induce mitochondrial swelling [143]. Other studies have shown that MG can damage mitochondria [144–146], induce ROS formation [147], and promote cell death [148] and aging [149, 150].

The mechanism(s) by which damaged mitochondria are selected for destruction are uncertain, but may well involve both ubiquitination and autophagy. The process of protein ubiquitination is one mechanism by which polypeptides are marked for selective proteolysis by either the proteasomes or lysosomes. It has been known for decades that selective elimination of mitochondria in fertilized ova [151], maturing reticulocytes [152], and yeast [153] requires organelle ubiquitination. Recent studies have confirmed that ubiquitin has a role in ensuring mitochondrial homeostasis, most probably by targeting dysfunctional organelles for selective autophagy [154–156], especially in neurodegenerative conditions where altered proteins accumulate [156–159]. Furthermore, mitochondria accumulate polyubiquitinated proteins upon impairment of the proteasome system [160, 161], a condition which promotes altered protein accumulation and inclusion body formation in mouse brain. Intriguingly, it has been found that decreasing bulk cytosolic

protein synthesis using cycloheximide can suppress mitochondrial degeneration in a yeast model of progressive external ophthalmoplegia [162], an adult-onset degenerative disease caused by a mutation which results in a reduced mitochondrial membrane potential. One interpretation is that biosynthetic error-proteins deleteriously interact with mitochondria, but the cycloheximide treatment reduces generation of biosynthetic error-proteins simply as a consequence of the decreased protein synthesis. Consistent with this proposal are the findings that a number of abnormal proteins and protein fragments such as β-amyloid, parkin, and huntingtin [156–159], as well as ubiquitinated cytosolic and presumably abnormal proteins [160], can bind to mitochondria to compromise function. Interestingly, β-amyloid toxicity has been shown to be attenuated by a mitochondrial transcription factor (Tfam) [163], possibly due to the upregulation of chaperone proteins which may interact with β-amyloid. Overall, these observations indicate interaction or cross talk between the cytosolic and mitochondrial compartments and suggest that various proteotoxic altered proteins bind to mitochondria and compromise their function. Indeed, Torres and Perez have suggested that proteasome failure leads to mitochondrial dysfunction and ROS production [118], possibly due to the presence of altered proteins, while dietary restriction-induced life span extension in *C. elegans* requires a functional ubiquitination system [164], presumably ensuring effective elimination of altered proteins.

8. Elimination of Toxic Proteins by Asymmetrical Cell Division

It is a truism that being young seems to suppress many symptoms of aging including the accumulation of altered (potentially toxic) polypeptides. The discussion so far has concentrated on those processes that facilitate proteolytic destruction (mostly proteasomal or lysosomal) of aberrant polypeptide chains. However, cross-linked proteins seem to resist proteolytic attack, possibly due to their relative insolubility. So it is necessary to address the problem of how being young seems to prevent the apparent intracellular accumulation of insoluble cross-linked proteins, assuming this form of aberrant polypeptide is generated. It appears that yeast cells possess a seemingly altruistic mechanism which involves an unequal or asymmetrical distribution of highly abnormal proteins during cell division [165, 166]. The result of this process being that the daughter cell, when released from the mother cell, is young, containing little or no altered polypeptides (measured as protein carbonyls, these being retained in the mother cell). Interestingly, this process is controlled by a sirtuin (Sir2); sirtuins also control life span extension in a number of organisms (discussed below). Similarly, in starving *Escherichia coli*, cytokinesis during cell division is asymmetric producing progeny, most

die while retaining the protein carbonyls, whereas the remaining cells remain viable. It is unsure whether this discriminatory facility has been evolutionarily retained in multicellular organisms; conceivably, during growth large amounts of aberrant polypeptide of any origin could be sequestered to cells that then undergo apoptosis, keeping the remainder biochemically young. This could help to suppress aging and accumulation of altered proteins in the young. Evidence showing differential sequestration of aggregated proteins has recently been obtained in mammalian cells [167, 168]. It is possible that apoptosis following leakage of cytochrome *c* from grossly damaged mitochondria into the cytoplasm could be analogous to the altruistic phenomenon observed in single-celled organisms.

9. Mitochondria, Metabolism and Life span extension

For many years the only reproducible method of life span extension was caloric restriction in which the calorie intake was decreased by around 30% or more. The effects of this were to increase life span by up to 40% in rodents and delay the onset of age-related pathology. Subsequently, it was found that every-other-day feeding protocols, while not decreasing overall calorie intake, induced similar effects [169–171]. These findings indicate that the antiaging effects might be a consequence of the occurrence of periods of fasting rather than decreased caloric intake [172–174].

Other studies revealed that among these dietary-induced consequences was the upregulation of mitochondrial activity and mitogenesis [19, 20] together with enhanced autophagy [55–66], changes which involved the activity of the so-called silent information regulators or sirtuins [175–177], in cooperation with a control center called the mammalian target of rapamycin (mTOR) [178, 179]. In fact, it has recently been shown that treatment of model aging systems with rapamycin, which inhibits the mTOR regulatory complex, mimics the effects of dietary restriction and delays aging onset [180, 181], by suppressing glycolysis and enhancing mitochondrial ATP generation and autophagy [182].

As discussed above, caloric restriction and every-other-day feeding induce periods of fasting in the animals, during which glycolysis will be suppressed to a major degree due to the lowered serum glucose levels. One consequence will be a decrease in formation of MG, which is generated by the spontaneous decomposition of triose-phosphate glycolytic intermediates (glyceraldehyde-3-phosphate and dihydroxyacetone-phosphate) [171, 173]. As mentioned above, MG is highly deleterious, hence decreasing its formation will lower the incidence of MG-induced protein modification, cross-linking, AGE formation, oxidative stress, and mitochondrial inactivation. It is also suggested

that the accompanying increase in mitochondrial activity and mitogenesis, induced by dietary restriction, will further improve cellular ability to oxidize NADH to NAD^+ [183]. This will, in turn, help ensure triose-phosphates conversion to phosphoglyceric acid and thereby decrease MG generation and consequent proteotoxicity [144–146]. The possibility that changes in MG generation are important in the dietary-induced life span modulation [173] is supported by the finding that life span was increased in *C. elegans* when elimination of MG was enhanced by increased glyoxalase-1 expression; glyoxalase-1 knockdown reduced life span however [184]. Subsequent studies showed that high glucose toxicity, ROS and AGE generation, mitochondrial modification, and effects on *C. elegans* life span are mediated via MG synthesis [185]. Furthermore, it has been demonstrated that oral glycotoxins (i.e., MG-modified proteins) can counteract the beneficial effects of dietary restriction on mouse life span [186]. These studies suggest that NAD^+ availability, by limiting glyceraldehyde dehydrogenase-mediated metabolism of MG precursors, can strongly influence proteotoxicity [173, 183]. It should also be noted that the activity of another enzyme, 2-oxoaldehyde dehydrogenase, which also participates in MG detoxification, is regulated by NAD^+ [187].

Not only may NAD^+ help to prevent synthesis of some altered (glycated) proteins, but NAD^+ availability may also help facilitate the selective elimination of altered proteins too [183]: NAD^+-dependent Sirt1 has been shown to promote expression of stress proteins [188], which should improve aberrant protein recognition, and also enhance autophagy [189–191].

It is interesting that insulin and insulin-like growth factors (I/IGF-1) seem to have ambivalent effects on proteotoxicity and aging [192]. Again a consideration of metabolism on NAD^+ availability may help provide an explanation. I/IGF-1 are normally anabolic effectors. Consequently, their actions include not only an upregulation of protein and nucleic acid synthesis but also the provision of macromolecular precursors. These effects can have opposite outcomes with respect to proteotoxicity. While insulin and IGF-1 can inhibit autophagy, the increase in protein biosynthesis will nevertheless include increased expression of the necessary chaperone proteins and proteolytic activities sufficient to ensure continued protein quality control, an effect which may help suppress accumulation of altered proteins. In contrast, the increased glycolysis, providing amino acid and nucleotide precursors, may also increase the potential for MG-mediated protein damage, especially should the pentose phosphate pathway be suppressed if synthesis of nucleic acids is not required. This will result in increased flux through the triose phosphates, which if not immediately converted to phosphoglyceric acid by the NAD^+-dependent enzyme glyceraldehyde-3-phosphate dehydrogenase, may spontaneously decompose into MG and increase protein glycation. The constitutive chaperone proteins, Hsc70 and Hsc90, are carrier proteins

required for the input of cytoplasmically synthesized proteins into mitochondria [193]. As previously mentioned, Hsc70 is a major target for intracellular glycation [135], and consequently any increase in MG levels promoted by I/IGF-1 actions could inhibit mitogenesis and result in dysfunctional mitochondria. As Hsc70 is also involved in macroautophagy, as well as proteasome-mediated elimination proteolysis [40, 66], its glycation could theoretically promote altered protein accumulation generally. Furthermore, as glycated proteins may also promote autophagic inefficiency, this may increase the retention of dysfunctional mitochondria, inducing either apoptosis or senescence [194]. Oxidation of mitochondrial Hsp70 (mortalin) has also been suggested to be a possible cause of mitochondrial dysfunction [195].

There is an additional route by which mitochondrial dysfunction might be induced by metabolic changes. It is known that variation in the relative concentrations of nucleotide triphosphates can influence mitochondrial mutations and deletions, most probably resulting from replication errors [196, 197]. Metabolically induced variation in synthesis of the four nucleotide triphosphates may therefore help explain tissue-specific differences in mtDNA mutation and mitochondrial dysfunction [198].

Excessive glycolysis may also be deleterious to neuronal viability. A recent study has revealed that control of a key glycolytic enzyme, 6-phosphofructo-2-kinase, is important for neuronal survival. Herrero-Mendez *et al.* [191] showed that ubiquitination and degradation of this enzyme is necessary to maintain the balance between the metabolism of fructose-6-phosphate down the glycolytic pathway and the pentose phosphate pathway in neurons. It was found that overexpression of 6-phosphofructo-2-kinase decreased metabolism via the pentose phosphate pathway, but increased neuronal ROS levels, promoting neuronal death. One possible interpretation of this observation is that not only would glutathione synthesis be decreased, but also the generation of the triose phosphates could be increased which then would increase MG formation, which as described above, can promote ROS formation, induce mitochondrial dysfunction, and promote cell death. Because the level of 6-phosphofructo-2-kinase is controlled by its ubiquitination and proteolysis, this proposal may also explain why compromised proteasome activity increases ROS generation, as observed by Torres and Perez [118] and discussed above.

10. Uncoupling, the Oxygen Paradox and Longevity

Uncoupling of ATP synthesis from NADH oxidation to NAD^+ is important for suppression of mitochondrial ROS formation and consequent dysfunction [22, 23, 199]. One consequence of uncoupling is to increase regeneration of NAD^+ from NADH and, as the NAD^+ is essential for

triose-phosphate metabolism, this will decrease MG formation and consequent proteotoxicity as discussed above.

It is generally observed that increased aerobic exercise is frequently beneficial toward much age-related pathology, and also induces increased mitogenesis and mitochondrial activity. If oxygen plays a causal role in aging, the association between raised mitochondrial oxygen usage and an increase in life span and delayed onset of age-related pathology is paradoxical. Below is an attempt to resolve this apparent paradox.

Both caloric restriction and every-other-day feeding induce periods of fasting in which glycolysis is likely to be suppressed and mitochondrial ATP generation stimulated (via fatty acid oxidation from fat reserves). Thus, it can be argued that beneficial effect of these regimes is to suppress glycolysis, induced by fasting or rapamycin which also delays aging. One possible consequence of this is to decrease MG formation from the glycolytic intermediates, glyceraldehyde-3-phosphate and dihydroxyacetone-phosphate. In hyperglycemic conditions, large amounts of MG are generated which result in increased ROS formation, protein glycation, and AGEs. As noted above, overexpression of the MG-detoxifying enzyme glyoxalase-1 increases life span in *C. elegans* [184, 185], while the beneficial effects of dietary restriction on rodent life span are countered by glycated proteins [163]. These observations support the idea that decreased MG generation may influence the onset of aging and related disorders [172, 173].

Increased mitochondrial activity, induced by aerobic exercise, fasting, or rapamycin, can directly affect MG formation by facilitating triose-phosphate conversion to 3-phospho-glyceric acid. The enzyme which catalyzes this step (glyceraldehyde-3-phosphate dehydrogenase) requires the coenzyme NAD^+ which is converted to NADH, which in turn can be reoxidized to NAD^+ by the mitochondria. Consequently, as there is not an unlimited cellular supply of NAD^+, suppression of mitochondrial metabolism by increased glycolysis may inhibit NADH oxidation. Whereas any process, such as aerobic exercise, which increases ATP demand which is satisfied by increased mitochondrial ATP generation, will also increase NADH conversion to NAD^+ and thereby facilitate triose-phosphate metabolism and thus lower the potential for MG formation. Consistent with this proposal is the observation that mitochondrial uncoupling (electron transport without ATP synthesis) also delays aging, etc., possibly by increasing NADH oxidation to NAD^+ [23, 200]. Thus, generation of proteotoxic proteins will be suppressed by improving NAD^+ availability [183].

Increased mitogenesis may also improve cellular ability to recognize and eliminate altered proteins. Synthesis of new mitochondria, stimulated by increased aerobic exercise or by dietary means, will increase protein biosynthesis both in the cytosol and within mitochondria. To ensure protein

quality, increased production of the necessary mitochondrial and chaperone proteins and proteases will also be required. This will improve overall cellular ability to recognize and eliminate altered proteins of any origin (biosynthetic error-proteins or those damaged postsynthetically) and thereby improve cellular stress resistance and delay aging onset [77, 126, 127].

Increased mitogenesis will also increase the demand for synthesis of DNA and RNA precursors via the pentose phosphate pathway from glucose-6-phosphate, the first step of which generates NADPH which is required for glutathione synthesis which will then improve antioxidant function. MG formation will also be decreased due to the diversion of glucose metabolism away from triose-phosphate formation.

Increased mitogenesis may be additionally beneficial because synthesis of humanin is also increased. This may be beneficial because tissue and sera levels of humanin decrease with age. It is interesting that humanin is transcribed from an open reading frame within mitochondrial 16S ribosomal RNA [201]. Humanin appears to increase tissue insulin sensitivity and exerts beneficial effects with respect to Alzheimer's disease and type-2 diabetes. Among other effects mediated by this 24 amino acid-residue molecule are suppression of apoptosis and decreased sera glucose levels due to increased glucose uptake by insulin-dependent tissues. Consequently, mitogenesis, which requires mitochondrial ribosome synthesis, could provide an opportunity for humanin synthesis from the increased synthesis of ribosomal RNA; this proposal provides another possible explanation of the beneficial effects of mitogenesis, whether induced by dietary means or by increased aerobic exercise.

It is therefore suggested that the apparent paradox of increased oxygen usage delaying aging might be explained by decreased MG formation, improved protease/chaperone synthesis, and increased humanin and glutathione synthesis.

11. Antiaging Effects of Dysfunctional Mitochondria

This title may seem contradictory to the theme of this chapter—dysfunctional mitochondria promote aging and senescence—but research has shown [32, 202] that life span is *increased* in mice possessing a heterozygous mutation ($Mclk1^{+/-}$) coding for a mitochondrial protein. The effects of this mutation are increased mitochondrial dysfunction, as evidenced by decreased ATP synthesis rate, increased hydrogen peroxide generation, and raised level of organelle protein carbonyls. These findings clearly question conventional views about the roles of ROS and mitochondrial dysfunction in organism aging. Analogously, life span is also extended in clk-1 mutants

of *C. elegans* [31, 203, 204]. Similarly, life span extension has been achieved in *C. elegans* by treating them with interfering RNAs (RNAi) corresponding to five genes encoding mitochondrial protein components of the electron transport chain; although high RNAi doses provoked a decreased life span [205].

Given that mitochondria undergo both fission and fusion to varying degrees [206–208], it is likely that cells obtained from mice heterozygous for the Mclk1 mutation contain mixtures of mitochondria with varying levels of normal and defective Mclk gene products; embryos homozygous for this mutation do not survive. It is therefore suggested that the presence of a heterogeneous mixture of mitochondria—some homogeneously normal, while others containing varying amounts of the normal and mutant gene products—exhibit varying degrees of dysfunction including increased ROS generation. It is likely that some mitochondria will be so dysfunctional that their autophagic destruction will be induced [60–63, 95, 131]. This increased mitochondrial destruction via the autophagic apparatus (mitophagy) may in turn stimulate an increase in mitogenesis to replace the dysfunctional organelles. Thus, the mitogenic response to the elimination of the defective mitochondria involves the upregulation of synthesis of mitochondrial proteins, which, as discussed above, will also include raising the levels of chaperone proteins and proteases to ensure maintenance of protein biosynthetic quality. However, because the cells are genetically heterozygous for the defective gene, any resultant newly synthesized mitochondria will likely be a mixture of the normal and aberrant, and so the cycle of mitophagy and mitogenesis will be repeated. Consequently, it can be expected that, on average, the cells of the Mclk1$^{+/-}$ mutant mice will contain a higher proportion of young normal mitochondria compared to wild-type cells. Additionally, the cytosol of mutant cells will, due to the upregulation of protein synthesis, etc., also contain increased levels of chaperone proteins and proteases which not only participate in the recognition and elimination of erroneously synthesized and misfolded proteins but also polypeptides modified postsynthetically by ROS, etc.. Furthermore, the stimulation of autophagic activities, chaperones and proteases, which may enhance the elimination of altered proteins (including protein carbonyls) of any origin, may be considered as a form of endogenous perpetual hormesis. This suggestion also explains the lower levels of protein carbonyls observed in the cytosol of the heterozygous cells, despite the increased generation of mitochondrial ROS and raised mitochondrial protein carbonyls [32]. Additionally, continuing reliance on normal/younger mitochondria for ATP synthesis, rather than glycolysis, will decrease the potential for MG generation [173, 185]. A further effect of continuous mitogenesis could be the increased synthesis of murine humanin from 16S ribosomal RNA [201] with consequential beneficial activity toward insulin sensitivity of insulin-dependent

tissues, accompanied by decreased blood glucose levels which would lower the potential for MG production in tissues that are freely permeable to the sugar (described above).

12. Mitochondrial Inactivity and ROS Generation

It has been apparently assumed by many gerontologists that increased oxygen utilization by mitochondria will automatically increase ROS formation. This assumption is reported to be incorrect [22–25, 209, 210]. Even from first principles one would predict that an incompletely reduced oxygen molecule (i.e., an oxygen free-radical) would be more likely to be produced when few electrons are passed down the electron transport chain (i.e., in resting mitochondria), compared to when there is a ready supply of electrons to the organelle (i.e., during active respiration). And this is what is observed [209, 210].

Mitochondrial ROS generation is inversely related to their respiration state; highly active mitochondria produce almost no ROS, whereas resting-state mitochondria produce much larger amounts of ROS despite lower oxygen consumption [22, 33]. These findings allow one to propose that inactive mitochondria are dangerous. Indeed, it has been shown that muscle immobilization is accompanied by increased ROS generation [211–214]. Furthermore, oxygen delivery to tissues is controlled in part by carbon dioxide levels (the Bohr effect) which decreases delivery of oxygen to inactive mitochondria and thereby help to suppress ROS formation in tissues whose mitochondria are not synthesizing ATP. It may also be significant that in highly trained aerobic muscle, mitochondrial removal is initiated quite rapidly (within 2 days) when aerobic training ceases [24, 25]. This could indicate that the excess resting mitochondria in the tissue are potentially deleterious, possibly due to their increased rate of ROS generation. Increased ROS generated by the inactive mitochondria could act as a trigger for selection of the organelle for mitophagy [130].

13. Conclusion

Aging is often associated with both mitochondrial dysfunction and accumulation of aberrant proteins, although it remains uncertain as to which, if either, is the initiating event. For example, it has recently been suggested that activation of mitochondrial biogenesis may delay aging [215] and furthermore that brain mitochondria should be targeted in treatment of Alzheimer's disease, as amyloid β-peptide-induced damage to these organelles is a

particularly early event in a mouse model of Alzheimer's disease [216]. It is also interesting to note that it has recently been shown that suppression of protein synthesis, by rapamycin-induced activation of the translation inhibitor 4E-BP, ameliorated mitochondrial defects in proteolytic-deficient cells obtained from individuals with Parkinson's disease [217]. This observation is again consistent with the idea that decreased generation of error proteins contributes to maintenance of mitochondrial function and integrity [162]. There is much evidence suggesting that ROS can damage mitochondria and proteins, but it can also be demonstrated that aberrant proteins and dysfunctional mitochondria can induce ROS generation. Indeed, it is possible that altered proteins deleteriously interact with mitochondria and induce ROS generation. Consequently, it is difficult to suggest with any certainty the precise causal relationship between these phenomena. Furthermore, regulation of energy metabolism by AMP-activated kinase activity [218] may influence life span, generation of altered protein, and mitochondrial activity, possibly by modulating NAD^+ availability. Indeed, it is becoming clear that NAD^+ may have a pivotal role in aging [219, 220], not only due to its effects on sirtuin activities but also because of its effects on MG formation which can induce mitochondrial dysfunction. The impact of dysregulation of proteostasis (cytosolic and mitochondrial) in relation to aging and related conditions has recently been formalized by Powers *et al.* [221], who also suggest that the capacity of proteostasis network is limited, only providing just sufficient activity for the error-protein and protein-folding load. However, as the proteostasis network is mediated by polypeptide chains which are themselves subject to stochastic insult and deleterious modification (e.g., by MG), it is likely that this may contribute to the proteostasis collapse which appears to be an early molecular aging event, at least in *C. elegans* [222]. Additionally, as decreased proteolytic activity not only affects protein quality but can also influence glycolytic flux [191], it is likely that the potential for MG formation will be raised and thereby increase ROS generation and damage to proteins and mitochondria. These findings illustrate the complex interrelationships between energy provision pathways and protein homeostasis (proteostasis), and their impact on mitochondria and cellular and organismal aging.

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