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Review

Mitochondria in organismal aging and degeneration

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Abstract

Several lines of experimentation support the view that the genetic, biochemical and bioenergetic functions of somatic mitochondria deteriorate during normal aging. Deletion mutations of the mitochondrial genome accumulate exponentially with age in nerve and muscle tissue of humans and multiple other species. In muscle, a tissue that undergoes age-related fiber loss and atrophy in humans, there is an exponential rise in the number of cytochrome-oxidase-deficient fibers, which is first detectable in the fourth decile of age. Most biochemical studies of animal mitochondrial activity indicate a decline in electron transport activity with age, as well as decreased bioenergetic capacity with age, as measured by mitochondrial membrane potential. Mitochondrial mutations may be both the result of mitochondrial oxidative stress, and cells bearing pure populations of pathogenic mitochondrial mutations are sensitized to oxidant stress. Oxidant stress to mitochondria is known to induce the mitochondrial permeability transition, which has recently been implicated in the release of cytochrome *c* and the initiation of apoptosis. Thus several lines of evidence support a contribution of mitochondrial dysfunction to the phenotypic changes associated with aging.

Contents

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1. The multifactorial deficits of function that accumulate with age can be understood in an evolutionary context

The multifactorial decrements of function that occur during human aging support the view that aging is the result of a multiple underlying causes. Aging can be understood in an evolutionary context, in which natural selection strongly favors genetic variation that leads to survival to the age of reproduction [1], whereas positive or negative natural selection for traits that appear long after the age of reproduction is weak or neutral [2–7]. Other support for the neutrality of genetic variation that underlies aging phenotypes comes from the consideration that for most of the history of modern humans (i.e. since about 140 000 years ago [8,9]) the mean human lifespan has been much shorter than it currently is, providing a relatively small pool of elderly individuals on which selection could act (Fig. 1). Since selection is likely to be effectively neutral on genetic polymorphisms that promote or attenuate survival long after the age of reproduction, the human gene pool is likely to contain substantial genetic variation that controls the rate of cellular maintenance [10,11]. Genes that provide for cellular maintenance are likely to include

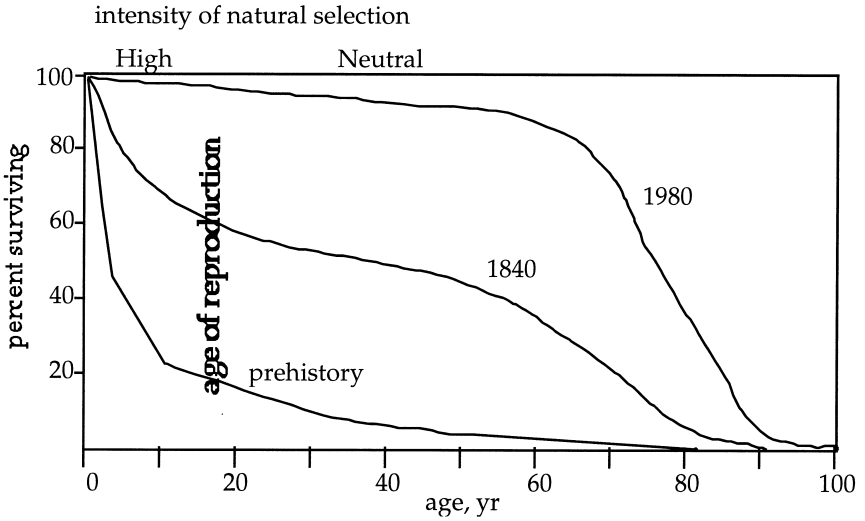

Fig. 1. Ideogram of the historical age structure of the human population and the intensity of natural selection on genotypes which affect aging phenotypes. US population census data from 1980 and 1840 are redrawn from J.F. Fries, L.M. Crapo, Vitality and Aging, W.H. Freeman, San Francisco., 1981. The age structure of pre-agricultural human hunter-gatherers is a matter of debate, but infant mortality (a major influence on mean lifespan) is likely to be at least twice as high as in 1840, and adult mortality is likely to be significantly increased as well.

those which protect molecules from damage, including genes for DNA repair and protection from oxidant stress. DNA repair of the nuclear and mitochondrial genomes, for example, is quite a costly process in terms of metabolic energy spent. Thus one might expect the 'set point' of DNA repair processes to provide that genomes should maintain integrity until the age of reproduction, but that natural selection for maintenance of genomes long after the age of reproduction should be weak. Thus a corollary prediction of the evolutionary theory of aging is that the somatic mutations and other defects resulting from incomplete cellular maintenance should rise with age.

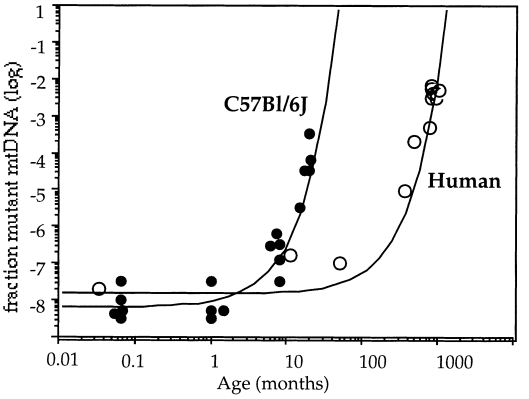

Fig. 2. Exponential accumulation of mitochondrial DNA deletion mutations in mice and humans. Data redrawn from [50].

2. Nuclear somatic mutations rise in human tissues with age

There is substantial evidence that the frequency of nuclear somatic mutations rises with age in non-oncogenes using phenotypic selection assays of mutagenesis [12]. Using a PCR-based method of genotypic selection, it has been demonstrated that somatic mutations also rise in known oncogenes both with age and with exposure to carcinogens [13–15]. In animal models, a two-fold rise in nuclear somatic mutations with age in somatic mutations with age has been reported [16].

3. The contribution of mitochondrial deficits to aging phenotypes has been suggested

A contribution of mitochondrial defects to age-related decline in cellular function of humans and other animals has been posited [17–25]. Support for a decline in mitochondrial function with age has come genetic assays of mitochondrial mutagenesis, and biochemical assays of mitochondrial function.

3.1. Age-related rise in mtDNA deletion mutagenesis

The mitochondrial genomes of humans [21,26–30], monkeys [31], rats [32,33], mice [34–36], and nematodes [37] accumulate deletion and/or rearrangement mutations with age. Some of the deletion mutations that accumulate in humans with age are identical with those that occur in human mitochondrial genetic disease (Fig. 2). In humans, mtDNA mutations accumulate preferentially in postmitotic tissues that are oxidatively active [26]; and aged brains cells of the substantia nigra accumulate the highest levels of mitochondrial mutation [38]. It is possible that the higher level of deletion mutagenesis in these tissues is the result of increased mitochondrial oxidative stress; however, this has never been directly demonstrated. These deletion mutations appear to be identical to those observed in the mitochondrial disease Kearns–Sayre's syndrome [39–42], and appear to rise exponentially with age [21,26,43–46].

4. Absolute frequency and distribution of age-related mtDNA mutations

The highest mean concentration of any particular deletion mutation, such as the 4977 deletion may be maximally about 1% in any particular tissue homogenate, and if the 4977 deletion were evenly distributed and the only mtDNA deletion to accumulate with age it is not clear if this would have a major physiological effect. However, it appears quite unlikely that mtDNA mutations are evenly distributed; data are more consistent with an uneven distribution of mtDNA deletion mutations [47–49], as one might expect from a somatic mutational process. Further support for uneven distribution of age-related mtDNA mutagenesis comes from a comparison of apparent mtDNA mutation frequencies in DNA prepared from cells vs. tissue homogenates using a

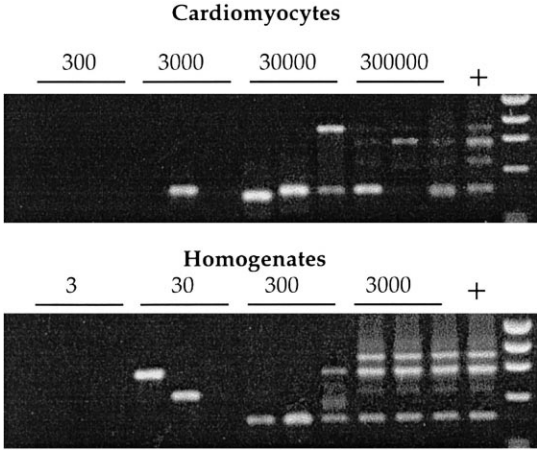

Fig. 3. Differential apparent concentration of mtDNA deletions in homogenates vs. cardiocytes is consistent with clustering of mtDNA mutations. E. Wang and G.A. Cortopassi, unpublished data. Upper panel: PCR of DNA extracted from cardiocytes and amplified by method described in [50]. Lower panel: PCR of DNA extracted from heart homogenates. The estimated number of cells is indicated on the top of each gel. +, positive control; M, size marker.

quantitative assay [50] (see Fig. 3), in which mutations were assayed from DNA prepared in two ways from the same aged mouse hearts. From one heart half DNA was prepared from homogenized tissue by the standard phenol/chloroform extraction method. From its sister half, cardiocyte cells were first disaggregated into pools of differing sizes, pools of 300–300 000 cardiocytes. The apparent deletion mutagenesis frequency is approximately 100-fold higher in the identical amount of DNA made from homogenates compared with cardiocytes, supporting the notion that mtDNA deletion mutations occur in groups or clusters.

5. Point mutations of mtDNA increase with age

Other deletion and/or rearrangement mutations in addition to the 4977 deletion mutation have been reported to rise with age (reviewed in [51]). Also, a new method for total mtDNA amplification has been developed that illustrates the rise of several mtDNA deletions with age [24,25]; however, the assay is not quantitative and thus could overestimate or underestimate the total fraction of mutant mtDNA genomes. Point mutations identical to pathogenic mtDNA mutations have been reported to rise with age [52–56], however, using a different mutation assay for a different point mutation; this increase was not observed [57].

6. Biochemical analyses of mitochondrial function with aging

Three major types of analysis of the contribution of mitochondrial biochemical defects with age have been carried out, including single enzyme analysis in aging muscle fibers, analysis of the complete electron transport chain or individual complexes, and analysis of membrane potential with age.

A rise in cytochrome-oxidase-negative muscle fibers in humans over the age of 40 years has been reported by multiple groups, [58–61], the data of Byrne and Dennett are redrawn below (Fig. 4). These data support an exponential rise in cytochrome-oxidase-negative fibers, which are not observable in individuals under age 40 years, and which rise sharply after age 40. Although it is known that muscle atrophy and dropout of muscle fibers does occur with age, it is not clear if this is the result of loss of cytochrome oxidase activity.

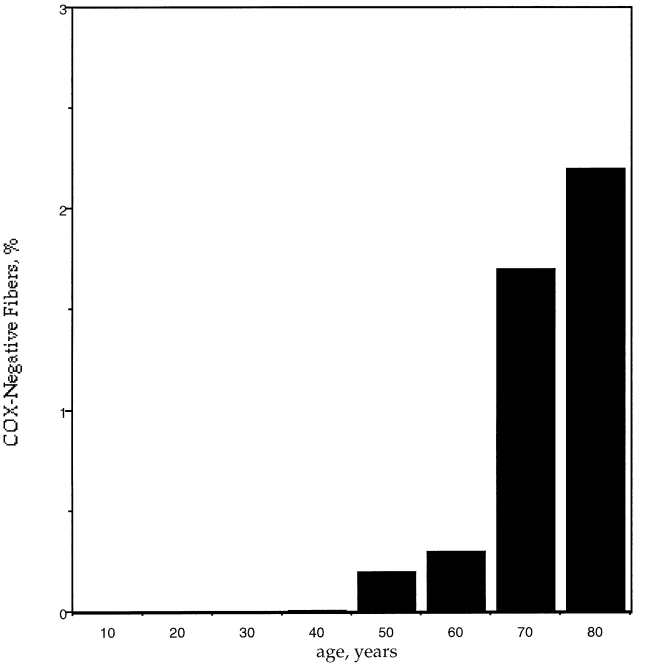

Fig. 4. The incidence of cytochrome *c* oxidase-negative muscle fibers increases exponentially after the fourth decile of age. Data redrawn from Byrne et al. [20,58].

The second type of analysis of mitochondrial function with respect to age is the analysis of enzymatic activity of the complete mitochondrial respiratory chain and/or its four major electron transport units, or the rate of ADP-stimulated molecular oxygen consumption (i.e. state 3 respiration). In this category, the majority of studies report an age-related decline in electron transport chain activity, in mice, rats and humans [62–70]. However two groups have observed no decline in electron transport chain activity in rodents [71–73].

The third type of analysis is to measure the mitochondrial membrane potential, the driving force for ATP synthesis, in young and old animals. Measurements of the mitochondrial membrane potential in young and old animals have supported the view that there is decreased mitochondrial membrane potential in mitochondria from aged species [74–76].

7. Oxidant stress and mitochondria

Because approximately 90% of cellular oxygen is consumed in mitochondria, and because approximately 3% of molecular oxygen is thought to escape complete reduction to water, mitochondria are considered to be the major intracellular contributor to superoxide generation, and perhaps to oxidant stress in general. The potential involvement of oxidative stress in aging has been suggested and reviewed elsewhere [17,77]. It has been demonstrated that there is an increased production of mitochondrial superoxide and hydrogen peroxide from aged animals ([78,79] and refs. therein). The results of knockout experiments in mice confirm the importance of protection from mitochondrially localized superoxide, in that knockouts of the mitochondrial superoxide dismutase are lethal [80,81].

Because the 13 polypeptides encoded by the mtDNA are each involved in respiration, the most likely effect of age-related mtDNA mutagenesis is a decrease or dysfunction of the respiratory chain. Inhibition of the electron transport chain late in the chain are expected to produce a more reduced electron transport chain. Since the majority of superoxide is thought to be generated by reaction of ubisemiquinone radical with molecular oxygen, it would

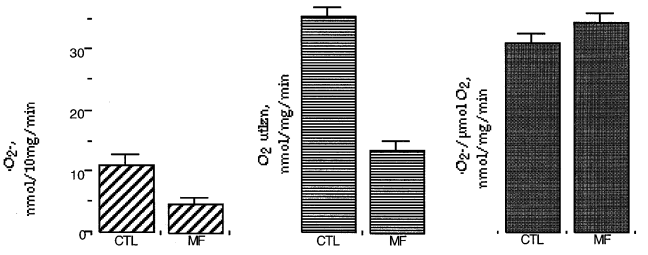

Fig. 5. Lower O_2 consumption and higher superoxide production from mutant MERRF than control mitochondria. T.P. Hutchin, G. Attardi and G.A. Cortopassi, unpublished data.

be predicted that inhibition of the electron transport chain should lead to increased superoxide generation. We observe, like others, that the MERRF mutation results in decreased oxygen consumption [82,83]. Using the reduction of cytochrome *c* to measure the production of superoxide from submitochondrial particles, we have measured the production of superoxide from submitochondrial particles prepared from 143B cells carrying the MERRF mtDNA vs. a control mtDNA. We observed that the MERRF mutation results in about an 11% increase in mitochondrial in mean mitochondrial superoxide production (Fig. 5, Hutchin, Attardi, and Cortopassi, unpublished results). Whether this 11% increase in superoxide production is relevant for MERRF pathophysiology is currently unknown.

8. Pure pathogenic MERRF, MELAS and LHON mtDNA mutations can confer sensitivity to oxidant stress

Although it is widely assumed that the phenotypic effects of inheritance of pathogenic mtDNA mutations result from deficits in bioenergetic functions, some alternative possibilities exist. For example, in MERRF, it has been observed that resting [ATP] levels are not any lower than in controls; however, that cytoplasmic levels of Ca^{2+} are higher in mutant vs. control cells [84]. Thus, alternative models for the pathophysiological mechanism of mitochondrial genetic diseases might be entertained, including one in which mitochondrial deficits sensitize cells to oxidant and/or Ca^{2+} stress. Thus, as a potential model of the mechanism of pathogenesis in germline mtDNA disease, and also potentially in aging of the mtDNA, we

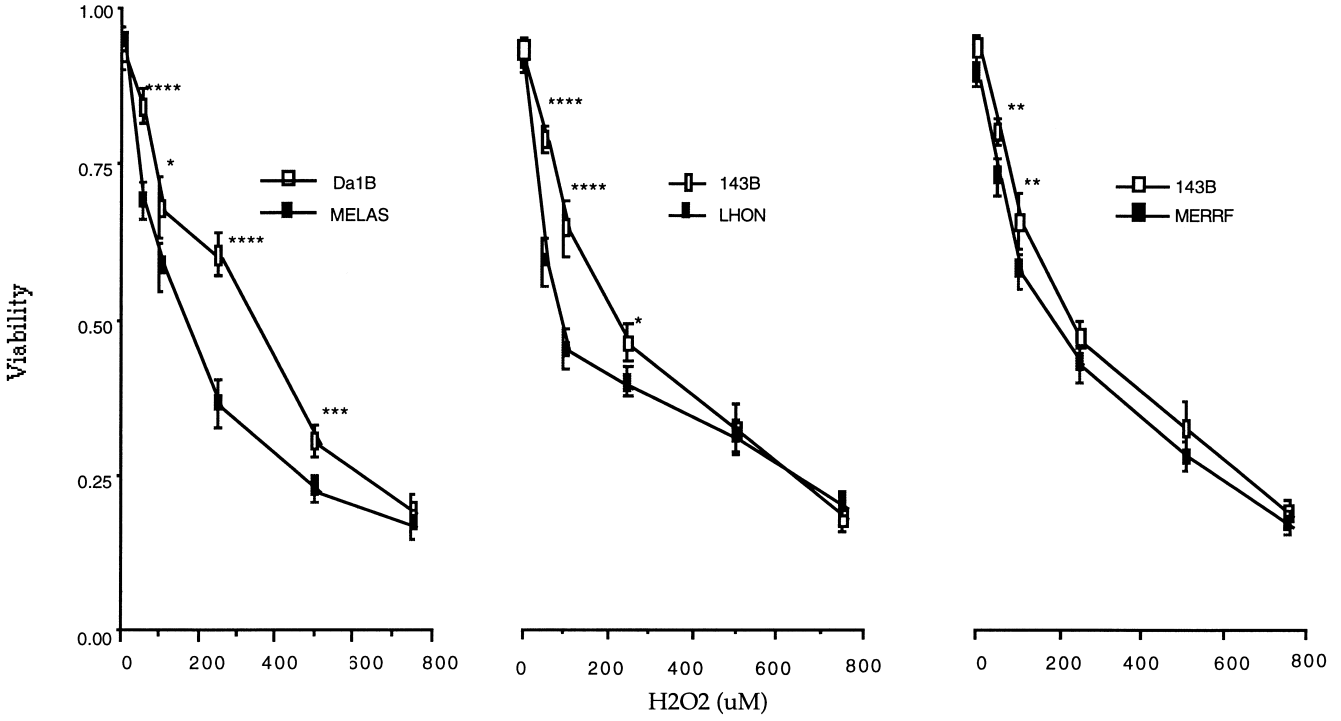

Fig. 6. Pathogenic mitochondrial mutations confer cellular sensitivity to oxidant stress. Redrawn from [85].

have tested the sensitivity of cells bearing the pathogenic mitochondrial mutations LHON, MERRF, and MELAS to an oxidative stress, exposure to hydrogen peroxide, as seen in Fig. 6 and [85]. The MERRF, MELAS and LHON mutations each conferred significant sensitivity to oxidant stress. It is important for these data to be confirmed in other transmitochondrial cell lines to know if this is a general phenomenon. It is possible that the acceleration of cell death by pathogenic mitochondrial mutations is through increased activity of the apoptotic pathway, which is known to involve mitochondria; however, there is not yet direct evidence for this possibility. We observed rescue from death by depletion by depletion of Ca^{2+} from the tissue culture medium and by depletion of intracellular Ca^{2+} by treatment with the intracellular Ca^{2+} chelator BAPTA-AM. Thus, it is possible that oxidant stress results in a rise in intracellular Ca^{2+} that is required for toxicity. A potential downstream target of oxidant stress is the mitochondrial permeability transition (see below), which is sensitive to Ca^{2+} and reactive oxygen species and is inhibited by CsA [86]. It was observed that CsA protected MERRF, MELAS and LHON fibroblasts from oxidant stress, and specifically more than control cells.

9. Multiple lines of evidence implicate mitochondria in cell death

Multiple lines of evidence have implicated mitochondria in the control of cell death, including the localization of the anti-apoptotic protein Bcl-2 to the outer mitochondrial membrane [87], and the requirement for mitochondria [88] and specifically mitochondrial cytochrome *c* in cell-free systems of apoptosis [89], the early loss of mitochondrial membrane potential in some forms of cell death [90,91], the regulation of mitochondrial Ca^{2+} by Bcl-2 [92], and the inhibition of release of cytochrome *c* by Bcl-2 [93,94]. Currently the mechanism(s) by which cytochrome *c* are released from mitochondria, and by which Bcl-2 potentially inhibits the release of cytochrome *c* from mitochondria are of significant interest.

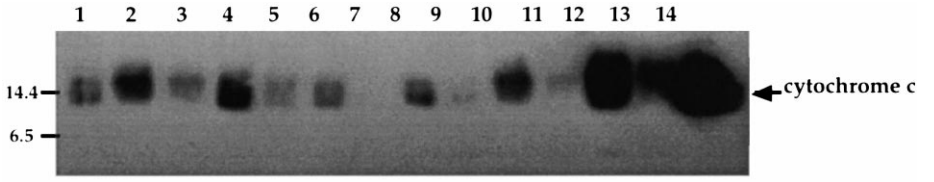

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Fig. 7. Induction of the mitochondrial permeability transition causes release of apoptogenic cytochrome *c*, redrawn from [99]. Cytochrome *c* is detected in supernatants from mitochondria induced for MPT by Western blot. Lane 1, control (uninduced). Lanes 2–13 are 6 pairs, the first sample of each pair is inducer alone, the second sample contains identical concentration of inducer plus 1 mM cyclosporin A. The six lanes and inducers were: 2 and 3, 100 mM Ca^{2+}; 4 and 5, 5 mM atractyloside; 6 and 7, 100 mM tBOOH; 8 and 9, 5 mM MPP^{+}; 10 and 11, 5 mM 1 mM H_2O_2; 12 and 13, 0.5 mM dATP. Lane 14, 0.3 mg of cytochrome *c*.

10. The mitochondrial permeability transition has been associated with cellular dysfunction and death

The mitochondrial permeability transition (MPT) is an abrupt rise in mitochondrial permeability that is induced by Ca^{2+} and reactive oxygen species, and several other agents [86,95,96]. Cyclosporin A inhibits MPT, presumably by inhibiting the peptidyl-prolyl-isomerase of the mitochondrial cyclophilin to which it binds [97]. Induction of the mitochondrial permeability transition has recently been associated with cell death [91,98]. Cytochrome *c* has recently been demonstrated to be required for induction of the biochemical hallmarks of apoptosis in some cell-free systems [89,93,99]. One potential issue is by what mechanism is cytochrome *c* released from mitochondria to initiate apoptosis. Given the known sensitivity of the MPT to several known toxins, a straightforward question is to ask whether the induction of the MPT results in the release of cytochrome *c*. We [99] and others [100] have observed that the induction of the mitochondrial permeability transition is associated with the release of cytochrome *c*. In Fig. 7, several known inducers of the MPT in purified mitochondria were demonstrated to induce the release of cytochrome *c* by Western blot. However, whether this release of cytochrome *c* occurs specifically, or by bursting of the outer mitochondrial membrane is a relevant issue. We have observed that osmotic support of purified mouse liver mitochondria with PEG-1000 completely inhibited the release of cytochrome *c* mediated by the standard MPT inducers tBOOH, Ca^{2+}, and atractyloside. These results are most simply explained by PEG-mediated suppression of mitochondrial bursting. However, MPT-dependent mitochondrial bursting (i.e. nonspecific release of cytochrome *c*) could be a physiologically relevant death mechanism, inasmuch as the MPT inhibitor cyclosporin A does mediate protection from cell death in many experimental paradigms. By contrast with the results with canonical MPT inducers, we observed that dATP induced a dose-dependent release of cytochrome *c* which was not strongly inhibited by PEG, but was inhibitable by cyclosporin A, which potentially suggests an independent release mechanism for cytochrome *c* [78].

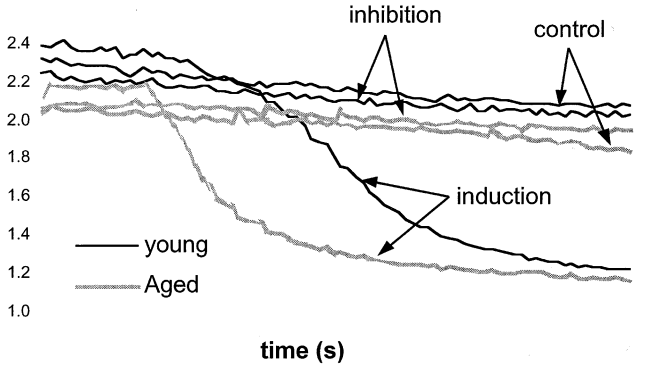

Fig. 8. Sample spectrophotometric recording showing the design used for a comparison of rate of mitochondrial permeability transition in young vs. old mice, redrawn from [78]. Numbers on *y*-axis are absorbance, the induction of the mitochondrial permeability transition results in increased light scattering. Induction mitochondria received Ca^{2+} stimulus, control mitochondria received no Ca^{2+} stimulus, and inhibition samples received Ca^{2+} stimulus and cyclosporin A, a specific inhibitor of the mitochondrial permeability transition.

11. Increased MPT inducibility in aged mouse hepatocytes

Given the association of the MPT with cellular pathophysiology, and the association between deficits of mitochondrial dysfunction with age, one potential issue is whether the MPT is more inducible with age. In a recent comparison of MPT in young (1 month) vs. old (36 month) liver mitochondria, lower state 3 respiration, higher state 4 respiration, and more inducible MPT were observed in the old mitochondria ([79] and Fig. 8 below) which provides the first experimental support for the concept that MPT may be more inducible in aged animals. Further research will demonstrate whether or not these observations are general in other strains and species. In Fig. 8 below, the inducibility of MPT was measured as the difference in the rate of induction of swelling, which is observed as an increased light scattering of the swollen mitochondria. The observed mouse liver MPT was quite variable from day to day, but paired same-day comparisons of old vs. young mice indicated a statistically significantly faster MPT induction in old mice. This MPT was both Ca^{2+} dependent and cyclosporin A inhibitable.

12. Future prospects

Several lines of evidence indicate that there is a decline in mitochondrial function with age at the genetic, biochemical and bioenergetic level; however, to what extent these deficits of mitochondrial function underlie phenotypic changes that occur with aging is still in an area of very active research. Although it is clear that mitochondrial deletion mutations rise exponentially with human age, and there is substantial support for an association between cox-negative fibers and mtDNA deletions, it is also clear that there is a substantial number of cox-negative fibers in muscle that do not have detectable mtDNA deletions. Although it is a reasonable hypothesis, it is not yet clear whether mitochondrial and or mtDNA damage is a cause of muscle atrophy, or atrophy and death of other cell types with age. Analytical techniques which globally and quantitatively assay mtDNA mutagenesis in a quantitative way are needed to resolve this issue, as well as the ability to assay such damage on aging human tissue in situ.

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