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## Role and Treatment of Mitochondrial DNA-Related Mitochondrial Dysfunction in Sporadic Neurodegenerative Diseases

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### Abstract

Several sporadic neurodegenerative diseases display phenomena that directly or indirectly relate to mitochondrial function. Data suggesting altered mitochondrial function in these diseases could arise from mitochondrial DNA (mtDNA) are reviewed. Approaches for manipulating mitochondrial function and minimizing the downstream consequences of mitochondrial dysfunction are discussed.

### Keywords

cybrids; endophenotype; mitochondria; mitochondrial DNA; mitochondrial medicine; neurodegenerative disease

## 1. INTRODUCTION

Mitochondrial function is altered in several late-onset neurodegenerative diseases including Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS) [1, 2]. When it comes to altered mitochondrial function in these conditions there are commonalities, such as the fact that changes are observed both within and beyond the nervous system and are therefore not simply a consequence of neurodegeneration [3, 4, 5]. There are also very specific differences that distinguish between the diseases. For example, AD subject mitochondria show reduced cytochrome oxidase (complex IV) activity, while PD subject mitochondria show reduced NADH:ubiquinone oxidoreductase (complex I) activity [6, 7, 8, 9].

Epidemiologic similarities also exist between AD, PD, and ALS. Each disease has relatively rare Mendelian variants and much more common sporadic variants [10, 11, 12]. It is important to note, though, that in this context "sporadic" does not mean genes do not contribute to disease risk or pathology. It simply means that recognizable autosomal dominant, autosomal recessive, or X-linked inheritance is not observed. Sporadic epidemiology also does not infer that a particular family is only allowed to have one affected member. It is well recognized that the offspring of an AD or PD-affected parent have an increased risk of developing AD or PD, and families in which there are both an affected parent and child are frequently ascertained [13, 14, 15]. Still, the percentage of the offspring of an AD parent that develop AD rarely reaches 50%, and the percentage of offspring of a PD parent that develop PD rarely reaches 50%. Autosomal dominant inheritance is therefore unlikely, or else if dominant mutations are responsible then they must have very low

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penetrance. Also, unless the responsible mutations are extremely common in the population, autosomal recessive inheritance would not readily account for kindreds that have affected members in different generations.

The non-autosomal, yet genetically-influenced epidemiology of the late-onset, sporadic neurodegenerative diseases suggest genetic factors that do not follow the rules of Mendelian genetics may play a role. Multiple lines of evidence indicate mtDNA may influence disease risk and also cause pathologic phenomena observed in the common sporadic variants of AD, PD, and ALS [16]. Understanding why and how mtDNA potentially contributes to these diseases is important because it yields mechanistic insights and identifies potential therapeutic targets. The first part of this review discusses evidence that argues mtDNA is relevant to various neurodegenerative diseases. The second part discusses therapeutic options from the perspective of an evolving field of medicine called mitochondrial medicine [17, 18, 19, 20].

## 2. MTDNA AND DISEASE

The human mtDNA consists of 16,569 nucleotides and includes 37 genes [21]. Thirteen of these genes encode electron transport chain (ETC) subunit proteins, two encode rRNA subunits used in mtDNA protein gene translation, and the remaining 22 encode tRNAs used in mtDNA protein gene translation. There is also a non-coding regulatory region that helps guide mtDNA replication and transcription [22].

Several diseases are recognized consequences of mtDNA mutations [1]. Some of the more common diseases include the mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes syndrome (MELAS); the myoclonic epilepsy and ragged red fiber disease syndrome (MERRF); the neuropathy, ataxia, and retinitis pigmentosa syndrome (NARP); and Leber's hereditary optic neuropathy (LHON) [23, 24, 25, 26, 27]. MELAS often arises from an A3243G substitution in the tRNA<sup>Leu(UUR)</sup> gene, MERRF often arises from a A8344G substitution in a tRNA<sup>Lys</sup> gene, NARP arises from a T8993G substitution in the ATPase 6 gene, and LHON can arise from mutation in several genes although the single most common mutation is a G11778A substitution in the ND4 gene. In most cases the causal mutations are present as high percentage heteroplasmies, meaning that the majority of mtDNA copies have the mutation, or the mutations are homoplasmic (all copies of mtDNA carry the mutation). Sometimes matrilineal inheritance patterns are discernable. Still, although these mutations are typically inherited from one generation to the next through maternal lines, matrilineal inheritance patterns are often not obvious.

Non-inherited mtDNA mutations may also mediate disease phenotypes. One example includes the mitochondrial neurogastrointestinal encephalopathy (MNGIE) syndrome, which is associated with mutations of the TYMP gene that encodes a thymidine phosphorylase enzyme [28]. TYMP mutation alters the intracellular nucleotide balance, which in turn causes the accumulation of mtDNA mutations and also mtDNA depletion. Although acquired mtDNA mutations are believed to drive MNGIE signs and symptoms, the disorder follows an autosomal recessive inheritance pattern since the ultimate cause is homozygous mutations in a nuclear gene. More recently, mutations in the nuclear gene that encodes the mtDNA polymerase gamma (POLG) protein required for mtDNA replication have been identified as a potential cause of PD [29, 30, 31, 32]. Impaired POLG function can cause an accelerated accumulation of mtDNA mutations, including point mutations and deletions [33, 34].

Several questions about the relationship between mtDNA mutation and human disease remain unresolved. One question relates to the issue of "threshold". After all, an individual cell can contain thousands of mtDNA copies, and diverse heteroplasmic ratios are possible.

A particular mtDNA mutation may reside in a cell at a very low level, and in some cases mutations may even reside on less than 1% of a cell's mtDNA copies. Whether or not a heteroplasmic mutation causes a clinical phenotype is likely influenced by a combination of the mutation's qualitative pathogenicity and its quantitative burden within an individual cell. For very deleterious mutations the burden of mutation required to affect biochemical function and thus surpass the disease threshold may be relatively low. For less deleterious mutations very high mutational burdens or perhaps even mutational homoplasmy may be needed to surpass the disease threshold. For the least deleterious mtDNA substitutions, potential clinical consequences may not always be obvious. For example, very mild mutations may only subtly affect a person's risk for a particular disease rather than exert a deterministic effect, and act in conjunction with other factors to simply modify risk. Very mild mutations might also contribute to phenotypes that don't readily manifest as a disease per se, but which instead manifest as accepted features of other conditions. Age-related functional changes might constitute one such example of this. It is in this context that mtDNA signatures may influence the risk of developing and also the progression of particular late-life neurodegenerative diseases.

A potential role for mtDNA in sporadic neurodegenerative diseases was almost simultaneously proposed by different investigators whose hypotheses were based on different assumptions. In the construct proposed by Parker, inherited mtDNA mutations were assumed to ultimately be critical [7, 35]. Parker postulated that in general mtDNA might influence the onset and progression of sporadic diseases that are nevertheless genetically influenced because mitochondrial genetic principles enable mtDNA to evade the rules of Mendelian genetics [16]. These unique mitochondrial genetic principles include maternal inheritance, heteroplasmy, threshold, and mitotic segregation; mitotic segregation refers to the fact that an individual's different tissues, and within a tissue different cells, may accumulate different amounts of a heteroplasmic mutation. Figure 1 illustrates how these mitochondrial genetic principles let mtDNA contribute to sporadic-appearing diseases. According to an alternative hypothesis championed by several investigators including Wallace, an age-associated acquisition of mtDNA mutations in the central nervous system was assumed to represent the primary cause of late-onset, sporadic neurodegenerative diseases [36].

In potential support of either view, it became increasingly apparent during the 1980s and 1990s that the mitochondrial ETC was altered in sporadic, late-onset neurodegenerative diseases and that these alterations were potentially relevant to the disease phenotype [2, 37, 38]. In some cases ETC dysfunction in a particular disease was rather specific. A systemic complex I defect was described in PD subjects, and the importance of this defect was emphasized by the fact that complex I inhibition had been found to cause parkinsonism in both humans and animals [3, 39, 40, 41, 42]. A systemic cytochrome oxidase defect was described in AD subjects, and the importance of this defect was emphasized through studies in which cytochrome oxidase inhibition was found to impair long term potentiation in rats [8, 38, 43]. The etiology of these disease-specific ETC defects, though, was not known. Exogenous toxins were considered as a possible cause, but no universal toxins that could explain the majority of disease cases were identified. Similarly, the possibility that endogenously generated ETC inhibitors might be responsible was considered but no such toxin could be reliably implicated. The fact that these ETC defects were systemic, and not simply a consequence of neurodegeneration, raised the question of whether they were gene-mediated.

This question could not be quickly answered through gene-sequencing studies. The ETC components are multi-unit holoenzymes; complex I alone contains 46 known subunits. Complexes I, III, IV, and V (complex V is not an ETC holoenzyme, although it is an

indispensable component of the oxidative phosphorylation apparatus) are also bi-genomically encoded as they have subunits that are transcribed and translated from genes present on both nuclear and mtDNA. Further, the inclusion of mtDNA as a potential source of ETC miscoding introduced its own unique set of problems. MtDNA is highly variable between individuals, so it is not unusual to find mtDNA sequence deviations in disease-affected subjects [44]. Because by definition these diseases are sporadic, it is difficult to know whether the co-occurrence of a particular sequence deviation with a clinical phenotype is coincidental or etiologically relevant. Indeed, when evaluated from the context of an extended family, it is very likely a particular mtDNA polymorphism will not reliably segregate with disease. This indicates common mtDNA variants are very unlikely to cause disease, although it does not rule out a role for such variants in influencing disease risk. Also, until recently screening for low abundance heteroplasmic mutations was not routinely conducted and even now such surveys are rarely performed [45, 46, 47, 48, 49]. In the 1990s an alternative strategy, the cytoplasmic hybrid (cybrid) strategy, was therefore used to functionally assess whether unique mtDNA signatures might account for the known ETC defects present in AD and PD subjects [50].

### 3. DATA ADDRESSING A ROLE FOR MTDNA IN SPORADIC NEURODEGENERATIVE DISEASES

#### 3.1 Cybrid Data

The cybrid technique was initially developed in the 1970s to assess the effects of particular mtDNAs on cell function [51]. In one of the earliest cybrid experiments, mtDNA from a chloromphenicol-resistant HeLa cell strain was transferred to a chloramphenicol-sensitive strain [52]. MtDNA transfer conferred chloramphenicol resistance, and indicated chloramphenicol resistance in HeLa cells is an mtDNA-determined trait. Over time, the technique was increasingly used to assess the consequences of known mtDNA mutations [53, 54, 55, 56, 57, 58]. This approach was assisted by the creation of mtDNA-depleted cell lines [59, 60, 61]. Since decades earlier mtDNA was commonly referred to as  $\rho$  DNA [62], these mtDNA-depleted cell lines were also called  $\rho 0$  cells [63].  $\rho 0$  cells lack a functional ETC but can survive and expand in culture if appropriate metabolic support is provided. By transferring a known mtDNA sequence from an exogenous source to  $\rho 0$  cells, investigators were able to determine that sequence's ability to restore ETC function and oxygen consumption rates [59]. The inability of a particular mutation-bearing mtDNA sequence to restore full function was assumed to reflect that mutation's deleterious effects and potentially also its disease-relevance. Figure 2 summarizes the cybrid technique.

Cybrid cell lines containing mtDNA from subjects with the common, sporadic forms of several late-onset neurodegenerative diseases have been generated and analyzed [50]. In most but not all of these studies subject mtDNA was obtained from platelets, since platelets are easily obtained and lack nuclei. Co-incubating platelets and  $\rho 0$  cells in the presence of the detergent polyethylene glycol allows platelet and  $\rho 0$  cell cytosolic contents to mix. After a brief period the detergent is quenched by dilution in a cell growth medium. A small percentage of cells that contain both a  $\rho 0$  cell nucleus and at least one platelet mitochondria will emerge. These cybrid cells are identified, isolated, and expanded. The result is a novel cell line that has the nucleus of the  $\rho 0$  cell line used to generate it, as well as the mtDNA from the platelet donor.

It is important to note the cytosolic transfer is not limited to mtDNA. Indeed, whole mitochondria transfer occurs. Because mitochondria undergo constant fusion and fission [64, 65], as the exogenous mtDNA replicates within the cell it reconstitutes the cell's mtDNA content. During this period, the new cybrid cell line develops a unique

mitochondrial population in which all its mitochondria's nuclear-encoded proteins are generated by the nucleus from the  $\rho 0$  cell line, and all its mtDNA-encoded proteins are generated by its new mtDNA. Theoretically, the only difference between cybrid cell lines containing mtDNA from different donors is their mtDNA sequence. If different cybrid cell lines are functionally analyzed and found to have functional differences, as was discussed above the differences presumably reflect differences between mtDNA sequences.

Sporadic, late-onset neurodegenerative diseases that have been studied in depth include AD, PD, ALS, and progressive supranuclear palsy (PSP). Compared to cybrid cell lines prepared from control individuals, AD cybrid cell lines show reduced cytochrome oxidase activity, which recapitulates the systemic cytochrome oxidase activity reduction that was already known to exist in AD subjects [66, 67, 68]. Cybrid cell lines prepared from PD subjects have reduced complex I activity, which recapitulates the known PD subject complex I defect [69, 70, 71, 72]. ALS cybrids were found to have at least reduced complex I activity [73], which recapitulates a complex I activity reduction in ALS subject muscle [74, 75]. PSP cybrids have reduced complex I activity [76]. In each of these diseases the mtDNA-dependent ETC functional changes give rise to other abnormalities, including oxidative stress and protein aggregation phenomena [68, 72, 73, 77, 78, 79, 80, 81, 82, 83]. Results from most of the many cybrid studies published over the past 15 years were previously summarized in a number of comprehensive review articles [3, 4, 50, 84]. Table 1 summarizes the diseases in which cybrid studies have demonstrated specific respiratory chain (ETC or complex V) defects [3, 4, 50, 84, 85, 86, 87].

### 3.2 MtDNA Sequence Analysis Data

One limitation of the cybrid approach used to study late-onset, sporadic neurodegenerative diseases is that while disease-associated differences between disease and control cybrid cell lines imply disease-associated differences in mtDNA, the responsible mtDNA signature is not revealed. Various mtDNA sequence analysis studies have tried to address this knowledge gap [1, 3, 4, 38, 84, 88]. These studies often produce discordant results or reach different conclusions. It is nevertheless possible to venture several take-home messages.

First, it does not appear that for any of the diseases studied a single homoplasmic or nearly homoplasmic mtDNA deterministic mutation accounts for a significant percentage of cases. Second, studies that test for low abundance, heteroplasmic mtDNA mutations are uniformly positive. Some studies show deletion mutations, and some show point mutations. Whether these mutations are acquired, inherited, or both is not entirely clear. Such mutations are also found in control subjects. While some studies conclude there are no differences in either mutational burden or distribution, others report unique quantitative or qualitative signatures [45, 46, 47, 48, 49, 89, 90, 91, 92, 93]. Third, although deterministic homoplasmic mutations are an unlikely cause of disease, it may turn out that common homoplasmic mtDNA variations influence disease risk [44]. This is supported by a number of association studies that report particular mtDNA polymorphisms or haplogroups are more or less common in certain disease populations than they are in control populations [94, 95, 96, 97, 98, 99]. As is often the case with disease association studies, though, not all surveys yield positive results.

The big question at this time is not whether homoplasmic mtDNA variations or low-abundance heteroplasmic mutations are found in persons with diseases such as AD, PD, and ALS. They clearly are. Rather, the question now is whether these mtDNA variations or mutations influence disease risk, cause disease, or mediate at least some disease-associated pathologies. The ability of mitochondrial toxins to cause disease phenocopies and the presence of systemic mitochondrial dysfunction in disease-affected subjects further suggests altered mitochondrial function in persons with late-onset, sporadic neurodegenerative diseases is not simply an epiphenomenon. The fact that these functional mitochondrial

alterations perpetuate in cultured cells following mtDNA transfer from affected subjects similarly argues mtDNA signatures are at least to some degree disease-relevant. In the case of AD a new line of investigation, endophenotype studies, is also consistent with a disease-relevant role for mtDNA.

### 3.3 Endophenotype Data

An “endophenotype” is a particular disease-associated characteristic or trait an individual demonstrates despite the fact that individual does not meet diagnostic criteria for the actual disease [4]. An endophenotype is typically demonstrable by biomarker studies or other types of approaches. The presence of a disease endophenotype does not necessarily mean the endophenotype carrier will develop the disease it is associated with, although it may imply the carrier individual has an increased risk of developing the disease. It often implies the affected individual has at least some genetic predisposition for the disease.

In AD, a family history of sporadic AD is a well-recognized risk factor as an individual’s risk of AD is increased if either parent has this disease [100]. Some degree of sporadic AD risk is certainly conferred via Mendelian genes. This is confirmed by the fact that particular Mendelian gene polymorphisms associate with AD. The most studied Mendelian associations are for two single nucleotide polymorphisms in the APOE gene on chromosome 19, which is found at the 19q13.2 region [101]. After almost 20 years of investigation it is still not entirely clear how the APOE gene product, apolipoprotein E protein, influences AD. Interestingly, polymorphisms in a neighboring gene that encodes the translocase of the outer mitochondrial membrane 40 (TOMM40) protein also reportedly influence AD risk [102, 103, 104, 105, 106, 107]. AD-associated TOMM40 and APOE gene polymorphisms are actually in linkage disequilibrium with each other. This means it is possible the TOMM40 protein, with its obvious role in mitochondrial function, is more relevant to AD than the apolipoprotein E protein.

Regardless of which gene or genes from the 19q13.2 drives AD risk, as a group middle aged, cognitively normal individuals with APOE4 alleles (the APOE variant associated with increased risk) have fluorodeoxyglucose positron emission tomography (FDG PET) scans that resemble those of AD subjects [108, 109]. Like the AD subjects, non-demented APOE4 carriers tend towards reduced glucose utilization in specific regions, including the parietal and posterior cingulate cortex. APOE4 carriers thus have a demonstrable AD endophenotype.

AD endophenotypes that are not observed in the children of AD-affected fathers are seen in the non-demented children of AD-diagnosed mothers [13]. Maternally inherited AD endophenotypes are demonstrable using various approaches. Compared to the children of AD fathers, the children of AD mothers have decreased glucose utilization on FDG PET [110, 111]. With brain amyloid imaging with Pittsburgh Compound B (PIB) PET, the children of AD mothers have more amyloid deposition [112]. Magnetic resonance imaging voxel based morphometry (MRI VBM) analyses reveal increased precuneus and parahippocampal atrophy and atrophy rates in those with a maternal AD family history [113, 114, 115]. As is the case with AD subjects, the children of AD mothers have low cerebrospinal fluid (CSF) A $\beta$ 42/A $\beta$ 40 ratios [116]. They also have increased CSF isoprostanes, which suggests increased oxidative stress is present [116]. Those with an AD maternal family history and an APOE4 allele have a unique AD endophenotype that is demonstrable through cognitive testing [117].

Maternally-inherited AD endophenotypes suggest a maternally inherited genetic factor such as mtDNA modifies AD risk. In this respect, it was recently reported that compared to those with an AD paternal family history, those with an AD maternal family history have lower

platelet mitochondria cytochrome oxidase activities. Since cytochrome oxidase is partly encoded by mtDNA, this finding further suggests the responsible maternally inherited factor may be mtDNA.

## 4. MITOCHONDRIAL MEDICINE FOR NEURODEGENERATIVE DISEASES

### 4.1 Manipulating the Electron Transport Chain and Cell Energy Supplies

It is assumed ATP levels are reduced in at least the degenerating regions of neurodegenerative disease-affected brains. It is further presumed low ATP levels may alter cell physiology in ways that promote cell dysfunction and death. If correct, increasing cell ATP levels would seem to represent a reasonable therapeutic target. Several pathways and cycles that contribute to ATP production are shown in Figure 3.

Increasing electron flux through the ETC constitutes one potential therapeutic strategy. Various approaches have been used to attempt this. Dichloroacetate (DCA) inhibits pyruvate dehydrogenase kinase, which in turn activates pyruvate dehydrogenase complex [118]. The enzymatic conversion of pyruvate to acetyl CoA directly produces an NADH reducing equivalent, and should also increase Krebs cycle flux. While DCA has the ability to reduce blood lactate levels in MELAS patients [119], in the largest trial clinical benefits were not obvious and toxic side effects were noted [120].

Administering alternative electron carriers has been tried. The goal of this approach is essentially to bypass a known or suspected ETC block. For example, menadione and ascorbate used in combination allow ETC electron flux to circumvent a defective complex III enzyme [121]. While occasional anecdotal reports note a potential benefit in selected persons, in general this appears to have little impact. Methylene blue, which is used in the treatment of methemoglobinemia, is able to enhance cytochrome oxidase activity, possibly through a mechanism that involves a direct electron donation [122, 123, 124]. More recently, methylene blue has been advocated for the treatment of AD, either as a mitochondrial enhancing agent [125] or as an anti-neurofibrillary tangle agent [126]. In either case, if methylene blue is proven efficacious for the treatment of AD its ability to transfer electrons to cytochrome oxidase will need to be considered as a potential mechanism. The electron carriers coenzyme Q and idebenone, a water soluble ubiquinone analog, may also be included in this intervention category. Neither appear to have a big impact in subjects with late-onset, sporadic neurodegenerative diseases although a PD phase II trial did report over the course of the study those on coenzyme Q declined less than those on placebo [127]. Development of idebenone for AD was discontinued after a clinical trial showed an insufficient degree of benefit [128]. Idebenone may reduce ventricular hypertrophy and possibly confer a slight amount of neurologic improvement in persons with Friedreich's ataxia (FA) [129, 130].

NADH and FADH<sub>2</sub> supply high-energy electrons to the ETC. Nicotinic acid and nicotinamide, precursors of NADH, and riboflavin, an FADH<sub>2</sub> precursor, have been tested in a variety of populations. The rationale is that supplementation with these precursors could increase mitochondrial reducing equivalents and ETC electron flux. Although riboflavin is sometimes used to reduce migraine frequency rates in persons with recurrent migraines [131], at this point none of these compounds have been shown to positively benefit persons with sporadic neurodegenerative diseases although results from clinical trials are either lacking or pending.

In terms of increasing energy stores, creatine supplementation has been tried in various neurodegenerative mitochondrialopathies [132, 133]. The underlying rationale is that increasing creatine levels will enable cells to generate and store greater amounts of creatine

phosphate. Under conditions of increased energy demand it is hoped the high energy phosphate of the creatine phosphate could be used to generate ATP. While creatine supplementation has not produced dramatic results in any clinical trials, and has had negative results in some [134, 135], at least in PD it currently cannot be concluded to constitute a futile approach [136, 137].

Supplementing brain bioenergetic metabolism with carbon fuels other than glucose represents yet another strategy for enhancing failing brain energy metabolism. The ketone bodies acetoacetate and  $\beta$ -hydroxybutyrate support neuron respiration [138]. It was empirically recognized decades ago that ketogenic diets, which boost acetoacetate and  $\beta$ -hydroxybutyrate levels, reduce seizure frequency in persons with epilepsy [139]. The effects this has on neuron respiration may contribute to this intervention's efficacy. Using  $\beta$ -hydroxybutyrate as a way to enhance failing brain bioenergetics in AD was actually proposed in 1989 [140]. More recently this hypothesis was clinically tested. Clinical data from AD subjects are consistent with a possible benefit for at least some individuals, including in particular those without an APOE4 allele [141]. A supplement that increases  $\beta$ -hydroxybutyrate levels is currently available for the management of AD [142].

## 4.2 Manipulating Oxidative Stress

Oxidative stress is observed in multiple neurodegenerative disease states [143, 144]. This is demonstrable in the form of protein, lipid, or DNA oxidative modifications. In these cases oxidative stress is believed to arise as a consequence of free radical overproduction rather than reduced free radical scavenging. Free radicals are often cited as a potential cause of cell dysfunction, damage, and death and the mitochondrial ETC constitutes the single greatest source of cell free radical production [145]. Therefore, reducing free radicals and free-radical mediated chemistry has long been considered a reasonable mitochondrial medicine approach.

The earliest attempts to reduce oxidative stress focused on antioxidant vitamin supplements. For example, during the 1990s daily administration of a vitamin E, vitamin C, and beta carotene represented an often-used treatment for ALS despite a lack of objective supporting evidence [146]. High-dose vitamin E supplements were tried in PD without success [147]. In one prospective study, a group of AD patients taking 2000 IU per day of vitamin E took longer to reach predefined decline endpoints, such as nursing home placement, than a placebo group did [148]. While this was interpreted as a positive study, it is important to note the potential benefit was not completely straightforward because at the start of the trial the mini mental state exam (MMSE) score in the vitamin E group was slightly lower than that of the control group. The raw data from the study were subsequently adjusted to correct for this inequity. Without this mathematical adjustment there was no apparent advantage to the vitamin E treatment.

Multiple other compounds that accept electrons and could potentially reduce superoxide production have been evaluated. Idebenone was tested, without success, in AD [128]. As mentioned above, coenzyme Q was tested in a PD phase II and in that study a small benefit could not be excluded [127]. Coenzyme Q has also been evaluated in PSP [149].

More sophisticated antioxidant approaches have been developed and some have entered clinical trials. One important modification of the antioxidant approach includes the design of mitochondrially-targeted antioxidants [150]. This is accomplished by combining a cationic moiety with a classic antioxidant. MitoQ is perhaps the best known example of this [151]. MitoQ has been tested in PD subjects, but results to date are not encouraging [152].



Future antioxidant-based approaches may involve manipulations that enhance antioxidant enzyme expression [153, 154]. Investigators from the aging research field have thus far evaluated this approach. Results are mixed. In one study, expression of a mitochondrially-targeted catalase was found to extend lifespan in mice [155]. Other studies, though, in which antioxidant enzymes are over-expressed in transgenic mice have not produced lifespan extension [156, 157].

### 4.3 Manipulating Apoptosis

Evidence suggests apoptosis or apoptosis-related pathways or physiology may mediate cell death in neurodegenerative diseases [158]. Apoptotic signaling in these cases is believed to originate from mitochondria (the “internal” apoptotic pathway) [159, 160, 161, 162]. Efforts have focused on preventing the release of mitochondria-localized proteins that initiate apoptosis, such as cytochrome c. Strategies considered include preventing activation of the mitochondrial permeability transition (MPT) [163, 164], and preventing mitochondrial swelling. Manipulating ratios of proteins that regulate the MPT, especially ratios of Bcl-2 family proteins, constitutes another approach [165, 166]. Compounds that accomplish these ends are also sometimes referred to as mitochondrial stabilizing agents.

In cell culture and animal studies minocycline blocks the pore that is formed during MPT activation [167, 168]. It has been tested in ALS, but in a human trial minocycline-treated subjects actually did worse than placebo-treated subjects [169]. Rasagaline, which is approved as an MAO B inhibitor for the treatment of PD, has been shown under *in vitro* conditions to increase Bcl2/Bax ratios [170]. This helps mitigate toxin-induced mitochondrial depolarization and apoptosis [171, 172, 173, 174]. For these reasons it has been postulated rasagaline may have possible neuroprotective, or “disease modifying”, effects in PD. This possibility was tested in a clinical trial, but the results were not clear-cut [175]. In this trial the results from the low-dose rasagaline treatment group were consistent with a disease modifying effect, but data from the high-dose treatment group were not. R+ pramipexole, another reported mitochondrial stabilizing agent, is being evaluated in ALS clinical trials [176].

Latrepidine, which was originally developed as an antihistamine, is reported by some to have mitochondrial stabilizing properties. This claim is based on a study that found latrepirdine reduced swelling of mitochondria exposed to A $\beta$ , and a study in cultured cells that found it helped preserve mitochondrial membrane potentials under stress conditions [177, 178]. Its ability to mediate such effects in humans has been questioned [179]. In a phase II AD clinical study latrepirdine-treated subjects demonstrated improved function over placebo-treated subjects [180], but in a larger AD phase III trial latrepirdine had no effect [181]. Results from other phase III trials of latrepirdine in AD have yet to be reported.

### 4.4 Manipulating Mitochondrial Autophagy

Mitochondrial autophagy, also called mitophagy, refers to the intracellular removal of mitochondria through phagosome formation and subsequent lysosomal digestion [65, 182]. It provides a mechanism through which a cell can rid itself of failed or dysfunctional mitochondria [183]. Increased and altered mitochondrial autophagy is observed in AD, and evidence suggests autophagy is likely perturbed in other neurodegenerative disorders as well [184, 185, 186]. Further, mutations that cause Mendelian PD affect proteins that mediate autophagy, which implies autophagy plays a role in preventing PD while impaired autophagy contributes to PD [187, 188, 189, 190, 191].

From a conceptual standpoint, mitochondrial autophagy constitutes an attractive mitochondrial medicine approach since enhancing autophagy may help with mitochondrial

quality control. Several years ago lithium was reported to prolong ALS patient survival, and the authors of that study also presented data that suggested its mechanism of action could involve mitophagy activation [192]. Unfortunately, additional clinical trials found lithium did not benefit ALS patients [193, 194]. Rapamycin, which has been shown to extend longevity in mice [195], is another drug being considered as a potential autophagy-inducing treatment for various neurodegenerative diseases [196, 197, 198]. Rapamycin activates autophagy through a mammalian target of rapamycin (mTOR)-dependent process [199, 200, 201].

#### 4.5 Manipulating Mitochondrial Mass

Quantifying how many mitochondria a cell contains is challenging because they are physically dynamic organelles. A single mitochondrion may fission into individual, smaller mitochondria. Conversely, individual mitochondria can fuse together and even form a syncytium that extends throughout the cytosol [64, 65]. Mitochondrial fission and fusion are altered in AD brain and also AD fibroblasts [202, 203].

For this reason, considering how *much* mitochondria a cell contains may constitute a more physiologically meaningful parameter than how *many* mitochondria it has. Mitochondrial mass has been evaluated in different sporadic, late-onset neurodegenerative diseases. While results of studies that quantify total mitochondrial mass can vary, the mass of intact mitochondria is reduced in AD brain and also ALS spinal cord [185, 204, 205, 206]. In terms of developing mitochondrial medicine for the treatment of these and other related disorders, increasing mitochondrial mass is therefore a reasonable goal.

Mitochondrial mass is increased through the process of mitochondrial biogenesis. Mitochondrial biogenesis requires the synthesis of new mitochondrial protein, membrane, and mtDNA. This is accomplished through the coordinated efforts of transcriptional programs located within the nucleus [207]. In particular, the peroxisomal proliferator activated receptor gamma coactivator 1 $\alpha$  (PGC1 $\alpha$ ) co-transcriptional factor in at least some tissues serves as a master regulator of mitochondrial mass [208, 209, 210]. PGC1 $\alpha$  is itself regulated by various enzymes, transcription factors, and intracellular signaling pathways that monitor cell energy levels and metabolic states. Silent information regulator 1 (SIRT1), AMP kinase (AMPK), cyclic AMP responsive element binding protein (CREB), mTOR, the forkhead transcription factors (FOXOs), and AKT all associate with PGC1 $\alpha$  in that they either directly or indirectly influence or are influenced by PGC1 $\alpha$  [211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227]. Several of these relationships are summarized in Figure 4.

Certain drugs are known to increase PGC1 $\alpha$  levels or increase its activity. Two thiazolidinedione drugs currently used to treat type II diabetes mellitus (DMII), pioglitazone and rosiglitazone, are examples [228, 229, 230]. In the case of these thiazolidinediones, PGC1 $\alpha$  activation may actually contribute to their mode of action, which is a decrease in peripheral insulin resistance [231, 232]. Both these agents have been tested in AD, with results that range from no demonstrable benefit to a possible small benefit in selected treatment groups [233, 234, 235]. One limitation of these trials is that the thiazolidinediones poorly penetrate the blood-brain barrier (BBB). It seems unlikely that doses used in humans would generate brain levels that are high enough to induce a robust mitochondrial biogenesis [228]. Bezafibrate, another drug that activates PGC1 $\alpha$ , has been tested in cell culture and animal models with mitochondrial dysfunction and in these models it appears to improve cell bioenergetics [236, 237].

When it comes to developing mitochondrial biogenesis induction approaches it may be useful to consider exercise and dietary restriction. Exercise is associated with improved

health, and dietary restriction extends lifespan in invertebrate and vertebrate species [238, 239, 240, 241, 242, 243, 244]. Exercise increases muscle mitochondrial mass, and this likely mediates to some extent its physiologic benefits [245, 246, 247, 248, 249]. Dietary restriction induces liver mitochondrial biogenesis, and also has been reported to induce mitochondrial biogenesis in other tissues although its extra-hepatic impact may be less robust [250, 251, 252, 253, 254].

It is not surprising that exercise and dietary restriction should impact non-brain tissues more than they impact the brain. The brain is a metabolically privileged organ in the sense that the rest of the body has evolved to keep the brain in a state of bioenergetic homeostasis. For example, falling blood glucose levels activates liver gluconeogenesis and this helps ensure a steady delivery of glucose to the brain. In states of more extreme nutritional stress the liver will produce and secrete the ketone bodies acetoacetate and  $\beta$ -hydroxybutyrate, which are used in the brain as a bioenergetic supplement that fuels neuron respiration [138, 255]. Tight regulation of brain homeostasis may limit the degree to which exercise and dietary restriction can benefit the brain. This is not to say these interventions do not have a brain impact, as data certainly indicate that they do [254, 256, 257, 258]. These benefits, though, may represent relatively indirect sequellae that arise as a consequence of direct affects on other tissues. For example, both exercise and dietary restriction increase peripheral insulin sensitivity and reduce insulin production. This, in turn, should reduce brain insulin levels and brain insulin signaling [259]. Obviously, when it comes to the brain effects of exercise and dietary restriction, very complex relationships may exist. In any case, identifying effective ways to induce brain mitochondrial biogenesis by mimicking the effects of exercise on muscle or of dietary restriction on liver may prove useful.

#### 4.6 Manipulating Redox State

For the purposes of this section redox state refers to the ratio of nicotinamide adenine dinucleotide's (NAD) oxidized (NAD<sup>+</sup>) and reduced (NADH) states. In the cytosol NAD<sup>+</sup> is reduced to form NADH during glycolysis, and in order for glycolysis to continue it is necessary to re-oxidize NADH back to NAD<sup>+</sup>. In the mitochondrial matrix NAD<sup>+</sup> is reduced to NADH during the conversion of pyruvate to acetyl CoA and each fully executed run through the Krebs cycle generates another three NADH. Mitochondrial NADH, in turn, is used to feed high energy electrons into the ETC at complex I. Energy from these high energy electrons is used to pump protons from the matrix to the intermembrane space. This creates an electrochemical gradient that drives the ATP synthase proton flux and ADP phosphorylation.

A case has been made for increasing the cytosolic NAD<sup>+</sup>/NADH ratio [18, 260]. Activation of SIR2 in *C. elegans* and its mammalian homolog, SIRT1, is associated with life extension in dietary restriction [261]. NAD<sup>+</sup> is consumed by sirtuin enzymes and an oxidized redox state activates sirtuins [262, 263, 264, 265]. It is therefore possible that increasing the cytosolic NAD<sup>+</sup>/NADH ratio may have sirtuin-mediated health benefits. Polyphenolic compounds such as resveratrol, which activate sirtuins, are currently being evaluated for the treatment of human diseases [266, 267, 268, 269, 270, 271]. Activating sirtuins could produce a number of downstream effects, including PGC1 $\alpha$  activation. Increasing the cytosolic NAD<sup>+</sup>/NADH ratio, either through bioenergetic manipulation or by supplying NAD<sup>+</sup> mimetics, should similarly accomplish this goal [272].

It is also possible that pushing the mitochondrial NAD pool to a more reduced state, or a lower NAD<sup>+</sup>/NADH ratio, could benefit brain bioenergetics in conditions where brain bioenergetics are impaired. In this respect Blass and Gibson have advocated malate, which may increase mitochondrial NADH levels, should constitute part of a bioenergetics-based

approach to treating AD [273]. Malate is transported from the cytosol to the mitochondrial matrix, and inside the matrix the oxidation of malate to oxaloacetate generates NADH.

#### 4.7 Manipulating mtDNA

The most direct way of fixing an mtDNA-induced bioenergetic problem would be to correct the responsible mtDNA defect. Approaches to accomplish this have been considered. In the case of a deleterious heteroplasmic point mutation, it is possible under *in vitro* conditions to shift the heteroplasmic ratio towards a greater percentage of wild type mtDNA by selectively blocking replication of mtDNA molecules that contain the mutation [274]. This requires the ability to distinguish between mtDNA sequences. In the past, sequence-specific peptide nucleic acids which can bind complementary DNA sequences but which do not prime extension and which block new strand synthesis have been used to accomplish this [275].

As is the case with nuclear DNA, mtDNA associates with proteins. Transcription factor A of the mitochondria (TFAM) is the most important of these proteins and plays an important role in both mtDNA replication and transcription [276, 277, 278]. Increasing TFAM levels reportedly enhances mtDNA levels, mitochondrial biogenesis, respiration rates, or a combination of these events. One study reported that when TFAM was overexpressed, cell mtDNA levels increased but mitochondrial mass and mitochondrial respiration did not [279]. Another study found TFAM overexpression reduced age-associated complex I and IV reductions in mouse brain, and this was associated with reduced brain oxidative stress and behavioral testing benefits [280].

Administering an exogenous TFAM that is linked to a mitochondrial leader sequence and protein transduction domain reportedly increased respiration and mitochondrial biogenesis in cultured cells and mouse tissues [281]. This strategy may therefore provide a pharmacologic approach for increasing mtDNA replication and transcription *in vivo*, and increase both immediate and long term respiration through these effects. Further, because TFAM readily binds mtDNA, it may be possible to use this recombinant TFAM to deliver exogenous mtDNA molecules to mitochondria [282]. Given that it is already possible to deliver restriction enzymes to mitochondria [283, 284, 285], this could prove to be a powerful way to remove even homoplasmic mtDNA mutations from cells and replace the mutated mtDNA with mtDNA that does not contain the mutation [286].

## 5. MITOCHONDRIAL MEDICINE CONSIDERATIONS

### 5.1 Pros and Cons of Manipulating Cell Energy Supplies

It is assumed that increasing ATP levels in bioenergetically compromised neurons should benefit those neurons. While this is a straightforward assumption, it is necessary to consider data from the dietary restriction literature that in general suggest some degree of bioenergetic stress, or hormesis, can prove advantageous [287, 288]. This is not to suggest that reducing ATP levels to sub-normal levels is good. Rather, forcing neurons towards a more aerobic state could have wide ranging consequences, such as AMPK and SIRT1 activation.

Another point to consider is that the brain is not a metabolically homogeneous entity. It consists of highly aerobic neurons and relatively anaerobic astrocytes. There is an emerging realization that neuron and astrocyte energy metabolism are intricately linked [289, 290, 291, 292, 293, 294]. As part of this relationship, glutamate release by active neurons is followed by astrocyte glutamate uptake. This uptake is coupled to and driven by sodium gradients. Exporting the acquired sodium is an energy dependent process that activates astrocyte aerobic glycolysis and produces lactate. The lactate is then exported to neurons

where it is used to support mitochondrial respiration. This “tripartite synapse” model is illustrated in Figure 5. How manipulating brain energy metabolism may influence this dynamic relationship requires consideration.

## 5.2 Pros and Cons of Manipulating Oxidative Stress

It is assumed that decreasing oxidative stress in bioenergetically compromised neurons should benefit those neurons. Again, although this seems quite reasonable cautionary notes must be taken from other lines of investigation. Oxidative stress actually plays a crucial role in various intracellular signaling pathways [295, 296]. Indeed, dampening oxidative stress has been shown to abrogate the lifespan extension that results in *C. elegans* when the worms are treated with 2-deoxyglucose [287]. Antioxidants also minimize the endurance benefits that result from aerobic training [297, 298, 299]. In both cases, these adverse antioxidant effects associate with reduced mitochondrial biogenesis. It is also worth noting nitric oxide and superoxide signaling have been implicated in learning and memory [300, 301].

## 5.3 Pros and Cons of Manipulating Apoptosis

While it is tempting to assume blocking apoptosis in cells with mitochondrial dysfunction that is severe enough to activate apoptosis cannot have a downside, it is important to keep in mind that the pathways that mediate apoptosis do not exist in an all-or-none state. Some degree of caspase enzyme activity occurs in normal cells and likely has physiologically important and necessary roles [302, 303, 304]. Also, in the case of mtDNA-induced mitochondrial dysfunction apoptosis should represent a relatively downstream event. Changes that occur in the run-up to apoptosis, when occurring below the threshold needed to initiate cell death, may mediate part of a compensatory stress response. Interfering with compensatory adaptations can cause more harm than good. In this respect, it is interesting that minocycline treatment of ALS subjects actually accelerated mortality [169].

## 5.4 Pros and Cons of Manipulating Autophagy

The ability to selectively dispose of damaged or dysfunctional mitochondria is particularly attractive. Moreover, in dietary restriction mitophagy is increased [251, 305, 306]. This would certainly support the view that enhancing mitophagy beyond baseline levels could have therapeutic benefits. Still, for cases in which mtDNA mutation drives mitochondrial dysfunction, it is not clear that mitophagy mechanisms are dysfunctional or that these mechanisms require manipulation. Also, the consequences of enhancing mitophagy in cells with uniformly impaired mitochondria could prove complex. This point is emphasized by the prior finding that mitochondrial respiration itself can differentially influence mitophagy regulation and its functional outcomes [189].

## 5.5 Pros and Cons of Manipulating Mitochondrial Mass

Data suggest that in some tissues, increasing mitochondrial mass may constitute a normal response to age-associated declines in mitochondrial function [185, 307, 308]. This includes the brain. It may even be the case that with age-related diseases in which a particular biochemical or molecular phenomenon represents an exaggeration of a particular age-associated phenomenon, the changeover from aging to disease occurs when compensatory mechanisms cease to compensate [4, 84, 309]. For example, brain mitochondrial mass may increase with normal aging, but in AD itself the number of intact mitochondria is reduced [185]. If this decline contributes to the disease, then inducing mitochondrial biogenesis would seem to represent a logical therapeutic goal.

One potential downside is that increasing the mass of dysfunctional mitochondria within a cell may have adverse consequences. Increasing the mass of dysfunctional mitochondria

within a compromised but still partly functional cell could push oxidative stress or apoptotic signaling above tolerable levels.

### 5.6 Pros and Cons of Manipulating Redox States

As discussed above, one line of reasoning presumes that increasing cytosolic NAD<sup>+</sup>/NADH ratios will enhance bioenergetic capacity. Increasing this ratio should increase glycolysis flux, which may in turn compensate for reduced mitochondrial ATP production. On the other hand, pushing cells that should be in a more aerobic state to a less aerobic state may have unintended consequences.

Another line of reasoning presumes that pushing the mitochondrial NAD pool towards reduction will benefit mitochondrial respiration. It is difficult to know, however, whether increasing mitochondrial NADH levels will increase respiration in functionally impaired mitochondria. A case can even be made for the opposite argument, which is that pushing the mitochondrial NAD pool towards oxidation could improve overall cell function by activating compensatory stress signaling pathways or compensation-enhancing transcription programs. A more oxidized mitochondrial NAD (or FAD) pool might also facilitate the transfer of reducing equivalents from the cytosol to the mitochondria, which could potentially increase glycolysis.

Finally, SIRT2 de-acetylates tubulin and this leads to microtubule depolymerization [310, 311, 312]. Excessive tubulin de-acetylation could potentially disrupt cytoskeletal infrastructure and secondarily impair mitochondrial axonal transport as well as the transport of aggregated proteins [313]. Indeed, in the PD cybrid model microtubule de-acetylation and alpha-synuclein oligomer levels directly correlate, and further data suggest tubulin de-acetylation may contribute to alpha synuclein oligomerization [314]. Consistent with this argument, reducing SIRT2 activity was found to be protective in cell and *Drosophila* PD models [315]. Similarly, an AD transgenic mouse model was shown to benefit from nicotinamide-induced sirtuin inhibition [316].

### 5.7 Pros and Cons of Manipulating mtDNA

It is possible that preventing replication of a particular mtDNA species, or eliminating a particular mtDNA species, could cause a transient or permanent state of relative mtDNA depletion. In such a case cleansing the cell of one form of mtDNA problem could introduce another type of mtDNA problem. Otherwise, when it comes to treating mtDNA-driven mitochondrial dysfunction through correction of a responsible mtDNA defect it is hard to think of a downside. Progress towards accomplishing this goal will hopefully continue.

## 6. CONCLUSIONS

A variety of pathologies observed in the late-onset, sporadic versions of several neurodegenerative diseases may arise from or relate to altered or deficient mitochondrial function. Some of these diseases, including AD, PD, and ALS, have relatively uncommon Mendelian forms in which nuclear gene mutations directly or indirectly induce mitochondrial dysfunction. What initiates mitochondrial dysfunction in the far more common sporadic versions is less clear, although data suggest in this circumstance mtDNA plays a role.

Given the existence of a mitochondrial nexus in common late-onset, sporadic neurodegenerative diseases, efforts targeted towards the therapeutic manipulation of mitochondria certainly seem reasonable. A number of approaches are probably worth pursuing, although two cautionary points are worth making. The first point is that when manipulating a specific aspect of mitochondrial function or mitochondria-related

physiology, it is probably important not to extinguish or reverse potentially compensatory adaptations. The second point is that interventions which seem eminently rationale may have unforeseen negative consequences. Thus, the efficacy of a mitochondrial medicine approach may be maximized and the potential risks minimized by manipulating the most upstream identifiable cause of the observed mitochondrial dysfunction.

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## ABBREVIATIONS

<b>AD</b>	Alzheimer's disease
<b>ALS</b>	amyotrophic lateral sclerosis
<b>AMPK</b>	AMP kinase
<b>APOE</b>	apolipoprotein E gene
<b>BBB</b>	blood-brain barrier
<b>CoQ</b>	coenzyme Q
<b>CREB</b>	cyclic AMP responsive element binding protein
<b>CrP</b>	creatine phosphate
<b>CSF</b>	cerebrospinal fluid
<b>Cybrid</b>	cytoplasmic hybrid
<b>Cyt. C</b>	cytochrome c
<b>DCA</b>	dichloroacetate
<b>DMII</b>	diabetes mellitus type II
<b>ETC</b>	electron transport chain
<b>FA</b>	Friedreich's ataxia
<b>FAD</b>	flavin adenine dinucleotide
<b>FDG</b>	fluorodeoxyglucose
<b>FKHR</b>	forkhead homologue in rhabdomyosarcoma
<b>FOXO</b>	forkhead O-box transcription factor
<b>LHON</b>	Leber's hereditary optic neuropathy
<b>MELAS</b>	mitochondrial encephalopathy, lactic acid, and stroke syndrome
<b>MERRF</b>	myoclonic epilepsy and ragged red fiber syndrome
<b>mLST8</b>	target of rapamycin complex subunit LST8
<b>MAPK</b>	mitogen activated protein kinase
<b>MMSE</b>	mini-mental state exam
<b>MNGIE</b>	mitochondrial neurogastrointestinal encephalopathy syndrome
<b>MPT</b>	mitochondrial permeability transition
<b>MRI</b>	magnetic resonance spectroscopy

<b>mtDNA</b>	mitochondrial DNA
<b>mTOR</b>	mammalian target of rapamycin
<b>NAD</b>	nicotinamide adenine dinucleotide
<b>NAD<sup>+</sup></b>	nicotinamide adenine dinucleotide, oxidized
<b>NADH</b>	nicotinamide adenine dinucleotide, reduced
<b>NARP</b>	neuropathy, ataxia, and retinitis pigmentosa syndrome
<b>PD</b>	Parkinson's disease
<b>PET</b>	positron emission tomography
<b>PGC1<math>\alpha</math></b>	peroxisomal proliferator activated receptor gamma coactivator 1 $\alpha$
<b>PIB</b>	Pittsburgh Compound B
<b>POLG</b>	polymerase gamma
<b>PSP</b>	progressive supranuclear palsy
<b>SIR(T)</b>	Silent information regulator
<b>TOMM40</b>	translocase of the outer mitochondrial membrane 40 gene
<b>TFAM</b>	transcription factor A of the mitochondria
<b>TORC1</b>	mTOR complex 1
<b>TYMP</b>	thymidine phosphorylase gene
<b>VBM</b>	voxel based morphometry
<b>YY1</b>	yin-yang 1

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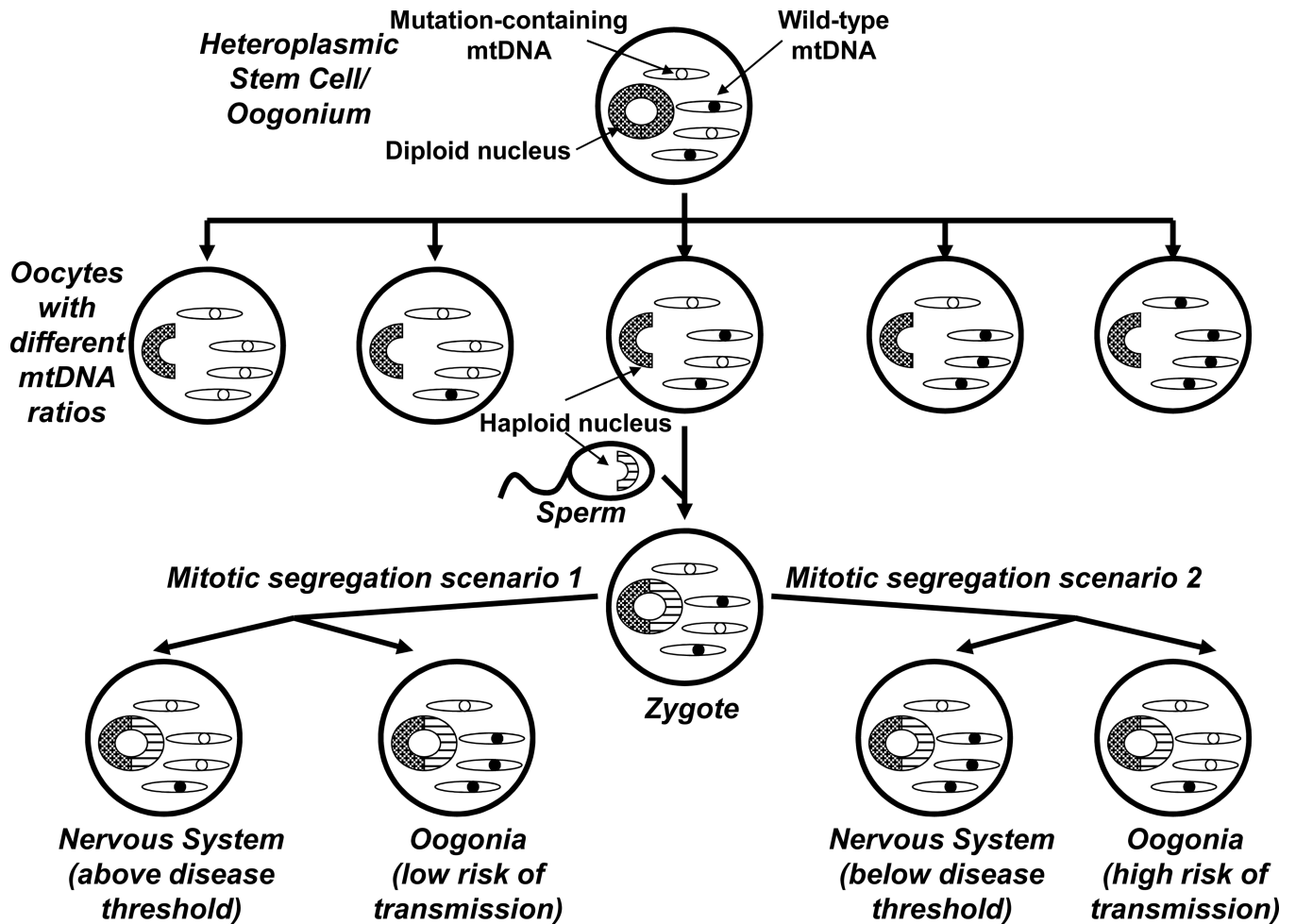
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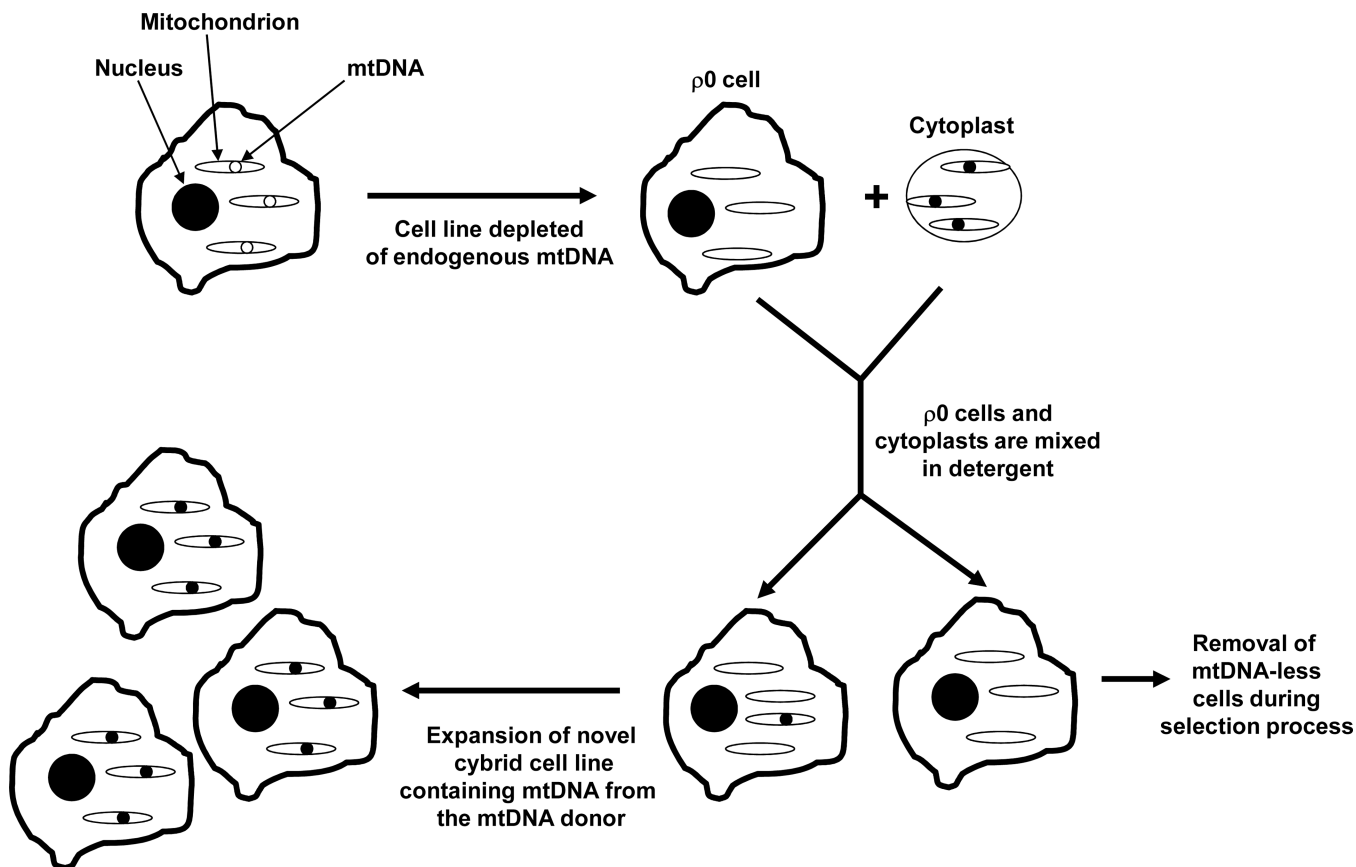
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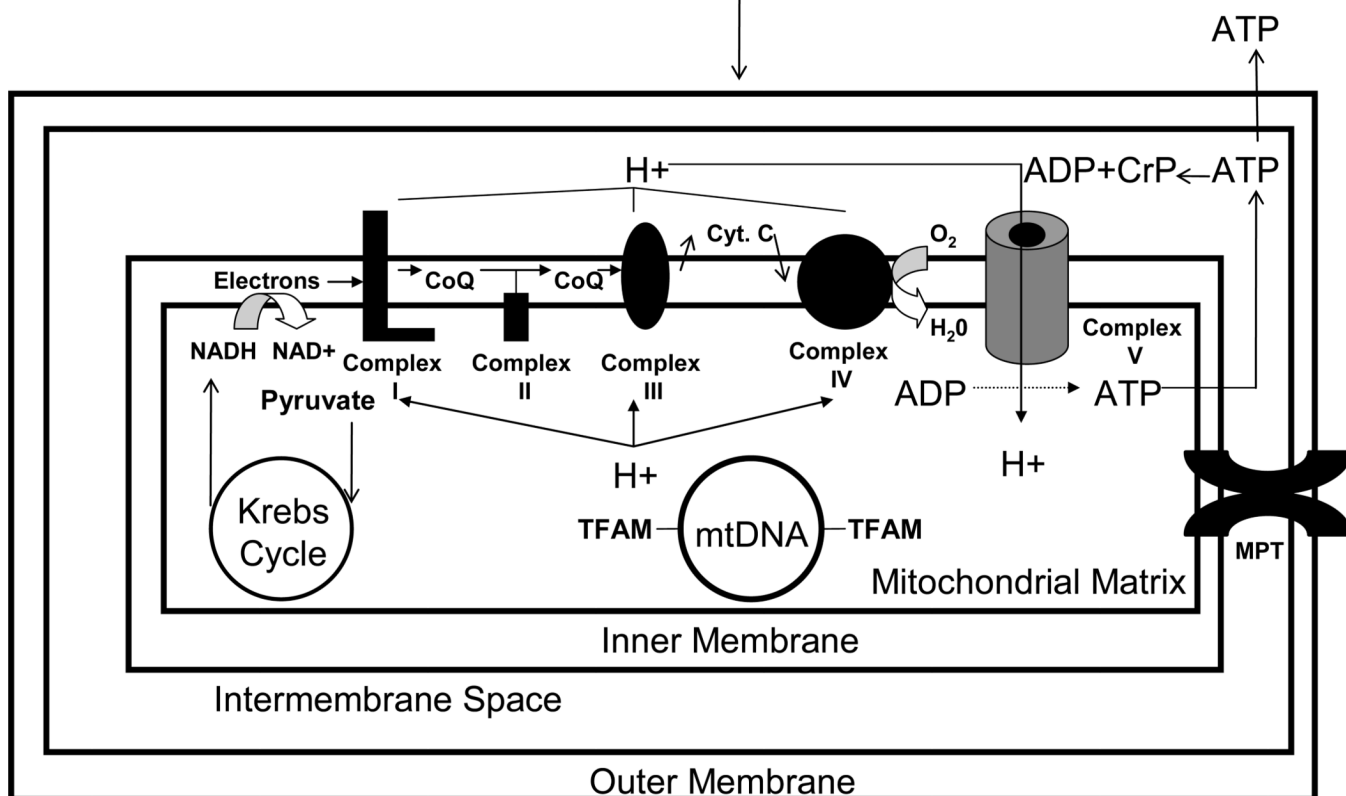
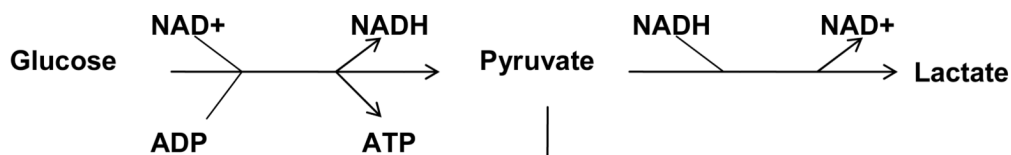
**Figure 1. Maternal inheritance, heteroplasmy, mitotic segregation, and threshold effects distinguish mitochondrial genetics from Mendelian genetics**

During embryogenesis a female generates diploid oogonia, which produce haploid oocytes. An oogonium that contains a heteroplasmic mtDNA mutation may produce oocytes with different mtDNA mutation burdens; oocytes with low mutation levels are less likely to produce disease-affected offspring, while oocytes with high mutation levels are more likely to do so. Fertilization then produces a zygote whose mtDNA comes from the oocyte, with little or no sperm contribution. Because of mitotic segregation, heteroplasmic mtDNA mutations may end up having unequal tissue distributions. In scenario 1, the developing individual has a high mutational burden in the nervous system and low mutational burden in their germ cells. This individual has an elevated neurologic disease risk (surpasses the threshold) and low risk of passing on disease risk (if the individual is a male, there is no chance of passing on disease risk). In scenario 2, the developing individual ends up with a low mutational burden in the nervous system and high mutational burden in their germ cells. This individual does not have an elevated neurologic disease risk (below the threshold), but if the individual is female there is an increased risk of passing on the mtDNA mutation. Therefore, although mtDNA is maternally inherited diseases influenced by mtDNA-inheritance can appear sporadic.

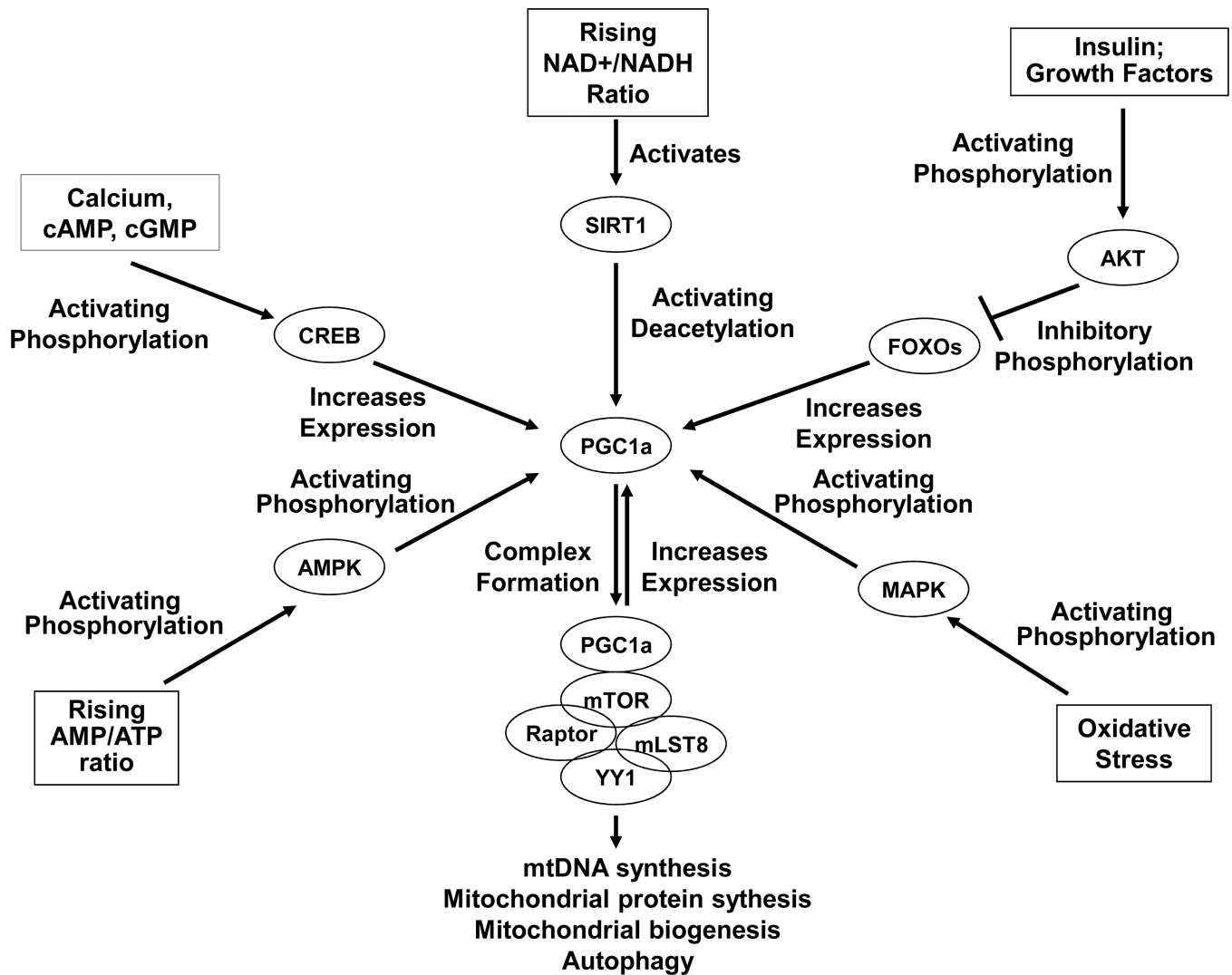


**Figure 2. The cybrid technique**

The cybrid technique typically uses an mtDNA-depleted ( $\rho 0$ ) cell line and non-nucleated cytoplasts (such as platelets). A cybrid cell lines is produced by mixing the cytosolic contents of the  $\rho 0$  cells and cytoplasts. The mixing procedure, which is often facilitated by the temporary addition of a detergent, creates a population of cells that contain both a nucleus (from the  $\rho 0$  cell line) and mtDNA (from the cytoplasm mitochondria). A selection process allows the isolation of these resulting cybrid cells, which can then be expanded in culture and used for biochemical and molecular analyses. The cybrid cell lines created from a common  $\rho 0$  cell nuclear background have equivalent nuclear genes, and the cell expansion environment is equivalent between cell lines. Therefore, biochemical or molecular differences between lines are expected to reflect differences between their mtDNA content.

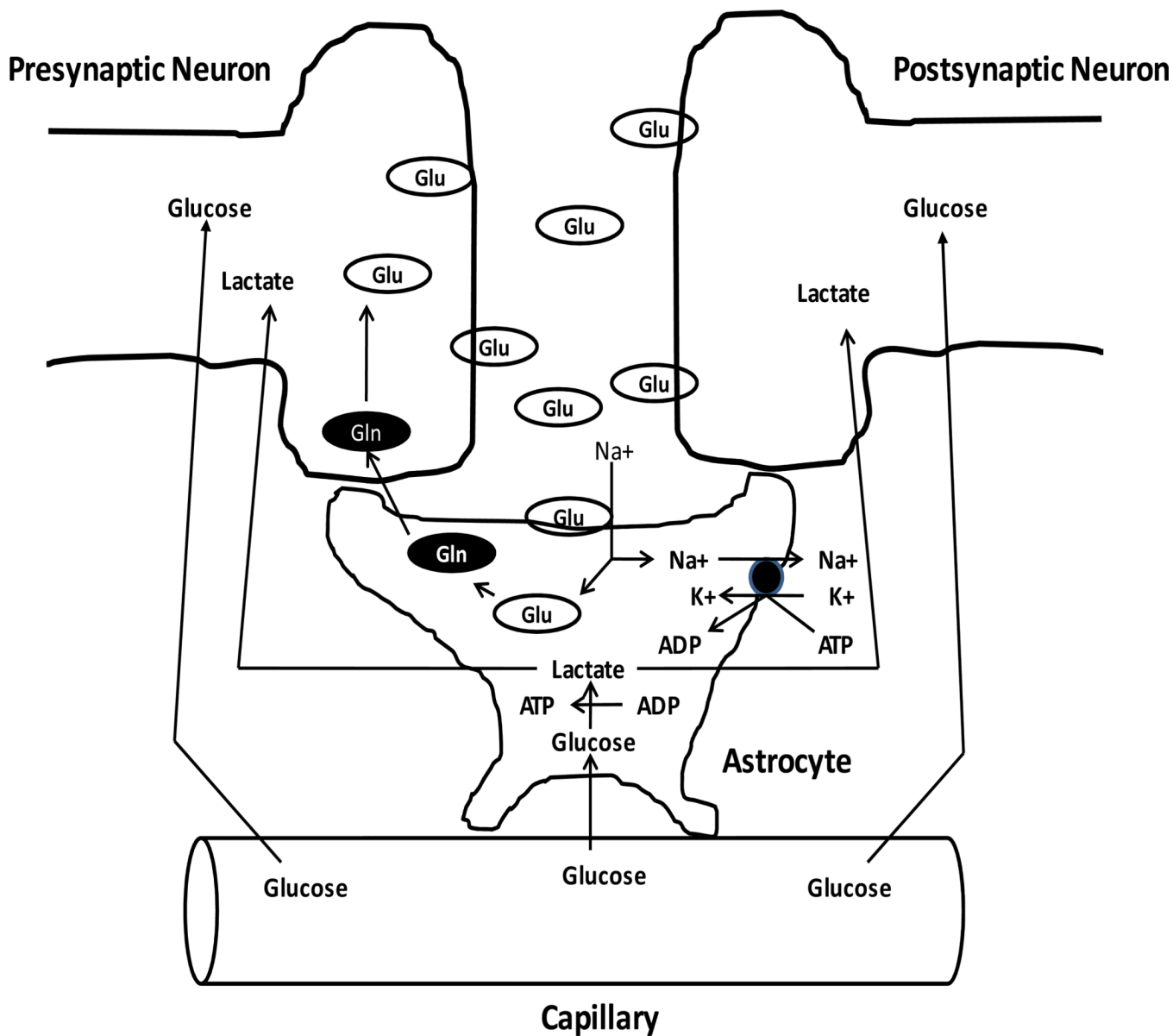


**Figure 3. Simplified schematic of some pathways and cycles involved in ATP production**  
 Carbon intermediates from various sources, especially glycolysis, are imported into mitochondria and consumed to produce high energy electrons. These high energy electrons are transiently stored as reduced NADH. Energy harvested from the electrons fed to the inner mitochondrial membrane's ETC creates a proton gradient; this energy is collected by the ATP synthase in the form of proton flux and used to phosphorylate ADP. High energy phosphate groups can also be stored as creatine phosphate. Also shown is the mtDNA, which is associated with TFAM protein (which helps mediate mtDNA replication and transcription), and the mitochondrial permeability transition (which is activated by mitochondrial membrane depolarization). CoQ=coenzyme; Cr-P=creatine phosphate; Cyt. C=cytochrome C.



**Figure 4. PGC1a, a key regulator of mitochondrial biogenesis, is regulated by various nutrient, energy, and stress-sensing pathways**

This diagram presents a schematic summary of different proteins and transcription factors that regulate PGC1a activity and levels. It is very simplified, and as more is learned about mitochondrial biogenesis the indicated relationships may change. Briefly, SIRT1-mediated PGC1a deacetylation and MAPK-mediated PGC1a phosphorylation induce PGC1a activation. AMPK also activates PGC1a through a direct phosphorylation event or indirectly through intermediates. The CREB transcription factor activates PGC1a expression. A FOXO transcription factor, FKHR, also appears to activate PGC1a expression; by retaining FOXOs in the cytosol, AKT phosphorylation of FOXOs would prevent this contribution. Activated PGC1a reportedly forms a super-complex with the mTOR-containing TORC1 complex (mTOR, raptor, and mLST8) and the YY1 transcription factor. This activated super-complex expresses genes needed to replicate mtDNA, produce mitochondrial proteins, accomplish mitochondrial biogenesis, and support autophagy. It also leads to the production of more PGC1a.



**Figure 5. The tripartite synapse**

An action potential induces glutamate release from a pre-synaptic neuron, which activates an excitatory post-synaptic potential. The glutamate signal is terminated by an astrocyte, which removes the glutamate from the synaptic cleft; this astrocyte glutamate uptake is driven by the plasma membrane sodium gradient. Astrocyte sodium-potassium ATPase pumps are activated by the sodium influx. The ATP this consumes is regenerated in the astrocyte through glycolysis, and generates lactate. The astrocyte lactate is exported by the astrocyte, imported by the neighboring neurons, and used by those neurons to fuel aerobic ATP production. This process also stimulates astrocyte uptake of capillary glucose, and provides for the return of the glutamate carbons, in the form of glutamine, to the presynaptic neuron.

**Table 1**  
**Specific respiratory chain defects observed in cybrid models of human diseases**

This table summarizes the most consistently reported results from disease-oriented cybrid studies performed by different groups. Please note for some diseases there have been studies that report detectable abnormalities in alternative complexes or no detectable abnormalities. See text for details and references.

Disease	Defect
Parkinson's disease	Complex I
Alzheimer's disease	Complex IV
Amyotrophic lateral sclerosis	Complex I
Progressive supranuclear palsy	Complex I
Leber's hereditary optic neuropathy	Complex I
MELAS	Complex I with lower heteroplasmic burdens; Complexes I, III, and IV with higher heteroplasmic burdens
MERRF	Can include Complexes I, III, and IV
NARP / maternally inherited Leigh's syndrome	Typically complex V; ETC enzymes may also be effected