Redox Biology 2 (2014) 619–631

Contents lists available at ScienceDirect

Redox Biology

journal homepage: www.elsevier.com/locate/redox

Review Article

Cytoplasmic hybrid (cybrid) cell lines as a practical model for mitochondriopathies

Heather M. Wilkins[a], Steven M. Carl[a], Russell H. Swerdlow[a,b,*]

[a]Department of Neurology and the University of Kansas Alzheimer's Disease Center, University of Kansas Medical Center, Kansas City, KS, United States of America
[b]Departments of Molecular and Integrative Physiology and Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, KS, United States of America

ARTICLE INFO

Article history:
Received 26 March 2014
Accepted 28 March 2014

Keywords:
Cybrid
Mitochondria
Mitochondrial DNA
Rho zero

ABSTRACT

Cytoplasmic hybrid (cybrid) cell lines can incorporate human subject mitochondria and perpetuate its mitochondrial DNA (mtDNA)-encoded components. Since the nuclear background of different cybrid lines can be kept constant, this technique allows investigators to study the influence of mtDNA on cell function. Prior use of cybrids has elucidated the contribution of mtDNA to a variety of biochemical parameters, including electron transport chain activities, bioenergetic fluxes, and free radical production. While the interpretation of data generated from cybrid cell lines has technical limitations, cybrids have contributed valuable insight into the relationship between mtDNA and phenotype alterations. This review discusses the creation of the cybrid technique and subsequent data obtained from cybrid applications.

© 2014 The Authors. Published by Elsevier B.V.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/).

Mitochondria are an essential cellular reticulum driving the molecular reduction of oxygen into the energy molecule ATP. The presence of a separate mitochondrial genome was discovered in 1963 [1,2]. The mitochondrial genome is comprised of circular double-stranded DNA containing 16,569 base pairs. While not all components of the electron transport chain (ETC) are encoded by the mitochondrial genome, its integrity is vastly important for ETC function. The mitochondrial genome encodes 11 components of the ETC and two subunits of ATP synthase (Fig. 1). In addition, the mitochondrial genome contains 24 tRNA/rRNA genes, which are required for translation of mitochondrial proteins (Fig. 1). The importance of mtDNA is highlighted by findings of mtDNA mutations and mitochondrial dysfunction in diseases associated with aging [3–5].

The cybrid technique

Rho zero cells

To facilitate cybrid generation, cells devoid of mtDNA are commonly utilized. These cells are termed ρ0 cell lines because prior to identification of the mitochondrial genome the DNA found in the cytoplasm of cells was termed "ρ-DNA" [6]. The ability to deplete mtDNA was first discovered in yeast models where natural mtDNA depletion occurred under conditions in which glycolysis was favored over mitochondrial respiration. This led to artificial techniques to deplete cells of mtDNA, the oldest of which is the use of the DNA-intercalating agent ethidium bromide (EtBr). EtBr, a positively charged aromatic compound, is attracted to negatively charged DNA molecules located within negatively charged mitochondrial matrices. Intercalation of EtBr into DNA results in the failure of DNA replication by DNA polymerase. The use of EtBr in yeast cells culminated in partial and complete mtDNA depletion [7–9].

When extended to vertebrate cells, the development of mtDNA depletion protocols proved more difficult. In one reported early attempt, while treatment with EtBr did result in almost complete depletion of mtDNA from the VA_2B human cell line, the high concentrations of EtBr that were used were toxic. Additionally, immediately following removal of EtBr, mtDNA was replenished [10].

The first successful vertebrate ρ0 cell lines were made in chicken embryo fibroblast cells using EtBr. The investigators determined the resulting cells were auxotrophic for the pyrimidine nucleoside uridine [11]. The basis for this can be explained by the function of dihydroorotate dehydrogenase. This mitochondrial enzyme is required for the synthesis of pyrimidines and relies upon the ETC to function [12]. Therefore, depletion of mtDNA and subsequent ETC failure will disrupt pyrimidine synthesis. Supplementing mtDNA- depleted cells with uridine bypasses the ETC-dependent, dihydroorotate dehydrogenase-catalyzed step in the pyrimidine synthesis pathway. An avian ρ0 cell line was subsequently generated using the same protocol, reducing mtDNA copy number from 300 per cell to undetectable levels [13]. It is important to emphasize, though, that ρ0 cells retain mitochondria [14]. These mitochondria are not respiration-competent, but they do retain a membrane potential, presumably by operating their ATP synthase in reverse. Although their structure

* Correspondence to: Department of Neurology, 3901 Rainbow Blvd MS 2012, University of Kansas Medical Center, Kansas City, KS 66160, USA.
E-mail address: rswerdlow@kumc.edu (R.H. Swerdlow).

2213-2317/$ - see front matter © 2014 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/).
http://dx.doi.org/10.1016/j.redox.2014.03.006

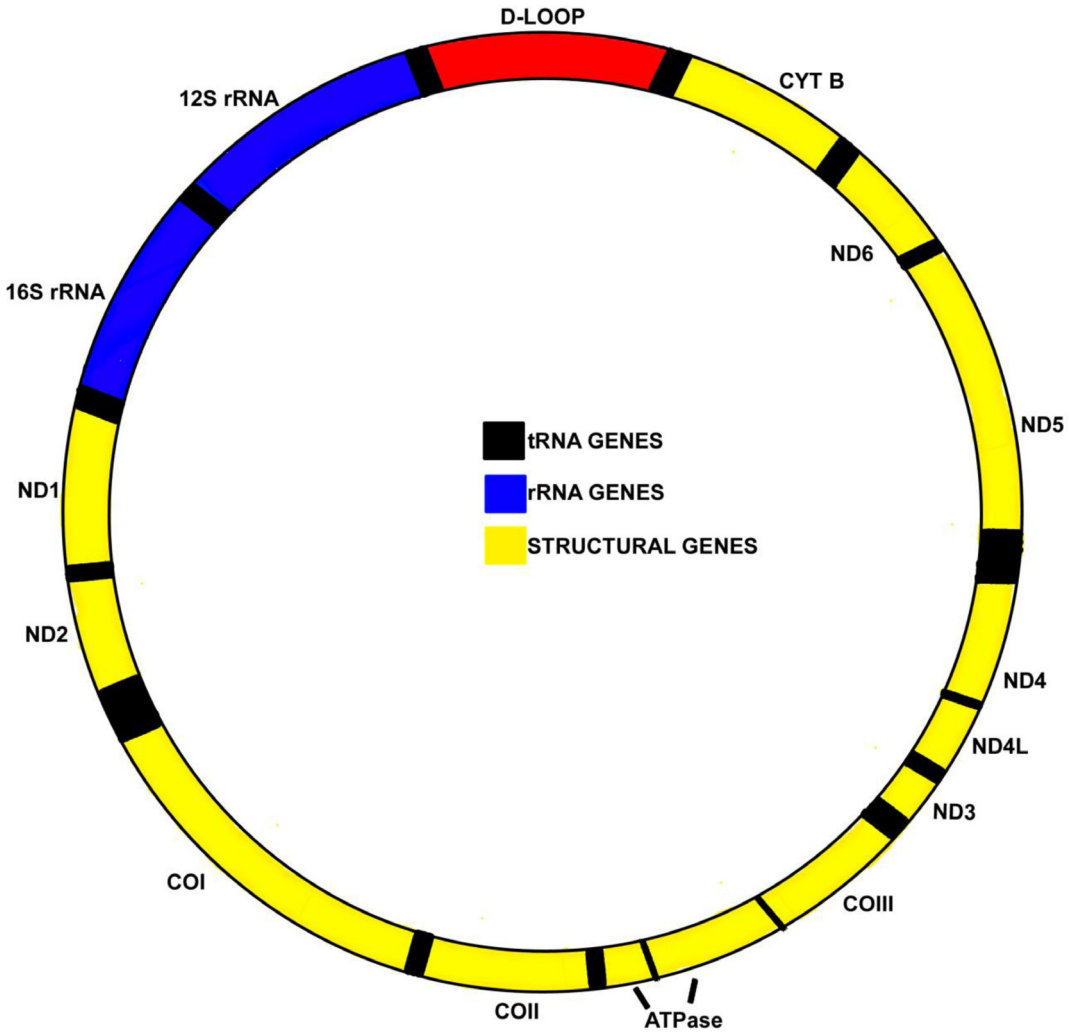

Fig. 1. The mitochondrial genome. This figure depicts the circular mitochondrial genome. Structural genes are shown in yellow. rRNA genes are shown in blue, while tRNA genes are shown in black. The regulatory region, D-loop, is shown in red. CO = cytochrome oxidase, ND = NADH dehydrogenase.

is altered they undergo proliferation and receive nuclear-encoded mitochondrial proteins. These respiration-incompetent mitochondria have been colloquially referred to by some authors as "mitoids" [15].

In 1989, the human osteosarcoma 143B cell line was depleted of mtDNA. The ρ0 clones (143B101 and 143B106) were auxotrophic for both uridine and pyruvate [16]. The necessity for pyruvate supplementation was hypothesized to be required for the maintenance of cellular redox status [17]. Cells devoid of mtDNA rely upon glycolysis for ATP production. Under physiological conditions, glycolysis increases NADH levels, while mitochondrial respiration produces NAD^+. Therefore, the absence of mitochondrial respiration significantly increases the NADH: NAD^+ ratio, interfering with glycolytic capacity. Supplementation with pyruvate leads to the formation of lactate, which generates NAD^+, thus allowing glycolytic function and ATP production. Additional cell lines have been depleted of mtDNA over the course of time. Table 1 provides a list of currently reported ρ0 cell lines.

To understand the mechanisms of mtDNA depletion, it is important to understand that the depletion of mtDNA occurs in two steps. First is the cessation of mtDNA replication. This step does not eliminate existing mtDNA molecules, but terminates their ability to replicate the mtDNA. As the cells divide, the mtDNA is divided among the new cells, leading to continuous dilution of the mtDNA pool. This creates a cell population in which some cells contain mtDNA while other cells lack the mtDNA—and are now "ρ0". The second step begins at this point, in which ρ0 cells are isolated and expanded, or mtDNA depletion continues until all mtDNA is degraded.

Table 1
Reported ρ0 cell lines.

ρ0 cell line	Reference
143B osteosarcoma	[16]
HeLa cervical carcinoma	[18]
A549 lung carcinoma	[19]
Lymphoblastoid, Wal2A	[20,21]
SH-SY5Y neuroblastoma	[22]
NT2 teratocarcinoma	[23,24]
U251 glioma	[25]
Fibroblast/transformed fibroblast	[26]
LL/2-m21 mouse	[27]
T-cell lymphoblastic leukemia, molt-4	[28]

Further development of techniques to deplete cells of mtDNA have moved away from the use of EtBr. The mitochondrial DNA polymerase γ inhibitor, ditercalinium, or expression of a dominant negative mitochondrial DNA polymerase γ construct have been used successfully to create ρ0 cell lines [29,30]. Exposure to dideoxynucleoside analogues, which interfere with mtDNA replication, leads to mtDNA depletion myopathy and is another technique for creating ρ0 cell lines [31,32].

Rhodamine 6-G, first noted for its ability to interfere with mitochondrial ETC function, is an additional tool for the generation ρ0 cell lines [33–35]. More recent techniques to deplete mtDNA have used the expression of mitochondrial targeted EcoRI, which is the focus of a biotech company, Rho Zero Technologies [36,37].

The generation of ρ0 cell lines requires techniques to verify depletion of the mtDNA. Several applications have been reported describing how to document ρ0 status. Techniques include southern blotting, PCR of mtDNA, competitive PCR to ratio mtDNA and nuclear pseudogenes, measurement of oxygen consumption and cytochrome c oxidase activity, pico green staining, and uridine/pyruvate auxotrophy [18,19,21–26]. The majority of these techniques depend upon the demonstration of a negative result. Most of these techniques, therefore, are limited by the sensitivity of the mtDNA detection protocol. Testing for uridine/pyruvate auxotrophy, however, is a practical approach which does not rely upon a negative result. A more recently developed technique, competitive PCR, is more labor intensive but also does not rely upon demonstration of a negative result. The competitive PCR approach provides a means to statistically analyze the probability of mtDNA depletion and that ρ0 status has been achieved [25].

mtDNA Transfer

During the development of mtDNA depletion techniques, methods for transferring mtDNA into cells were simultaneously being established. The first attempt, in 1972, led to the creation of a heterokaryon hybrid cell, which used the Sendai virus to fuse cells together [38]. Two years later, a separate technique was carried out using mixtures of nucleated cells and cytoplasts (non-nucleated cells) [39]. Bunn et al. called the resulting cells "cybrid" cells, distinguishing them from the term "hybrid", which implies the mixture of two nucleated cells.

The cybrid technique was first utilized in 1975, to determine if chloramphenicol resistance was mediated by the cytoplasm in a human cell line [40]. Cytoplasts were generated from a chloramphenicol resistant HeLa cell line fused with a chloramphenicol susceptible HeLa cell line that was also thymidine kinase negative (TK^-). The difference in TK content allowed for a selection process to eliminate intact chloramphenicol resistant cells which may have failed the enucleation procedure. This is important because both HeLa cell lines perpetuate, and contamination with intact chloramphenicol-resistant cells could lead to confounded results. It was ultimately concluded that the cybrid cells were chloramphenicol resistant, and that this characteristic was dependent upon the cytoplasm of the cell. Fig. 2 depicts this type of cybrid generation strategy, in which mitochondria are transferred from one nuclear partner to another, even though the recipient nucleated cell is not a ρ0 cell.

A separate cybrid technique—first reported in 1989—fuses enucleated cytoplasts with ρ0 cells. Cytoplasmic transfer to the ρ0 cell line was mediated through the use of polyethylene glycol, as well as direct injection of cytoplast mitochondria [16]. Repopulation of the ρ0 cell line with mtDNA was depicted through loss of uridine/pyruvate auxotrophy and restoration of oxidative phosphorylation biochemical measurements. Fig. 2 shows a schematic for this cybrid generation strategy. To further simplify the cybrid technique, Chomyn et al. [41] used a procedure in which platelets were employed as cytoplasts. Platelets do not contain a nucleus, therefore pre-fusion enucleation of the cytoplasmic donor cell is not required.

Two separate studies subsequently reported that synaptosomes from brain tissue could also serve as cytoplasts for mtDNA fusion [42,43]. However, the use of synaptosomes has overall proved challenging, because the efficiency of mtDNA transfer is low when fresh brain extracts are used from mice and dismal when post-mortem human brain tissue are used [44].

The use of cybrids to study mitochondrial genetics and mtDNA-nuclear DNA compatibility

Early cybrid studies used fibroblast mitochondria which contained mtDNA that harbored known pathogenic mutations. The major goal of these initial studies was to understand the pathological mechanisms underlying diseases associated with mtDNA mutations. To better understand the power of cybrids for this application, it is worth reviewing some basic mitochondrial genetic tenets.

Cells contain numerous copies of mtDNA. Homoplasmy defines a state in which the mtDNA sequence of different mtDNA molecules within a cell is always the same. Homoplasmy is the mitochondrial equivalent of nuclear homozygosity. However, it is reasonably common for mtDNA molecules within a cell, tissue, or organism to exhibit sequence heterogeneity. This state is called heteroplasmy. Heteroplasmy can be considered to loosely correspond to nuclear heterozygosity, but in reality it is far more complex. Because mtDNA is not a binary system like nuclear DNA, different ratios of wild-type and mutant mtDNA molecules can reside within a single mitochondrion or a single cell. Therefore, a heteroplasmic mutation can be present in varying degrees, and in either low or high abundance.

Threshold refers to the mtDNA mutational load that is required within a cell to cause a biochemical or phenotypic consequence. It is reasonable to hypothesize that the more pathogenic the mtDNA mutation, the lower abundance is necessary to reach a threshold that changes mitochondrial and, therefore, cell physiology. A schematic depicting the use of cybrids to study mtDNA heteroplasmy is shown in Fig. 3A.

The A3243G mtDNA mutation associated with the mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes syndrome (MELAS) was studied using the cybrid model. mtDNA nucleotide 3243 is located within one of the two mtDNA genes that encodes a leucine tRNA (tRNA Leu^{UUR}). This mutation does not produce an aberrant protein because the gene product is synthetic and not structural in nature. MELAS cybrids were made using osteosarcoma ρ0 cell lines, and found that a high mutational burden of 85% was required before phenotypic consequences were observed [45–48]. The mechanism leading to biochemical changes with this mtDNA mutation is related to translation deficiency of mtDNA-encoded structural proteins [45,49]. Following these initial studies, it became apparent that the nuclear background of the ρ0 cell used influenced the mitochondrial genotype–phenotype relationship. In the osteosarcoma MELAS cybrid model, a 90% mutational burden resulted in a 30% decrease in cytochrome oxidase activity, while an A549 lung carcinoma MELAS cybrid model required only a 55% mutational burden to observe a 50% reduction in cytochrome oxidase activity [50]. Both cybrid models required substantial mtDNA mutation load to alter biochemical endpoints compared to those required in human muscle [51,52]. Finally, nuclear differences in mutational drift were observed over time. For example, one nuclear background led to drift away from the mutant mtDNA, while a separate nuclear background led to drift toward the mutant mtDNA [50,53,54].

Other cybrid studies have examined mtDNA transfer from people with Leber's hereditary optic neuropathy (LHON). LHON is associated with mutations in mtDNA structural genes, unlike the synthetic gene mutations observed in MELAS. No single mutation leads to the LHON phenotype, as several distinct mutations have been reported. All mutations that lead to the LHON phenotype are found in structural genes, and mutations within the NADH dehydrogenase (ND) genes, which encode for Complex I subunits, are most common. The most frequently reported mutation is G11778A, which resides in the ND4 gene. LHON cybrid data suggests that biochemical consequences depend upon the mutation and nuclear background of the ρ0 cell line that is utilized. For example, LHON mutations that show no Complex I defects in one nuclear background can show Complex I defects in a separate nuclear background. The only consistent parameters

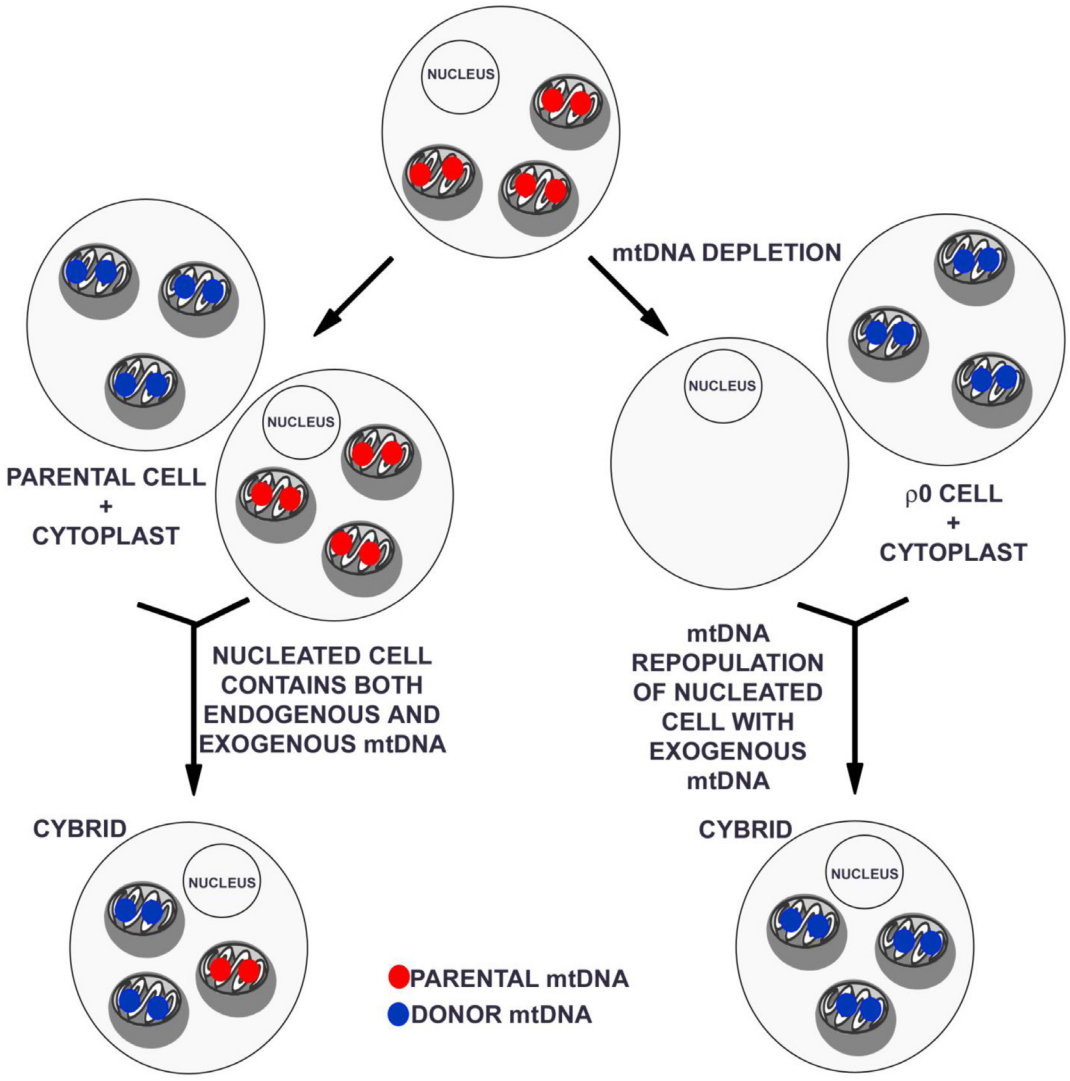

Fig. 2. Cybrid generation techniques. Cybrids are produced by combining cytoplasm from nucleated cells with non-nucleated cells or cytoplasts. The nucleated cell can be unaltered, or could have undergone depletion of endogenous mtDNA before cytoplasmic mixing. In either case, the goal is to populate the nucleated cell with mtDNA from the non-nucleated cell.

among various LHON cybrids are oxygen consumption deficits and a reduction in Complex I-dependent ATP synthesis. All phenotypic differences required a high mutational load, which is consistent with what is observed in the clinical setting, where people afflicted with LHON are homoplasmic or the mtDNA mutation is present at a high copy number [21,55–61].

LHON cybrid studies using an osteosarcoma nuclear background more recently reported that mitochondrial haplogroup status is also an important determining factor for the pathogenic potential of different LHON mutations [62–64]. Mitochondrial haplogroups are determined by ancestral hereditary. Mitochondrial haplogroup J and Uk are found at higher levels in persons afflicted with LHON, however haplogroup H is under represented in the incidence of LHON. Cybrids from different human mitochondrial haplogroups were generated using platelets fused on the osteosarcoma nuclear background. Haplogroup Uk and J have decreased mtDNA, mitochondrial RNA and mitochondrial protein translation products compared to haplogroup H. In addition, haplogroups Uk and J have lower oxygen consumption, ATP production, and mitochondrial membrane potential when compared to haplogroup H. In particular, LHON osteosarcoma cybrids had accumulations of low-molecular weight sub-complexes, particularly from Complex I structural proteins. However, polymorphisms in mtDNA, or specific mitochondrial haplogroups, influenced the assembly rates and stability of Complex I, III, and IV [62]. Overall, cybrid studies have contributed and will continue to be important to the understanding of how haplogroups influence multifactorial diseases, such as LHON.

Further LHON cybrid studies used the teratocarcinoma NT2 cell line as a nuclear background donor [35]. From a modeling perspective, the use of this ρ0 cell line to generate LHON cybrids may be argued to be more closely related to the disease itself, since NT2 cells display “neuronal-like” features and can be differentiated into neurons using retinoic acid [65]. It was found that undifferentiated LHON NT2 cybrids did not have altered biochemical parameters, but upon differentiation parametric differences could be observed. The phenotypic differences observed in the LHON NT2 cybrids were diminished efficiency in the differentiation protocol and production of higher levels of reactive oxygen species (ROS) when compared to control NT2 cybrids. An additional study using LHON NT2 cybrids depicted these cells were more sensitive to H_2O_2, displayed no change in superoxide dismutase (SOD) activity, but had decreased glutathione (GSH) levels [66]. However, these data again depict the importance of nuclear background, as similar LHON cybrids generated on an osteosarcoma

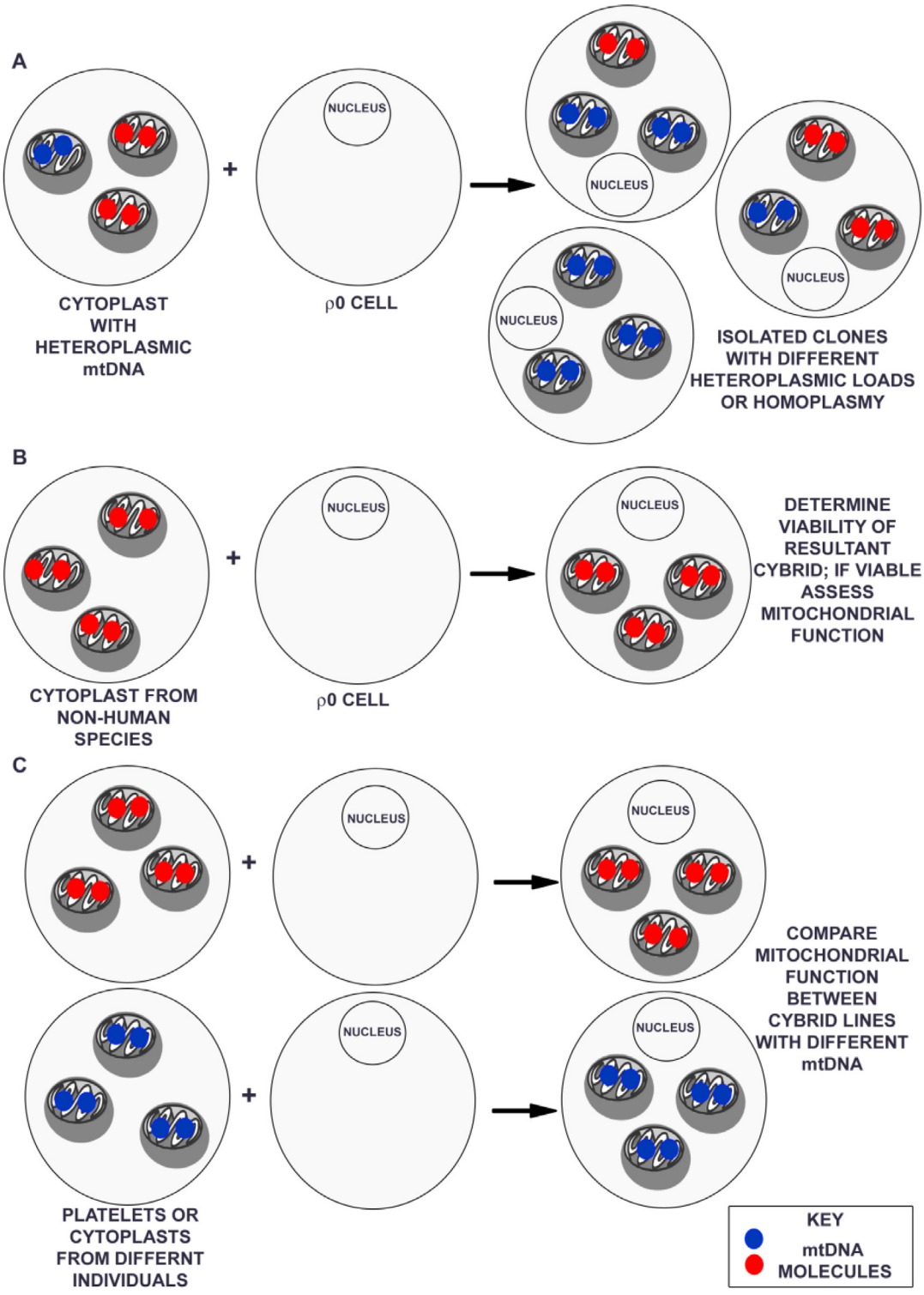

Fig. 3. Applications of the cybrid model. Cybrids can be used to (A) evaluate heteroplasmy-threshold corrections. It is unknown whether heteroplasmic mtDNA changes are found at the cellular level or the level of each mitochondrion. Such that, a single mitochondria could contain both wild-type and mutated mtDNA, or a cell could contain a population of mitochondria which have wild-type mtDNA and a population of mitochondria which have mutant mtDNA. Beyond that, a particular cell could contain all wild-type mtDNA, while a separate cell contains all mutant mtDNA. (B) Test mtDNA–nuclear DNA compatibility and (C) screen for mtDNA-determined functional differences between cell lines containing mtDNA from different sources.

background showed increased ROS production, decreased SOD activity, but no reduction in GSH levels [67].

Other diseases with known mtDNA mutations have been studied using the cybrid technique. These include myoclonic epilepsy and ragged red fiber disease (MERRF), Leigh's disease due to mtDNA mutation/neuropathy, ataxia, retinitis pigmentosa (NARP), and Kearns–Sayre Syndrome [41,68–73]. Kearns–Sayre Syndrome involves deletions of mtDNA, and cybrid work has resulted in notable findings [72]. One important finding is that large mtDNA deletions result in mitochondrial respiration failure, but mtDNA duplications do not. In addition, osteosarcoma cybrid cell lines, it appears, prefer to maintain a stable mtDNA total amount, as opposed to maintaining a constant mtDNA copy number. When osteosarcoma cells are predominately populated with mtDNA that contains large deletions, mtDNA copy number increases to compensate, which ultimately results in an amount of total mtDNA that is equivalent, in mass to that of cells without large deletions.

Studies examining mtDNA-nuclear DNA compatibility have also utilized the cybrid approach. These studies generated xenomitochondrial cell lines, where cytoplasts from primate fibroblasts were fused with a human osteosarcoma ρ0 cell line [74,75]. This mtDNA transfer gave rise to viable cybrid cell lines when the transferred mtDNA was derived from common chimpanzee, pigmy chimpanzee or gorilla fibroblasts. Orangutan, Old-world monkey, New-world monkey, and Lemur mtDNA were not compatible with the human osteosarcoma nuclear background, as no viable cybrid cells resulted. Cybrids expressing common chimpanzee mtDNA displayed a 20% lower oxygen consumption rate, while cybrids expressing pigmy chimpanzee mtDNA had 34% reduced oxygen consumption, and cybrids expressing gorilla mtDNA had 27% less oxygen consumption. No deficits were found for any of the xenomitochondrial cybrids with respect to Complex II, III, or IV activities. However, cybrids expressing common chimpanzee mtDNA showed a 41% reduction in Complex I activity, while cybrids expressing pigmy chimpanzee mtDNA had a 43% reduction in Complex I activity, and cybrids expressing gorilla mtDNA had 45% lower complex I activity. These are findings of interest because there are 159 amino acid differences between human and common chimpanzee mtDNA coding, 155 amino acid differences between human and pigmy chimpanzee mtDNA coding, 195 amino acid differences between human and gorilla mtDNA coding, and 411 amino acid differences between human and orangutan mtDNA coding. The conclusions drawn from this study are that primates which evolved longer than approx. 18 million years ago cannot function to replace human mtDNA. Fig. 3B shows a schematic of this cybrid application.

The use of cybrids as a Model for Mitochondriopathy-Associated sporadic diseases

Cybrid models have also been applied to test mitochondrial and cell physiology alterations arising from un-sequenced mtDNA. Here, mtDNA is transferred from an individual with a particular condition or disease, as shown in Fig. 3C. The resulting cybrid cell lines are expanded and used to measure biochemical and molecular endpoints, and compared to cybrid cell lines derived from control patients. Due to the nuclear background and environment (i.e. cell culture parameters) being constant, the differences observed in measured indices are assumed to reflect differences in mtDNA [76]. However, several criticisms of this cybrid application have arisen.

Most controversy surrounding this cybrid application is due to its assumption that the functional differences in cybrid cell lines are due to differences in mtDNA. Because the cybrid technique does not solely transfer isolated mtDNA, this assumption of differences being solely due to mtDNA is a leap of faith in some aspects. At a minimum, whole mitochondrial transfer occurs from donor platelets or cytoplasts. However, during the time it takes to select for cells repopulated with mtDNA, the cybrids pass through multiple cell division cycles, during which any possible non-perpetuating materials that were transferred are repeatedly diluted. For a case in which a doubling time of 24 h is expected, several weeks of cybrid selection and expansion would result in over a billion-fold dilution of any non-perpetuating material. Therefore, theoretically the only transferred cytoplast or platelet component that is self-perpetuating is mtDNA.

Another common criticism of this cybrid application is verification of results. Presumably, if a goal of using cybrid models is to implicate the presence of mtDNA differences in structural, synthetic, or regulatory genes (such as the D-loop), then actual nucleotide changes should be demonstrable. While this notion is conceptually correct, it is important to note that correlating specific nucleotide deviations to specific phenotype changes is challenging because mtDNA sequences between individuals tend to vary substantially. The question, therefore, is often not whether a particular mtDNA donor has sequence variations, but whether deviations that can be detected are functionally relevant. This differs from studies of Mendelian diseases, in which the functional relevance of a particular DNA sequence is generally resolved by tracking the suspect variation through family pedigrees; addressing whether the sequence variation is exclusive to a particular phenotype is relatively straightforward. However, the mitochondrial genome is not inherited through Mendelian patterns. mtDNA is normally inherited maternally, although rare cases of mutant mtDNA inheritance via sperm have been reported [77]. While some mtDNA-associated diseases do show recognizable maternal inheritance patterns [78], it is increasingly suspected that a majority of the time mtDNA influences disease risk or development in a primarily sporadic or pseudo-sporadic fashion [79,80]. Overall, this limits the ability to associate mtDNA sequence variations with a particular disease state.

When it comes to establishing mtDNA sequence-phenotype associations, though, the greatest limiting factor may turn out to be mtDNA heteroplasmy. The traditional Sanger-based sequencing approach is not designed to detect low abundance heteroplasmic mutations; if the mutational load is less than 20–30%, the ability to detect the mtDNA change is unreliable [81,82]. Further, recent data indicate mtDNA contains large numbers of low percentage, "microheteroplasmic" sequence variations [3,83–86]. It has been postulated that these microheteroplasmic variations are present at 1–2% abundance [87]. Microheteroplasmic variations within tissue also exist, and the presence of "compound microheteroplasmy", once postulated [88], has since been verified using next generation sequencing [86].

In addition to questions over the functional significance of mtDNA sequence deviations, the origin of especially heteroplasmic sequencing deviations may be difficult to prove. mtDNA sequence mutations that are anatomically limited within an individual may arise from inherited mutations via mitotic segregation (the mtDNA correlate of mosaicism), or represent somatically acquired mutations. Therefore, we will consider cybrid studies that depict biochemical differences between mtDNA donors as simply supporting the notion that functionally relevant mtDNA differences exist between the mtDNA donors, without concluding whether those differences are inherited or acquired.

Cybrid models have been used to screen for mtDNA aberrancy in several neurodegenerative diseases, particularly Parkinson's disease (PD) and Alzheimer's disease (AD). When the first cybrid studies of PD and AD were performed, it was already known that people afflicted with these diseases had specific ETC deficits [89,90]. Many argue that mitochondrial defects are secondary pathologies and/or irrelevant to the phenotype of these diseases. However, the fact remains that mitochondrial function within these diseases is not normal. This has led to these diseases being classified as "neurodegenerative mitochondriopathies" [91].

It has been repeatedly shown that Complex IV activity is reduced in AD subjects, and that Complex I activity is reduced in PD patients [76,92–95]. These deficits are not only found in the brains of those

afflicted with AD and PD, but also systemically in platelet mitochondria. The sporadic and systemic nature of these diseases makes the cybrid model a good application for study [96]. The cybrid technique seemed a reasonable approach to address the question of whether mtDNA accounted for at least part of the lesions observed in AD and PD.

The first PD cybrid study used the human SH-SY5Y neuroblastoma ("neuron-like") ρ0 cell line as the nuclear background. Platelets from 24 PD subjects and 28 age-matched control subjects provided mtDNA [15]. ETC Vmax measurements were taken from post-nuclear mitochondria enriched fractions from each cell line, 52 in total. Absolute Vmax levels were corrected for total protein content of mitochondrial-enriched fractions. The Complex I Vmax was decreased by 20% in the PD group compared to the control group. No difference was observed in Complex IV Vmax measurements between groups. This indicated mitochondrial repopulation was equivalent between PD and control groups. This study supports the notion that Complex I deficits observed in sporadic PD patients are at least partially determined by mtDNA.

Deficits in Complex I activity in PD cybrids compared to control cybrids were verified by four independent studies. One study used the SH-SY5Y ρ0 cell line as the nuclear background, while the other studies used the A549 lung carcinoma ρ0 cell line or the NT2 teratocarcinoma cell line, respectively [97–100]. A separate study was completed in which cybrids were generated from a large family in which multiple members had PD [78]. The pattern of PD inheritance of this family was consistent with maternal transmission. Cybrids generated from family members afflicted with PD had decreased Complex I activity when compared to cybrids generated from family members not afflicted with PD. Furthermore, family members who had strict maternal inheritance, but were younger than the age of onset for PD within this family, had lower mean Complex I activity than their paternally-descended cousins. In contrast, cybrids containing platelet mtDNA from members of the Contursi kindred did not show reduced Complex I activity [101]. In the Contursi kindred PD arises due to an autosomal dominant mutation in the α-synuclein gene. This inheritance pattern implies that the genetic etiology differs between those with nuclear gene mutations and those with the more common sporadic form or forms. mtDNA would be more likely to contribute to risk or onset in the sporadic cases.

PD cybrids have been analyzed for a variety of biochemical and molecular parameters. Increased ROS production and upregulation of antioxidant enzyme activity have been observed [15,99,102]. PD cybrids are more sensitive to H_2O_2 exposure and have decreased GSH content [103]. Bcl-2 and Bcl-XL levels are upregulated, NFkB is activated, there is enhanced p38 and JNK activity, and PARP cleavage is increased [100,104–106]. These findings are consistent with increased oxidative stress, as signaling pathways involved in both cell death and survival stress responses are enhanced in PD cybrids. Furthermore, mitochondrial calcium levels are reduced [107]. Maximum respiration capacity was lowered in PD cybrids, and enhanced proton leak was apparent [100]. Mitochondrial membrane potentials were relatively depolarized [99]. ATP content was decreased and PD cybrid cell lines released more LDH [99]. PD cybrids are more susceptible to MPP+ induced cell death [15,100]. The ultrastructure of mitochondria is altered and PD cybrids develop synuclein aggregation indicative of the Lewy bodies observed in the substantia nigra of PD subjects [78,108–110].

One study isolated clones from PD cybrids which developed Lewy Bodies, leading to the subcloning of three PD cybrid cell lines [110]. This approach yielded inconsistent results, as each subcloned PD cybrid exhibited different phenotypes. For example, in one clone mitochondrial respiration was decreased, in the second clone mitochondrial respiration was increased, while in the third clone mitochondrial respiration went unchanged. However, the data did correspond to the nucleoid content of each clone.

A study of PD cybrids generated on the HeLa cervical carcinoma ρ0 nuclear background did not find any reduction in Complex I activity, $n = 10$ [111]. It is important to note this study, and it is fair to assume data obtained from it are accurate. However, the results from this study have little bearing on the numerous SH-SY5Y and A549 ρ0 based PD cybrid studies, which have elucidated biochemical differences between control and PD cybrid cell lines.

In 1997, Davis et al. reported that, as a group, cybrid cell lines generated from AD subjects had a lower Complex IV activity than cybrid cell lines generated from a demographically matched group of control subjects [112]. This report was subsequently retracted due to an interpretive error involving non-cybrid data that this manuscript also contained [113]. The cybrids in this study were generated on an SH-SY5Y neuroblastoma ρ0 nuclear background. Complex IV Vmax measurements were completed using post-nuclear, mitochondrial enriched fractions from AD 45 and 20 control cybrid cell lines. The AD cybrid cell lines had a 20% reduction in Complex IV activity when compared to the control subjects. Complex I Vmax was equivalent between the two groups, suggesting that mitochondrial repopulation was comparable.

These initial AD cybrid data were recapitulated in subsequent studies. In AD 76 and 111 control cybrid lines also generated against an SH-SY5Y nuclear background, a 15% reduction in AD cybrid Complex IV activity was observed [114]. Smaller AD cybrid studies that used the SH-SY5Y nuclear background have also reported reduced Complex IV activity [107,115–117]. Two separate studies using the NT2 ρ0 cell line to generate AD cybrids also found decreased Complex IV activity. The first study compared AD 15 cybrid lines to 9 control cybrid lines, and reported a 16% decrease in Complex IV activity [23]. The second study compared AD 6 cybrid lines to 5 control cybrid lines, and reported a 22% decrease in Complex IV activity [118].

Cybrids generated from AD patients on both the SH-SY5Y and NT2 nuclear background show biochemical and molecular changes. Increased ROS production was demonstrated using fluorescence-based peroxide measurements [23,112,118]. AD cybrids generated on the NT2 nuclear background displayed increased antioxidant enzyme activities, protein carbonyl markers, and lipid peroxidation markers [23,118]. AD cybrids on both the NT2 and SH-SY5Y nuclear backgrounds showed activation of MAPK, Akt, and NFkB with increased caspase-3 activation and cytochrome c cytoplasmic levels [118–121]. These findings are consistent with pathways associated with cell death and stress responses. AD cybrids generated on the SH-SY5Y nuclear background had decreased basal heat shock factor I (HSFI) binding activity, increased phosphoinositide signaling, and low AP1 binding [122,123].

Decreased ATP levels (NT2 and SH-SY5Y AD cybrids), reduced mitochondrial movement in neurites (SH-SY5Y AD cybrids), reduced mitochondrial calcium (SH-SY5Y AD cybrids), reduced mitochondrial membrane potential (both SH-SY5Y and NT2 AD cybrids), and reduced mitochondrial "flickering" (SH-SY5Y AD cybrids) have been demonstrated [115,117–119,124–126]. AD cybrids generated on the NT2 nuclear background were more susceptible to cell death induced by β-amyloid exposure [118]. Mitochondrial ultrastructure is affected in SH-SY5Y AD cybrid cell lines, and over time increased mitochondrial proliferation is observed [108,116]. Finally, both intracellular and extracellular β-amyloid levels were increased in SH-SY5Y AD cybrid cell lines [119]. Dense extracellular aggregates which stained positive for $A\beta_{1-40}$ and $A\beta_{1-42}$ were also observed in the same study.

A recently published, detailed examination of AD cybrid cell lines also reported bioenergetic changes. In this study, 8 AD, 7 mild-cognitive impairment (MCI, frequently a precursor AD state), and 7 age-matched control cybrid cell lines were generated using platelets from human subjects and the SH-SY5Y ρ0 nuclear background [127]. Complex IV activity was significantly decreased when normalized to cytochrome oxidase subunit 4 isoform 1 (COX4I1) protein in the AD group. Both the MCI and AD cybrids displayed less glycolytic activity,

while the MCI cybrids had decreased basal mitochondrial oxygen consumption and the AD cybrids showed an increased respiratory leak rate. Both AD and MCI cybrid cell lines had decreased NAD^+/NADH ratios and the AD cybrids showed increased ADP/ATP ratios. Expression of proteins involved in mitochondrial biogenesis and bioenergetic fluxes were found to be altered. In both the MCI and AD cybrid cell lines, HIF1α protein was decreased, while its mRNA levels were elevated. In contrast, PGC1-α expression was reduced in both MCI and AD cybrids. Sirt1 cellular localization was altered, and phosphorylated Sirt1 protein was decreased in AD cybrids. The AD cybrid cell lines displayed upregulated expression of phosphorylated AMPK, phosphorylated p38, Bax, DRP1, TFAM, cytochrome c, and Complex IV subunits, and mtDNA levels were increased. mTOR expression was reduced and phosphorylated DRP1 levels were decreased in the AD cybrid cell lines. Overall, bioenergetic fluxes, mitochondrial mass, mitochondrial morphology dynamics, and cell signaling pathways were altered in the MCI and AD cybrid cell lines.

An additional study independently examined the same set of AD and control cybrid cell lines [128]. For the AD cybrid cell lines, a reduction in Complex I, Complex III, and Complex IV activities, as well as reduced ATP levels, was reported. ROS production, phosphorylated ERK, and mitochondrial DLP-1 levels were also increased in AD cybrids. Mitochondrial density and length were decreased.

A separate study generated AD, MCI, and age-matched control cybrid cell lines from human platelets and the NT2 ρ0 nuclear background [117]. It was found that both AD and MCI cybrid cell lines had decreased Complex IV activity and mitochondrial membrane potential. Increased lipid peroxidation, superoxide production, and protein carbonyls were observed in both AD and MCI cybrids.

One published study of AD cybrids generated on the HeLa nuclear background is notable for not detecting differences between AD and control cybrid line Complex IV activities [44]. Results from this study are confounded by the fact that mtDNA transfer from platelet, fibroblast, and synaptosomes were attempted, and the sources of mtDNA were not matched between AD and control cybrid cell lines. When AD and control cybrids generated from platelet mtDNA transfer are compared, data from only three cell lines from each group were provided. It is reasonable to take into account this study's small sample size when considering its conclusions. Regardless, even if this report's preliminary findings were to be confirmed in an adequately powered HeLa-based cybrid study, the relevance of this to the numerous positive SH-SY5Y and NT2-based AD cybrid studies would be unclear.

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that has autosomal dominant and sporadic etiology. Sporadic ALS accounts for approximately 90% of ALS cases and has a later age of onset. Based on the idea that mtDNA could act as a late onset, sporadic neurodegenerative disease risk factor, the cybrid model was used to study mtDNA in ALS [129,130]. Platelets from ALS patients were used as the mtDNA donor and SH-SY5Y neuroblastoma ρ0 cells provided the nuclear background. Eleven ALS cybrid cell lines and 12 age-matched control cybrid cell lines were generated. Relative to the control cybrid lines, post-nuclear mitochondrial enriched fractions from the ALS cybrid lines displayed trends for reductions in Complex III and Complex IV activities, and a statistically significant reduction in Complex I activity [131]. A subsequent study, interestingly, reported reduced Complex I activity in muscle tissue from ALS patients [132]. Although ROS production in the form of cytoplasmic peroxide levels was not increased in ALS cybrids, an increase in antioxidant enzyme activities was apparent [129]. ALS cybrid mitochondria also demonstrated reduced mitochondrial calcium concentrations.

A second ALS cybrid study used osteosarcoma ρ0 cells and platelets as the mitochondrial donor source [133]. This study generated 13 ALS cybrids and 10 control cybrids. While no difference in mitochondrial respiration was reported, this study did not directly assay the Complex I Vmax (the parameter demonstrated to be different in the Swerdlow et al. study [129]). Similarly, no change in ROS production in the form of cytoplasmic peroxide was noted; antioxidant enzyme levels were not reported. It is difficult to generalize the findings of these studies because there is a lack of overlap between experimental parameters, and cybrids were generated using different nuclear backgrounds.

Another late onset, sporadic neurodegenerative disease in which the cybrid model has been used is progressive supranuclear palsy (PSP). As early as 1994, PSP subjects were found to have reduced muscle oxidative metabolism [134]. In the initial PSP cybrid study, lines were generated using the SH-SY5Y nuclear background and platelet mitochondria from 15 PSP and 17 age-matched control subjects [135]. Compared to control cybrid lines, in the PSP cybrid lines the Complex I Vmax was reduced by 12.4%, while Complex IV activities were equivalent between the groups. Antioxidant enzyme activities were elevated (presumably upregulated) in the PSP cybrid group. Other investigators have used the same cybrid lines and have reported reductions in oxidative respiration, activity of Complexes I + III, and ATP levels in the PSP cybrids. Aconitase activity is reduced, suggesting increased mitochondrial oxidative stress. Exposure to ETC inhibitors also caused greater mitochondrial membrane depolarization in the PSP cybrids when compared to control cybrids. Treatment of cybrids with a Complex I inhibitor, rotenone, caused a more robust increase in PSP cybrid malondialdehyde (MDA) and hydroxynonenal (HNE) production than it did in control cybrid lines. Increased MDA and HNE in the PSP lines are consistent with a greater degree of rotenone-induced oxidative stress. On the other hand, MDA and HNE differences were not observed after treatment with malonate (a Complex II inhibitor) or cyanide (a Complex IV inhibitor) [136,137].

The cybrid model was used to study Huntington's disease (HD), a strictly Mendelian, autosomal dominant neurodegenerative disease [138]. While mitochondrial function is perturbed in HD, thereby qualifying it as a mitochondriopathy, based on its genetics there is no compelling reason to suspect the presence of functionally meaningful, disease-specific mtDNA variations (at least not from the perspective of inherited mtDNA sequence deviations). Consistent with this prediction, while direct measurements of HD subject platelets show decreased Complex I activity [139], HD cybrid cell lines do not [138,140,141]. On the other hand, Fereira et al. did report HD cybrids showed increased glycolysis, increased ATP production, increased peroxide production, and decreased mitochondrial NADH:NAD^+ ratios [140,141]. If correct, the presence of a mutant Huntingtin gene may, therefore, give rise to somatic yet perpetually-sustained mtDNA modifications.

Finally, the cybrid model has also been used to study mtDNA contributions to aging. In an initial study, fibroblast mtDNA was used to generate cybrids from 21 individuals on an osteosarcoma nuclear background [142]. The cybrid lines generated from these 21 individuals were subsequently sub-cloned, ultimately giving rise to a total of 356 uniquely expanded cybrid cell lines. Among these lines an inverse correlation between mitochondrial respiration and the age of the mtDNA donor was observed; the older the mtDNA donor, the lower the respiration capacity of the resulting cybrid line. In contrast to this, though, a separate, much smaller study using HeLa ρ0 cells as the nuclear background did not report correlations between mtDNA donor age and respiration [143]. In this negative study, two cybrid cells lines were generated. In one line, mtDNA was obtained from fibroblasts from a 97-year old, and in the other mtDNA was obtained from fibroblasts from a fetus. This study also used a clonal approach, and a total of five clones were analyzed. Complex IV activity constituted the experimental endpoint, and among these five clones comparable Complex IV activities were observed.

Aging cybrid clones were also generated on an osteosarcoma nuclear background from a 100 year-old donor bearing a T414G mutation. This particular mutation, which is quantitatively associated with aging, is located within the mtDNA control region [144]. No differences in oxygen consumption or ETC activities were observed.

However, two different mutations in the 16S rRNA gene (T1843C and A1940G) did associate with reduced oxygen consumption and reduced ETC activities. Lastly, another study that used mouse muscle and synaptosomes to generate cybrid cell lines on a mouse LL/2-m21 nuclear background [27] found differences in cybrid lines derived from young and old mtDNA donors. When muscle cytoplasts were used, cybrids containing mtDNA from the old mice showed decreased Complex I and Complex III activities, less ATP production, and reduced oxygen consumption (but no change in Complex IV activity). When synaptosomes were used, cybrids containing mtDNA form the old mice showed decreased Complex I activity and lowered ATP levels.

Methodological considerations

It is necessary to clarify the point that cybrid methodology varies significantly between individual studies, because these methodological variations may potentially account for and/or contribute to conflicting findings. This section discusses these methodological disparities.

Overall, the nuclear background of the ρ0 cell line used to generate cybrid cell lines influences study outcomes [50,59,71,145,146]. This finding is not surprising because mitochondrial physiology varies among tissue types and nuclear DNA largely determines these variations [147,148]. There is no guarantee that mitochondria obtained from a common source will similarly function within the context of different nuclear backgrounds, or that mitochondria obtained from different sources will consistently function in similar patterns when expressed within the context of different nuclear backgrounds.

The majority of ρ0 cell lines generated for use in cybrid models are tumor cell lines. Tumor cell lines exhibit aneuploidy, and therefore do not contain a normal chromosome complement. Thus, pieces of chromosomes may be missing or translocated, or additional chromosomes may be present. Aneuploidy varies significantly between different ρ0 cell lines [149–153]. While it has not been resolved exactly how aneuploidy impacts mitochondrial function, it could potentially affect nuclear DNA-mtDNA ETC subunit stoichiometry. This could cause some ρ0 cell lines to become more or less sensitive in measurements of mtDNA genotype–phenotype correlations. Moreover, it is important to consider that these tumor cells lines are anaerobic at baseline. Whether or not the aerobic versus anaerobic nature of the host cell influences mtDNA genotype–phenotype correlations should be taken into account. Data regarding this are limited, yet it seems reasonable to assume cybrid models using ρ0 cell lines derived from more aerobic tumor lines may yield inconsistent results when compared with cybrid studies in which ρ0 cells derive from more anaerobic tumor lines.

The majority of cybrid studies have utilized parental cell lines depleted of mtDNA by exposure to EtBr. However, new techniques for depleting mtDNA are becoming commercially available. Therefore, in future cybrid studies it will be imperative to take into account the method by which the parental cell lines were depleted of mtDNA. The source of the mtDNA donation, pre-fusion handling of the mtDNA-donating material, the post-fusion selection process, and variability in cell culture methods can all potentially influence findings. Finally, assay methodology, assay selection and sample sizes are all potential variables that need to be considered.

Reported findings using the cybrid model were obtained from studies that used isolated cybrid clones and mixed populations of cybrid clones. When a particular assessment requires a homogeneous population of cybrid cells, the preparation of clonal cybrids is a more appropriate choice. Examples of such studies include those examining heteroplasmy-threshold relationships of known mtDNA mutations. When cybrids are used to screen for functionally relevant mtDNA differences between mtDNA from different mtDNA donors, or when individually expanded clones from a cybrid line are biochemically assayed, the mean value obtained from the independent assay approximates the value obtained when the same cybrid is assayed using a mixed culture[114]. In conclusion, when using the cybrid model to screen for mtDNA genotype–biochemical phenotype correlations from unsequenced mtDNA, the use of mixed cybrid populations would seem to be the more logical approach.

The “best” endpoint to show functional differences in mtDNA-related cybrid lines is unclear and not universal. However, data suggests that radical production is a useful biochemical measure [154]. In clinical manifestations associated with oxidative stress, the cybrid model can aid in addressing the origin of free radical generation. If cybrid cell lines expressing mtDNA from a group of subjects perpetuate oxidative stress, this suggests mitochondria, and very likely mtDNA, contribute to that oxidative stress.

2. Conclusions

Data generated using cybrid models have enabled investigators to address questions related to mtDNA-induced mitochondrial biology. Furthermore, cybrid studies have provided important insight into how altered mitochondrial function influences cell physiology. Future investigators should find the cybrid model useful when interrogating nuclear–mitochondrial interactions. For studies investigating mtDNA heteroplasmy-threshold analysis, the cybrid model provides straightforward data that appropriately illustrate fundamental mitochondrial genetic principles. However, generalizing data to non-artificial biological models must proceed with caution, because critical differences exist between experimentally generated cybrid cell lines and the actual systems they serve to model.

Functional assessments of mitochondria with un-sequenced mtDNA show mitochondria from different sources are functionally unique. For several sporadic neurodegenerative diseases that do not show Mendelian inheritance, and where mitochondrial dysfunction occurs, mitochondrial transfer from individuals afflicted with those diseases can generate cybrid cell lines which recapitulate and perpetuate the same biochemical defects. The most straightforward interpretation for these findings is that mtDNA at least partly drives functional alterations observed in both the disease state and in cybrid cell lines prepared via the transfer of mitochondria obtained from the human subjects. While this interpretation has not been conclusively confirmed, data to date do not support an alternative explanation.

While cybrids remain a valuable technique and will continue to inform mtDNA-oriented studies, it is worth noting that animal-based models are also increasingly being used to elucidate mtDNA genotype–phenotype correlations. In one example, female progeny were backcrossed repeatedly with their original paternal strain [155]. Since mtDNA is maternally inherited, the mouse line that resulted was one in which the nuclear background was almost exclusively from the paternal strain, while the mtDNA was entirely from the maternal strain. These mouse studies, which use the same nuclear background with varying mtDNA show differences in oxidative phosphorylation phenotypes, which may be relevant to multifactorial diseases [63,156–158]. Other mouse models include those that express mutated versions of genes used in mtDNA replication and maintenance [159–161]. Mitochondrial DNA transgenic mice have also been generated, and review articles describing these advances have been published [162–165]. Finally, recent advances that allow for the transfer of mitochondria between different mouse strains will very likely advance our understanding of nuclear DNA–mtDNA functional relationships, as well as the impact of mtDNA variation on cell physiology [166].

Acknowledgments

The authors are supported by P30AG035982 (University of Kansas Alzheimer's Disease Center) and the Frank and Evangeline Thompson Alzheimer's Treatment Program Fund.

References

[1] M.M. Nass, S. Nass, Intramitochondrial fibers with DNA characteristics. I. Fixation and electron staining reactions, Journal of Cell Biology 19 (1963) 593–611. http://dx.doi.org/10.1083/jcb.19.3.593, 14086138.
[2] S. Nass, M.M. Nass, Intramitochondrial fibers with DNA characteristics. II. Enzymatic and other hydrolytic treatments, Journal of Cell Biology 19 (1963) 613–629. http://dx.doi.org/10.1083/jcb.19.3.613, 14086139.
[3] M.T. Lin, D.K. Simon, C.H. Ahn, L.M. Kim, M.F. Beal, High aggregate burden of somatic mtDNA point mutations in aging and Alzheimer's disease brain, Human Molecular Genetics 11 (2002) 133–145. http://dx.doi.org/10.1093/hmg/11.2.133, 38 11809722.
[4] N.G. Larsson, Somatic mitochondrial DNA mutations in mammalian aging, Annual Review of Biochemistry 79 (2010) 683–706. http://dx.doi.org/10.1146/annurev-biochem-060408-093701, 20350166.
[5] A. Bratic, N.G. Larsson, The role of mitochondria in aging, Journal of Clinical Investigation 123 (2013) 951–957. http://dx.doi.org/10.1172/JCI64125, 23454757.
[6] B. Ephrussi, H. Hottinger, A.M. Chimenes, Action de l'acriflavine sur les levures, I: la mutation "petite clonie.", Annales de l'Institut Pasteur (Paris) 76 (1949) 531.
[7] P.P. Slonimski, G. Perrodin, J.H. Croft, Ethidium bromide induced mutation of yeast mitochondria: complete transformation of cells into respiratory deficient non-chromosomal "petites", Biochemical and Biophysical Research Communications 30 (1968) 232–239. http://dx.doi.org/10.1016/0006-291X(68)90440-3, 5647224.
[8] E.S. Goldring, L.I. Grossman, D. Krupnick, D.R. Cryer, J. Marmur, The petite mutation in yeast. Loss of mitochondrial deoxyribonucleic acid during induction of petites with ethidium bromide, Journal of Molecular Biology 52 (1970) 323–335. http://dx.doi.org/10.1016/0022-2836(70)90033-1, 5485912.
[9] Nagley, W. Linnane, Mitochondrial DNA deficient petite mutants of yeast 39 (1970) 989–996. http://dx.doi.org/10.1016/0006-291X(70)90422-5, 5423835.
[10] A. Wiseman, G. Attardi, Reversible tenfold reduction in mitochondria DNA content of human cells treated with ethidium bromide, Molecular & General Genetics 167 (1978) 51–63, 739978.
[11] P. Desjardins, E. Frost, R. Morais, Ethidium bromide-induced loss of mitochondrial DNA from primary chicken embryo fibroblasts, Molecular and Cellular Biology 5 (1985) 1163–1169, 2987677.
[12] M. Gregoire, R. Morais, M.A. Quilliam, D. Gravel, On auxotrophy for pyrimidines of respiration-deficient chick embryo cells, European Journal of Biochemistry / FEBS 142 (1984) 49–55. http://dx.doi.org/10.1111/j.1432-1033.1984.tb08249.x, 6086342.
[13] P. Desjardins, J.M. de Muys, R. Morais, An established avian fibroblast cell line without mitochondrial DNA, Somatic Cell and Molecular Genetics 12 (1986) 133–139. http://dx.doi.org/10.1007/BF01560660, 3008349.
[14] R. Morais, P. Desjardins, C. Turmel, K. Zinkewich-Peotti, Development and characterization of continuous avian cell lines depleted of mitochondrial DNA, In Vitro Cellular & Developmental Biology: Journal of the Tissue Culture Association 24 (1988) 649–658. http://dx.doi.org/10.1007/BF02623602, 2840430.
[15] R.H. Swerdlow, J.K. Parks, S.W. Miller, J.B. Tuttle, P.A. Trimmer, J.P. Sheehan, et al, Origin and functional consequences of the complex I defect in Parkinson's disease, Annals of Neurology 40 (1996) 663–671. http://dx.doi.org/10.1002/ana.410400417, 8871587.
[16] M.P. King, G. Attardi, Human cells lacking mtDNA: Repopulation with exogenous mitochondria by complementation, Science 246 (1989) 500–503. http://dx.doi.org/10.1126/science.2814477, 2814477.
[17] M.P. King, G. Attardi, Isolation of human cell lines lacking mitochondrial DNA, Methods in Enzymology 264 (1996) 304–313. http://dx.doi.org/10.1016/S0076-6879(96)64029-4, 8965704.
[18] J. Hayashi, S. Ohta, A. Kikuchi, M. Takemitsu, Y. Goto, I. Nonaka, Introduction of disease-related mitochondrial DNA deletions into HeLa cells lacking mitochondrial DNA results in mitochondrial dysfunction, Proceedings of the National Academy of Sciences of the United States of America 88 (1991) 10614–10618. http://dx.doi.org/10.1073/pnas.88.23.10614, 1720544.
[19] A.G. Bodnar, J.M. Cooper, I.J. Holt, J.V. Leonard, A.H. Schapira, Nuclear complementation restores mtDNA levels in cultured cells from a patient with mtDNA depletion, American Journal of Human Genetics 53 (1993) 663–669, 8394647.
[20] S. Suissa, Z. Wang, J. Poole, S. Wittkopp, J. Feder, T.E. Shutt, et al, Ancient mtDNA genetic variants modulate mtDNA transcription and replication, PLoS Genetics 5 (2009) e1000474. http://dx.doi.org/10.1371/journal.pgen.1000474, 19424428.
[21] A.S. Jun, I.A. Trounce, M.D. Brown, J.M. Shoffner, D.C. Wallace, Use of transmitochondrial cybrids to assign a complex I defect to the mitochondrial DNA-encoded NADH dehydrogenase subunit 6 gene mutation at nucleotide pair 14459 that causes Leber hereditary optic neuropathy and dystonia, Molecular and Cellular Biology 16 (1996) 771–777, 8622678.
[22] S.W. Miller, P.A. Trimmer, W.D. Parker Jr, R.E. Davis, Creation and characterization of mitochondrial DNA-depleted cell lines with "neuronal-like" properties, Journal of Neurochemistry 67 (1996) 1897–1907, 8863494.
[23] R.H. Swerdlow, J.K. Parks, D.S. Cassarino, D.J. Maguire, R.S. Maguire, J.P. Bennett Jr, et al, Cybrids in Alzheimer's disease: a cellular model of the disease? Neurology 49 (1997) 918–925. http://dx.doi.org/10.1212/WNL.49.4.918, 9339668.
[24] D.R. Binder, W.H. Dunn Jr, R.H. Swerdlow, Molecular characterization of mtDNA depleted and repleted NT2 cell lines, Mitochondrion 5 (2005) 255–265. http://dx.doi.org/10.1016/j.mito.2005.04.003, 16050988.
[25] R.H. Swerdlow, G.T. Redpath, D.S. Binder, J.N. Davis II, S.R. VandenBerg, Mitochondrial DNA depletion analysis by pseudogene ratioing, Journal of Neuroscience Methods 150 (2006) 265–271. http://dx.doi.org/10.1016/j.jneumeth.2005.06.023, 16118020.
[26] S. Malik, H. Sudoyo, P. Pramoonjago, H. Suryadi, T. Sukarna, M. Njunting, et al, Nuclear mitochondrial interplay in the modulation of the homopolymeric tract length heteroplasmy in the control (D-loop) region of the mitochondrial DNA, Human Genetics 110 (2002) 402–411. http://dx.doi.org/10.1007/s00439-002-0717-3, 12073009.
[27] H. Li, Sharma L. Kumar, Y. Li, P. Hu, A. Idowu, D. Liu, et al, Comparative bioenergetic study of neuronal and muscle mitochondria during aging, Free Radical Biology and Medicine 63 (2013) 30–40. http://dx.doi.org/10.1016/j.freeradbiomed.2013.04.030, 23643721.
[28] A.J. Olszanski, M.L. LaCroix-Fralish, R. Armand, P. Perez, L.D. Lewis, Molt-4 Rho(0) cells up regulated stress-induced survival genes in an attempt to overcome the potentially lethal consequence of mitochondrial DNA depletion, Clinical Pharmacology and Therapeutics 75(2) (2004) 1–142.
[29] K. Inoue, D. Takai, H. Hosakia, S. Ito, H. Shitara, K. Isobe, et al, Isolation and characterization of mitochondrial DNA-less lines from various mammalian cell lines by application of an anticancer drug, ditercalinium, Biochemical and Biophysical Research Communications 239 (1997) 257–260, 9345305.
[30] M. Jazayeri, A. Andreyev, Y. Will, M. Ward, C.M. Anderson, W. Clevenger, Inducible expression of a dominant negative DNA polymerase-gamma depletes mitochondrial DNA and produces a rho0 phenotype, Journal of Biological Chemistry 278 (2003) 9823–9830. http://dx.doi.org/10.1074/jbc.M211730200, 12645575.
[31] I. Nelson, M.G. Hanna, N.W. Wood, A.E. Harding, Depletion of mitochondrial DNA by ddC in untransformed human cell lines, Somatic Cell and Molecular Genetics 23 (1997) 287–290. http://dx.doi.org/10.1007/BF02674419, 9542530.
[32] N. Ashley, D. Harris, J. Poulton, Detection of mitochondrial DNA depletion in living human cells using pico green staining, Experimental Cell Research 303 (2005) 432–446. http://dx.doi.org/10.1016/j.yexcr.2004.10.013, 15652355.
[33] A.R. Gear, Rhodamine 6G. A potent inhibitor of mitochondrial oxidative phosphorylation, Journal of Biological Chemistry 249 (1974) 3628–3637, 4275428.
[34] A.J. Williams, M. Murrell, S. Brammah, J. Minchenko, J. Christodoulou, A novel system for assigning the mode of inheritance in mitochondrial disorders using cybrids and rhodamine 6G, Human Molecular Genetics 8 (1999) 1691–1697. http://dx.doi.org/10.1093/hmg/8.9.1691, 10441332.
[35] A. Wong, L. Cavelier, H.E. Collins-Schramm, M.F. Seldin, M. McGrogan, M.L. Savontaus, et al, Differentiation-specific effects of LHON mutations introduced into neuronal NT2 cells, Human Molecular Genetics 11 (2002) 431–438. http://dx.doi.org/10.1093/hmg/11.4.431, 11854175.
[36] A. Kukat, C. Kukat, J. Brocher, I. Schäfer, G. Krohne, I.A. Trounce, et al, Generation of rho0 cells utilizing a mitochondrially targeted restriction endonuclease and comparative analyses, Nucleic Acids Research 36 (2008) e44. http://dx.doi.org/10.1093/nar/gkn124, 18353857.
[37] S. Heller, S. Schubert, M. Krehan, I. Schäfer, M. Seibel, D. Latorre, et al, Efficient repopulation of genetically derived rho zero cells with exogenous mitochondria, PLOS One 8 (2013) e73207. http://dx.doi.org/10.1371/journal.pone.0073207, 24019909.
[38] G. Poste, P. Reeve, Enucleation of mammalian cells by cytochalasin B. II. Formation of hybrid cells and heterokaryons by fusion of anucleate and nucleated cells, Experimental Cell Research 73 (1972) 287–294. http://dx.doi.org/10.1016/0014-4827(72)90050-X, 4341004.
[39] C.L. Bunn, D.C. Wallace, J.M. Eisenstadt, Cytoplasmic inheritance of chloramphenicol resistance in mouse tissue culture cells, Proceedings of the National Academy of Sciences of the United States of America 71 (1974) 1681–1685. http://dx.doi.org/10.1073/pnas.71.5.1681, 4525288.
[40] D.C. Wallace, C.L. Bunn, J.M. Eisenstadt, Cytoplasmic transfer of chloramphenicol resistance in human tissue culture cells, Journal of Cell Biology 67 (1975) 174–188. http://dx.doi.org/10.1083/jcb.67.1.174, 1176530.
[41] A. Chomyn, S.T. Lai, R. Shakeley, N. Bresolin, G. Scarlato, G. Attardi, Platelet-mediated transformation of mtDNA-less human cells: analysis of phenotypic variability among clones from normal individuals—and complementation behavior of the tRNALys mutation causing myoclonic epilepsy and ragged red fibers, American Journal of Human Genetics 54 (1994) 966–974, 8198140.
[42] K. Inoue, S. Ito, D. Takai, A. Soejima, H. Shisa, J.B. LePecq, et al, Isolation of mitochondrial DNAless mouse cell lines and their application for trapping mouse synaptosomal mitochondrial DNA with deletion mutations, Journal of Biological Chemistry 272 (1997) 15510–15515. http://dx.doi.org/10.1074/jbc.272.24.15510.
[43] I. Trounce, J. Schmiedel, H.C. Yen, S. Hosseini, M.D. Brown, J.J. Olson, et al, Cloning of neuronal mtDNA variants in cultured cells by synaptosome fusion with mtDNA-less cells, Nucleic Acids Research 28 (2000) 2164–2170. http://dx.doi.org/10.1093/nar/28.10.2164, 10773087.
[44] S. Ito, S. Ohta, K. Nishimaki, Y. Kagawa, R. Soma, S.Y. Kuno, et al, Functional integrity of mitochondrial genomes in human platelets and autopsied brain

tissues from elderly patients with Alzheimer's disease, Proceedings of the National Academy of Sciences of the United States of America 96 (1999) 2099–2103. http://dx.doi.org/10.1073/pnas.96.5.2099, 10051601.
[45] A. Chomyn, A. Martinuzzi, M. Yoneda, A. Daga, O. Hurko, D. Johns, et al, MELAS mutation inmtDNA binding site for transcription termination factor causes defects in protein synthesis and in respiration but no change in levels of upstream and downstream mature transcripts, Proceedings of the National Academy of Sciences of the United States of America 89 (1992) 4221–4225. http://dx.doi.org/10.1073/pnas.89.10.4221, 1584755.
[46] M.P. King, Y. Koga, M. Davidson, E.A. Schon, Defects in mitochondrial protein synthesis and respiratory chain activity segregate with the tRNA(Leu(UUR)) mutation associated with mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes, Molecular and Cellular Biology 12 (1992) 480–490, 1732728.
[47] Y. Koga, M. Davidson, E.A. Schon, M.P. King, Analysis of cybridsharboring MELAS mutations in the mitochondrial tRNA(Leu(UUR)) gene, Muscleand Nerve 3(Suppl.) (1995) S119–S123, 7603512.
[48] E.A. Shoubridge, Segregation of mitochondrial DNAs carrying a pathogenic point mutation (tRNA(leu3243)) in cybrid cells, Biochemical and Biophysical Research Communications 213 (1995) 189–195. http://dx.doi.org/10.1006/bbrc.1995.2115, 7639735.
[49] E.A. Schon, Y. Koga, M. Davidson, C.T. Moraes, M.P. King, The mitochondrial tRNA Leu(UUR) mutation in MELAS:a model for pathogenesis, Biochimica et Biophysica Acta 1101 (1992) 206–208. http://dx.doi.org/10.1016/0005-2728(92)90226-R10.1016/S0005-2728(05)80021-0.
[50] D.R. Dunbar, P.A. Moonie, H.T. Jacobs, I.J. Holt, Different cellular backgrounds confer a marked advantage to either mutant or wild-type mitochondrial genomes, Proceedings of the National Academy of Sciences of the United States of America 92 (1995) 6562–6566. http://dx.doi.org/10.1073/pnas.92.14.6562, 7604033.
[51] P.F. Chinnery, N. Howell, R.N. Lightowlers, D.M. Turnbull, Molecular pathology of MELAS and MERRF. The relationship between mutation load and clinical phenotypes, Brain:A Journal of Neurology 120 (1997) 1713–1721. http://dx.doi.org/10.1093/brain/120.10.1713, 9365365.
[52] P.F. Chinnery, D.J. Taylor, D.T. Brown, D. Manners, P. Styles, R. Lodi, Very low levels of the mtDNA A3243G mutation associated with mitochondrial dysfunction in vivo, Annals of Neurology 47 (2000) 381–384. http://dx.doi.org/10.1002/1531-8249(200003)47:3<381::AID-ANA17>3.0.CO;2-2, 10716261.
[53] M. Yoneda, A. Chomyn, A. Martinuzzi, O. Hurko, G. Attardi, Marked replicative advantage of human mtDNA carrying a point mutation that causes the MELAS encephalomyopathy, Proceedings of the National Academy of Sciences of the United States of America 89 (1992) 11164–11168. http://dx.doi.org/10.1073/pnas.89.23.11164, 1454794.
[54] C.J. Turner, C. Granycome, R. Hurst, E. Pohler, M.K. Juhola, M.I. Juhola, et al, Systematic segregation to mutant mitochondrial DNA and accompanying loss of mitochondrial DNA in human NT2 teratocarcinoma cybrids, Genetics 170 (2005) 1879–1885. http://dx.doi.org/10.1534/genetics.105.043653, 15944344.
[55] D.C. Wallace, G. Singh, M.T. Lott, J.A. Hodge, T.G. Schurr, A.M. Lezza, et al, Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy, Science 242 (1988) 1427–1430. http://dx.doi.org/10.1126/science.3201231, 3201231.
[56] D.R. Johns, S. Lessell, N.R. Miller, Molecularly confirmed Leber's hereditary optic neuropathy, Neurology 41 (1991) 347.
[57] L. Vergani, A. Martinuzzi, V. Carelli, P. Cortelli, P. Montagna, G. Schievano, et al, MtDNA mutations associated with Leber's hereditary optic neuropathy: studies on cytoplasmic hybrid (cybrid) cells, Biochemical and Biophysical Research Communications 210 (1995) 880–888. http://dx.doi.org/10.1006/bbrc.1995.1740, 7763260.
[58] G. Hofhaus, D.R. Johns, O. Hurko, G. Attardi, A. Chomyn, Respiration and growth defects in transmitochondrial cell lines carrying the 11778 mutation associated with Leber's hereditary optic neuropathy, Journal of Biological Chemistry 271 (1996) 13155–13161. http://dx.doi.org/10.1074/jbc.271.22.13155, 8662757.
[59] H.R. Cock, S.J. Tabrizi, J.M. Cooper, A.H. Schapira, The influence of nuclear background on the biochemical expression of 3460 Leber's hereditary optic neuropathy, Annals of Neurology 44 (1998) 187–193. http://dx.doi.org/10.1002/ana.410440208, 9708540.
[60] M.D. Brown, I.A. Trounce, A.S. Jun, J.C. Allen, D.C. Wallace, Functional analysis of lymphoblast and cybrid mitochondria containing the 3460, 11778, or 14484 Leber's hereditary optic neuropathy mitochondrial DNA mutation, Journal of Biological Chemistry 275 (2000) 39831–39836. http://dx.doi.org/10.1074/jbc.M006476200, 10976107.
[61] A. Baracca, G. Solaini, G. Sgarbi, G. Lenaz, A. Baruzzi, A.H.V. Schapira, et al, Severe impairment of complex I-driven adenosine triphosphate synthesis in Leber hereditary optic neuropathy cybrids, Archives of Neurology 62 (2005) 730–736. http://dx.doi.org/10.1001/archneur.62.5.730, 15883259.
[62] R. Pello, M.A. Martín, V. Carelli, L.G. Nijtmans, A. Achilli, M. Pala, et al, Mitochondrial DNA background modulates the assembly kinetics of OXPHOS complexes in a cellular model of mitochondrial disease, Human Molecular Genetics 15 (2008) 4001–4011.
[63] A. Gómez-Durán, D. Pacheu-Grau, E. López-Gallardo, C. Díez-Sánchez, J. Montoya, M.J. López-Pérez, et al, Unmasking the causes of multifactorial disorders: OXPHOS differences between mitochondrial haplogroups, Human Molecular Genetics 19 (2010) 3343–3353. http://dx.doi.org/10.1093/hmg/ddq246, 20566709.
[64] A. Gómez-Durán, D. Pacheu-Grau, I. Martínez-Romero, E. López-Gallardo, M.J. López-Pérez, J. Montoya, et al, Oxidative phosphorylation differences between mitochondrial DNA haplogroups modify the risk of Leber's hereditary optic neuropathy, Biochimica et Biophysica Acta 1822 (2012) 1216–1222. http://dx.doi.org/10.1016/j.bbadis.2012.04.014, 22561905.
[65] S.J. Pleasure, C. Page, V.M.Y. Lee, Pure, postmitotic, polarized human neurons derived from NTera 2 cells provide a system for expressing exogenous proteins in terminally differentiated neurons, Journal of Neuroscience: The Official Journal of the Society for Neuroscience 12 (1992) 1802–1815, 1578271.
[66] S. Schoeler, K. Winkler-Stuck, R. Szibor, M.F. Haroon, F.N. Gellerich, K. Chamaon, et al, Glutathione depletion in antioxidant defense of differentiated NT2-LHON cybrids, Neurobiology of Disease 25 (2007) 536–544. http://dx.doi.org/10.1016/j.nbd.2006.10.014, 17169568.
[67] M. Floreani, E. Napoli, A. Martinuzzi, G. Pantano, Riva V. De, R. Trevisan, et al, Antioxidant defences in cybrids harboring mtDNA mutations associated with Leber's hereditary optic neuropathy, FEBS Journal 272 (2005) 1124–1135. http://dx.doi.org/10.1111/j.1742-4658.2004.04542.x, 15720387.
[68] I. Trounce, S. Neill, D.C. Wallace, Cytoplasmic transfer of the mtDNA nt 8993 T→G (ATP6) point mutation associated with Leigh syndrome into mtDNA-less cells demonstrates cosegregation with a decrease in state III respiration and ADP/O ratio, Proceedings of the National Academy of Sciences of the United States of America 91 (1994) 8334–8338. http://dx.doi.org/10.1073/pnas.91.18.8334, 8078883.
[69] J.A. Enriquez, A. Chomyn, G. Attardi, MtDNA mutation in MERRF syndrome causes defective aminoacylation of tRNA(Lys) and premature translation termination, Nature Genetics 10 (1995) 47–55. http://dx.doi.org/10.1038/ng0595-47, 7647790.
[70] J.P. Masucci, M. Davidson, Y. Koga, E.A. Schon, M.P. King, In vitro analysis of mutations causing myoclonus epilepsy with ragged-red fibers in the mitochondrial tRNA(Lys)gene: two genotypes produce similar phenotypes, Molecular and Cellular Biology 15 (1995) 2872–2881, 7739567.
[71] L. Vergani, R. Rossi, C.H. Brierly, M. Hanna, I.J. Holt, Introduction of heteroplasmic mitochondrial DNA (mtDNA) from a patient with NARP into two human q0 cell lines is associated with either selection and maintenance of NARP mutant mtDNA or failure to maintain mtDNA, Human Molecular Genetics 8 (1999) 1751–1755. http://dx.doi.org/10.1093/hmg/8.9.1751.
[72] Y. Tang, E.A. Schon, E. Wilichowski, M.E. Vazquez-Memije, E. Davidson, M.P. King, Rearrangements of humanmitochondrialDNA (mtDNA): new insights into the regulation of mtDNA copy number and gene expression, Molecular Biology of the Cell 11 (2000) 1471–1485. http://dx.doi.org/10.1091/mbc.11.4.1471, 10749943.
[73] F. Palotti, A. Baracca, E. Hernandez-Rosa, W.F. Walker, G. Solaini, G. Lenaz, et al, Biochemical analysis of respiratory function in cybrid cell lines harbouring mitochondrial DNA mutations, Biochemical Journal 384 (2004) 287–293. http://dx.doi.org/10.1042/BJ20040561, 15324306.
[74] L. Kenyon, C.T. Moraes, Expanding the functional human mitochondrial DNA database by the establishment of primate xenomitochondrial cybrids, Proceedings of the National Academy of Sciences of the United States of America 94 (1997) 9131–9135. http://dx.doi.org/10.1073/pnas.94.17.9131, 9256447.
[75] A. Barrientos, L. Kenyon, C.T. Moraes, Human xenomitochondrial cybrids. Cellular models of mitochondrial complex I deficiency, Journal of Biological Chemistry 273 (1998) 14210–14217. http://dx.doi.org/10.1074/jbc.273.23.14210, 9603924.
[76] R.H. Swerdlow, Mitochondrial DNA-related mitochondrial dysfunction in neurodegenerative diseases, Archives of Pathology and Laboratory Medicine 126 (2002) 271–280.
[77] M. Schwartz, J. Vissing, Paternal inheritance of mitochondrial DNA, New England Journal of Medicine 347 (2002) 576–580. http://dx.doi.org/10.1056/NEJMoa020350, 12192017.
[78] R.H. Swerdlow, J.K. Parks, J.N. Davis, D.S. Cassarino, P.A. Trimmer, L.J. Currie, et al, Matrilineal inheritance of complex I dysfunction in a multigenerational Parkinson's disease family, Annals of Neurology 44 (1998) 873–881. http://dx.doi.org/10.1002/ana.410440605.
[79] W.D. Parker Jr, R.H. Swerdlow, Mitochondrial dysfunction in idiopathic Parkinson disease, American Journal of Human Genetics 62 (1998) 758–762. http://dx.doi.org/10.1086/301812, 9529370.
[80] D.T. Brown, M. Herbert, V.K. Lamb, P.F. Chinnery, R.W. Taylor, R.N. Lightowlers, et al, Transmission of mitochondrial DNA disorders: possibilities for the future, Lancet 368 (2006) 87–89. http://dx.doi.org/10.1016/S0140-6736(06)68972-1, 16815383.
[81] J. Jakupciak, New microarray improves sensitivity of cancer detection by pinpointing small changes in mitochondrial DNA, Affymetrix Microarray Bulletin (2005).
[82] J.P. Jakupciak, W. Wang, M.E. Markowitz, D. Ally, M. Coble, S. Srivastava, et al, Mitochondrial DNA as a cancer biomarker, Journal of Molecular Diagnostics: JMD 7 (2005) 258–267. http://dx.doi.org/10.1016/S1525-1578(10)60553-3, 15858150.
[83] Y. Michikawa, F. Mazzucchelli, N. Bresolin, G. Scarlato, G. Attardi, Aging-dependent large accumulation of point mutations in the human mtDNA control region for replication, Science 286 (1999) 774–779. http://dx.doi.org/10.1126/science.286.5440.774, 10531063.
[84] P.E. Coskun, M.F. Beal, D.C. Wallace, Alzheimer's brains harbor somatic mtDNA control- region mutations that suppress mitochondrial transcription and replication, Proceedings of the National Academy of Sciences of the United States of America 101 (2004) 10726–10731. http://dx.doi.org/10.1073/pnas.0403649101, 15247418.
[85] W.D. Parker Jr, J.K. Parks, Mitochondrial ND5 mutations in idiopathic Parkinson's disease, Biochemical and Biophysical Research Communications

326 (2005) 667–669. http://dx.doi.org/10.1016/j.bbrc.2004.11.093, 15596151.
[86] B.A. Payne, I.J. Wilson, P. Yu-Wai-Man, J. Coxhead, D. Deehan, R. Horvath, et al, Universal heteroplasmy of human mitochondrial DNA, Human Molecular Genetics 22 (2013) 384–390. http://dx.doi.org/10.1093/hmg/dds435, 23077218.
[87] R.M. Smigrodzki, S.M. Khan, Mitochondrial microheteroplasmy and a theory of aging and age-related disease, Rejuvenation Research 8 (2005) 172–198. http://dx.doi.org/10.1089/rej.2005.8.172, 16144471.
[88] R.H. Swerdlow, S.M. Khan, The Alzheimer's disease mitochondrial cascade hypothesis: an update, Experimental Neurology 218 (2009) 308–315. http://dx.doi.org/10.1016/j.expneurol.2009.01.011, 19416677.
[89] W.D. Parker Jr, S.J. Boyson, J.K. Parks, Abnormalities of the electron transport chain in idiopathic Parkinson's disease, Annals of Neurology 26 (1989) 1231–1234, 2557792.
[90] W.D. Parker Jr, C.M. Filley, J.K. Parks, Cytochrome oxidase deficiency in Alzheimer's disease, Neurology 40 (1990) 1302–1303. http://dx.doi.org/10.1212/WNL.40.8.1302, 16637162166249.
[91] R.H. Swerdlow, The neurodegenerative mitochondriopathies, Journal of Alzheimer's Disease 17 (2009) 737–751, 19542616.
[92] R.H. Swerdlow, Role of mitochondria in Parkinson's disease, in: M.F. Chesselet (Ed.), Molecular Mechanisms of Neurodegenerative Diseases. Totowa, NJ, Humana Press, 2000, pp. 233–270.
[93] R.H. Swerdlow, S.J. Kish, Mitochondria in Alzheimer's disease, International Review of Neurobiology 53 (2002) 341–385. http://dx.doi.org/10.1016/S0074-7742(02)53013-0.
[94] R.H. Swerdlow, Mitochondria and cell bioenergetics: Increasingly recognized components and a possible etiologic cause of Alzheimer's disease, Antioxidants & Redox Signaling 16 (2012) 1434–1455. http://dx.doi.org/10.1089/ars.2011.4149, 21902597.
[95] R.H. Swerdlow, Does mitochondrial DNA play a role in Parkinson's disease? A review of cybrid and other supportive evidence, Antioxidants & Redox Signaling 16 (2012) 950–964. http://dx.doi.org/10.1089/ars.2011.3948, 21338319.
[96] W.D. Parker, Sporadic neurologic disease and the electron transport chain: A hypothesis, in: R.M. Pascuzzi (Ed.), Proceedings of the 1989 Scientific Meeting of the American Society for Neurological Investigation: New Developments in Neuromuscular Disease. Bloomington, IN, Indiana University Printing Services, 1990.
[97] M. Gu, J.M. Cooper, J.W. Taanman, A.H. Schapira, Mitochondrial DNA transmission of the mitochondrial defect in Parkinson's disease, Annals of Neurology 44 (1998) 177–186. http://dx.doi.org/10.1002/ana.410440207, 9708539.
[98] C.W. Shults, S.W. Miller, Reduced complex I activity in parkinsonian cybrids, Movement Disorders 13(Suppl.) (1998) S217.
[99] A.R. Esteves, A.F. Domingues, I.L. Ferreira, C. Januário, R.H. Swerdlow, C.R. Oliveira, et al, Mitochondrial function in Parkinson's disease cybrids containing an nt2 neuron-like nuclear background, Mitochondrion 8 (2008) 219–228. http://dx.doi.org/10.1016/j.mito.2008.03.004, 18495557.
[100] A.R. Esteves, J. Lu, M. Rodova, I. Onyango, E. Lezi, R. Dubinsky, et al, Mitochondrial respiration and respiration-associated proteins in cell lines created through Parkinson's subject mitochondrial transfer, Journal of Neurochemistry 113 (2010) 674–682. http://dx.doi.org/10.1111/j.1471-4159.2010.06631.x, 20132468.
[101] R.H. Swerdlow, J.K. Parks, D.S. Cassarino, D.R. Binder, J.P. Bennett Jr, G. DiIorio, et al, Biochemical analysis of cybrids expressing mitochondrial DNA from Contursi kindred Parkinson's subjects, Experimental Neurology 169 (2001) 479–485. http://dx.doi.org/10.1006/exnr.2001.7674, 11358461.
[102] D.S. Cassarino, C.P. Fall, R.H. Swerdlow, T.S. Smith, E.M. Halvorsen, S.W. Miller, et al, Elevated reactive oxygen species and antioxidant enzyme activities in animal and cellular models of Parkinson's disease, Biochimica et Biophysica Acta 1362 (1997) 77–86. http://dx.doi.org/10.1016/S0925-4439(97)00070-7, 9434102.
[103] I.G. Onyango, J.B. Tuttle, J.P. Bennett Jr., Brain-derived growth factor and glial cell line-derived growth factor use distinct intracellular signaling pathways to protect PD cybrids from H_2O_2-induced neuronal death, Neurobiology of Disease 20 (2005) 141–154. http://dx.doi.org/10.1016/j.nbd.2005.02.009, 16137575.
[104] I.G. Onyango, J.B. Tuttle, J.P. Bennett Jr., Activation of p38 and N-acetylcysteine-sensitive c-Jun NH2-terminal kinase signaling cascades is required for induction of apoptosis in Parkinson's disease cybrids, Molecular and Cellular Neuroscience 28 (2005) 452–461. http://dx.doi.org/10.1016/j.mcn.2004.10.006.
[105] D.S. Cassarino, E.M. Halvorsen, R.H. Swerdlow, N.N. Abramova, W.D. Parker Jr, T.W. Sturgill, et al, Interaction among mitochondria, mitogen-activated protein kinases, and nuclear factor-kappaB in cellular models of Parkinson's disease, Journal of Neurochemistry 74 (2000) 1384–1392, 10737593.
[106] G.A. Veech, J. Dennis, P.M. Keeney, C.P. Fall, R.H. Swerdlow, W.D. Parker Jr, et al, Disrupted mitochondrial electron transport function increases expression of anti-apoptotic bcl-2 and bcl-X(L) proteins in SH-SY5Y neuroblastoma and in Parkinson disease cybrid cells through oxidative stress, Journal of Neuroscience Research 61 (2000) 693–700. http://dx.doi.org/10.1002/1097-4547(20000915)61:6<693::AID-JNR13>3.3.CO;2-W, 10972966.
[107] J.P. Sheehan, R.H. Swerdlow, W.D. Parker, S.W. Miller, R.E. Davis, J.B. Tuttle, Altered calcium homeostasis in cells transformed by mitochondria from individuals with Parkinson's disease, Journal of Neurochemistry 68 (1997) 1221–1233, 9048769.
[108] P.A. Trimmer, R.H. Swerdlow, J.K. Parks, P. Keeney, J.P. Bennett Jr, S.W. Miller, et al, Abnormal mitochondrial morphology in sporadic Parkinson's and Alzheimer's disease cybrid cell lines, Experimental Neurology 162 (2000) 37–50. http://dx.doi.org/10.1006/exnr.2000.7333, 10716887.
[109] P.A. Trimmer, M.K. Borland, P.M. Keeney, J.P. Bennett Jr, W.D. Parker Jr., Parkinson's disease transgenic mitochondrial cybrids generate Lewy inclusion bodies, Journal of Neurochemistry 88 (2004) 800–812. http://dx.doi.org/10.1046/j.1471-4159.2003.02168.x.
[110] E.N. Cronin-Furman, M.K. Borland, K.E. Bergquist, J.P. Bennett Jr, P.A. Trimmer, Mitochondrial quality, dynamics and functional capacity in Parkinson's disease cybrid cell lines selected for Lewybody expression, Molecular Neurodegeneration 26 (2013) 6.
[111] Y. Aomi, C.S. Chen, K. Nakada, S. Ito, K. Isobe, H. Murakami, et al, Cytoplasmic transfer of platelet mtDNA from elderly patients with Parkinson's disease to mtDNA-less HeLa cells restores complete mitochondrial respiratory function, Biochemical and Biophysical Research Communications 280 (2001) 265–273. http://dx.doi.org/10.1006/bbrc.2000.4113, 11162509.
[112] R.E. Davis, S. Miller, C. Herrnstadt, S.S. Ghosh, E. Fahy, L.A. Shinobu, et al, Mutations in mitochondrial cytochrome c oxidase genes segregate with late-onset Alzheimer disease, Proceedings of the National Academy of Sciences of the United States of America 94 (1997) 4526–4531. http://dx.doi.org/10.1073/pnas.94.9.4526, 9114023.
[113] R.E. Davis, S. Miller, C. Herrnstadt, S.S. Ghosh, E. Fahy, L.A. Shinobu, et al, Retraction, Proceedings of the National Academy of Sciences of the United States of America 95 (1998) 12069. http://dx.doi.org/10.1073/pnas.95.20.12069-b, 165788579786782.
[114] S.S. Ghosh, R.H. Swerdlow, S.W. Miller, B. Sheeman, W.D. Parker Jr, R.E. Davis, Use of cytoplasmic hybrid cell lines for elucidating the role of mitochondrial dysfunction in Alzheimer's disease and Parkinson's disease, Annals of the New York Academy of Sciences 893 (1999) 176–191. http://dx.doi.org/10.1111/j.1749-6632.1999.tb07825.x, 10672237.
[115] J.P. Sheehan, R.H. Swerdlow, S.W. Miller, R.E. Davis, J.K. Parks, W.D. Parker, et al, Calcium homeostasis and reactive oxygen species production in cells transformed by mitochondria from individuals with sporadic Alzheimer's disease, Journal of Neuroscience: the Official Journal of the Society for Neuroscience 17 (1997) 4612–4622, 9169522.
[116] P.A. Trimmer, P.M. Keeney, M.K. Borland, F.A. Simon, J. Almeida, R.H. Swerdlow, et al, Mitochondrial abnormalities in cybrid cell models of sporadic Alzheimer's disease worsen with passage in culture, Neurobiology of Disease 15 (2004) 29–39. http://dx.doi.org/10.1016/j.nbd.2003.09.011, 14751768.
[117] D.F. Silva, I. Santana, A.R. Esteves, I. Baldeiras, D.M. Arduino, C.R. Oliveira, et al, Prodromal metabolic phenotype in MCI cybrids: implications for Alzheimer's disease, Current Alzheimer Research 10 (2013) 180–190. http://dx.doi.org/10.2174/1567205011310020008, 22746213.
[118] S.M. Cardoso, I. Santana, R.H. Swerdlow, C.R. Oliveira, Mitochondria dysfunction of Alzheimer's disease cybrids enhances Abeta toxicity, Journal of Neurochemistry 89 (2004) 1417–1426. http://dx.doi.org/10.1111/j.1471-4159.2004.02438.x, 15189344.
[119] S.M. Khan, D.S. Cassarino, N.N. Abramova, P.M. Keeney, M.K. Borland, P.A. Trimmer, et al, Alzheimer's disease cybrids replicate beta-amyloid abnormalities through cell death pathways, Annals of Neurology 48 (2000) 148–155. http://dx.doi.org/10.1002/1531-8249(200008)48:2<148::AID-ANA3>3.3.CO;2-Z, 10939564.
[120] I.G. Onyango, J.P. Bennett Jr, J.B. Tuttle, Endogenous oxidative stress in sporadic Alzheimer's disease neuronal cybrids reduces viability by increasing apoptosis through pro-death signaling pathways and is mimicked by oxidant exposure of control cybrids, Neurobiology of Disease 19 (2005) 312–322. http://dx.doi.org/10.1016/j.nbd.2005.01.026, 15837587.
[121] I.G. Onyango, J.B. Tuttle, J.P. Bennett Jr., Altered intracellular signaling and reduced viability of Alzheimer's disease neuronal cybrids is reproduced by beta-amyloid peptide acting through receptor for advanced glycation end products (RAGE), Molecular and Cellular Neurosciences 29 (2005) 333–343. http://dx.doi.org/10.1016/j.mcn.2005.02.012, 15911356.
[122] G.N. Bijur, R.E. Davis, R.S. Jope, Rapid activation of heat shock factor- 1 DNA binding by H_2O_2 and modulation by glutathione inhuman neuroblastoma and Alzheimer's disease cybrid cells, Brain Research. Molecular Brain Research 71 (1999) 69–77. http://dx.doi.org/10.1016/S0169-328X(99)00168-0, 10407188.
[123] Sarno P. De, G.N. Bijur, R. Lu, R.E. Davis, R.S. Jope, Alterations in muscarinic receptor-coupled phosphoinositide hydrolysis and AP-1 activation in Alzheimer's disease cybrid cells, Neurobiology of Aging 21 (2000) 31–38, 10794846.
[124] D.S. Cassarino, R.H. Swerdlow, J.K. Parks, W.D. Parker Jr, J.P. Bennett Jr., Cyclosporin A increases resting mitochondrial membrane potential in SY5Y cells and reverses the depressed mitochondrial membrane potential of Alzheimer's disease cybrids, Biochemical and Biophysical Research Communications 248 (1998) 168–173. http://dx.doi.org/10.1006/bbrc.1998.8866, 9675105.
[125] C. Thiffault, J.P. Bennett Jr., Cyclical mitochondrial deltapsiM fluctuations linked to electron transport, F0F1 ATP-synthase and mitochondrial Na^{+}/Ca^{+2} exchange are reduced in Alzheimer's disease cybrids, Mitochondrion 5 (2005) 109–119. http://dx.doi.org/10.1016/j.mito.2004.12.002, 16050977.
[126] P.A. Trimmer, M.K. Borland, Differentiated Alzheimer's disease transmitochondrial cybrid cell lines exhibit reduced organelle movement, Antioxidants & Redox Signaling 7 (2005) 1101–1109. http://dx.doi.org/10.1089/ars.2005.7.1101, 16115014.
[127] D.F. Silva, J.E. Selfridge, J. Lu, L. E, N. Roy, L. Hutfles, et al, Bioenergetic flux, mitochondrial mass and mitochondrial morphology dynamics in AD and MCI cybrid cell lines, Human Molecular Genetics 1 (2013) 3931–3943, 23740939.
[128] X. Gan, S. Huang, L. Wu, Y. Wang, G. Hu, G. Li, et al, Inhibition of ERK-DLP1 signaling and mitochondrial division alleviates mitochondrial dysfunction in

Alzheimer'sdisease cybrid cell, Biochimica et Biophysica Acta 1842 (2014) 220–231. http://dx.doi.org/10.1016/j.bbadis.2013.11.009, 24252614.
[129] R.H. Swerdlow, J.K. Parks, D.S. Cassarino, P.A. Trimmer, S.W. Miller, D.J. Maguire, et al, Mitochondrian sporadic amyotrophic lateral sclerosis, Experimental Neurology 153 (1998) 135–142. http://dx.doi.org/10.1006/exnr.1998.6866.
[130] R.H. Swerdlow, J.K. Parks, G. Pattee, W.D. Parker Jr., Role of mitochondria in amyotrophic lateral sclerosis, Amyotrophic Lateral Sclerosis and Other Motor Neuron Disorders: Official Publication of the World Federation of Neurology, Research Group on Motor Neuron Diseases 1 (2000) 185–190. http://dx.doi.org/10.1080/14660820050515179, 11464951.
[131] R.H. Swerdlow, J.K. Parks, S.W. Miller, G. Pattee, R.E. Davis, W.D. Parker, Evidence of genetic mitochondrial pathology in sporadic amyotrophic lateral sclerosis, Society for Neuroscience Abstracts 22 (1996) 2143.
[132] F.R. Wiedemann, K. Winkler, A.V. Kuznetsov, C. Bartels, S. Vielhaver, H. Feistner, et al, Impairment of mitochondrial function in skeletal muscle of patients with amyotrophic lateral sclerosis, Journal of the Neurological Sciences 156 (1998) 65–72. http://dx.doi.org/10.1016/S0022-510X(98)00008-2, 9559989.
[133] C.D. Gajewski, M.T. Lin, M.E. Cudkowicz, M.F. Beal, G. Manfredi, Mitochondrial DNA from platelets of sporadic ALS patients restores normal respiratory functions in rho(0) cells, Experimental Neurology 179 (2003) 229–235. http://dx.doi.org/10.1016/S0014-4886(02)00022-5, 12618129.
[134] Monte D.A. Di, Y. Harati, J. Jankovic, M.S. Sandy, S.A. Jewell, J.W. Langston, Muscle mitochondrial ATP production in progressive supranuclear palsy, Journal of Neurochemistry 62 (1994) 1631–1634, 8133289.
[135] R.H. Swerdlow, L.I. Golbe, J.K. Parks, D.S. Cassarino, D.R. Binder, A.E. Grawey, et al, Mitochondrial dysfunction in cybrid lines expressing mitochondrial genes from patients with progressive supranuclear palsy, Journal of Neurochemistry 75 (2000) 1681–1684, 10987850.
[136] D.S. Albers, R.H. Swerdlow, G. Manfredi, C. Gajewski, L. Yang, W.D. Parker Jr, et al, Further evidence for mitochondrial dysfunction in progressive supranuclear palsy, Experimental Neurology 168 (2001) 196–198. http://dx.doi.org/10.1006/exnr.2000.7607, 11170735.
[137] J.W. Chirichigno, G. Manfredi, M.F. Beal, D.S. Albers, Stress-induced mitochondrial depolarization and oxidative damage in PSP cybrids, Brain Research 951 (2002) 31–35. http://dx.doi.org/10.1016/S0006-8993(02)03101-3, 12231453.
[138] R.H. Swerdlow, J.K. Parks, D.S. Cassarino, A.T. Shilling, J.P. Bennett Jr, M.B. Harrison, et al, Characterization of cybrid cell lines containing mtDNA from Huntington's disease patients, Biochemical and Biophysical Research Communications 261 (1999) 701–704, 10441489.
[139] W.D. Parker Jr, S.J. Boyson, A.S. Luder, J.K. Parks, Evidence for a defect in NADH: Ubiquinone oxidoreductase (complex I) in Huntington's disease, Neurology 40 (1990) 1231–1234. http://dx.doi.org/10.1212/WNL.40.8.1231, 2143271.
[140] I.L. Ferreira, M.V. Nascimento, M. Ribeiro, S. Almeida, S.M. Cardoso, M. Grazina, et al, Mitochondrial-dependent apoptosis in Huntington's disease human cybrids, Experimental Neurology 222 (2010) 243–255. http://dx.doi.org/10.1016/j.expneurol.2010.01.002, 20079354.
[141] I.L. Ferreira, T. Cunha-Oliveira, M.V. Nascimento, M. Ribeiro, M.T. Proença, C. Januário, et al, Bioenergetic dysfunction in Huntington's disease human cybrids, Experimental Neurology 23 (2011) 127–134, 21684277.
[142] K.A. Laderman, J.R. Penny, F. Mazzucchelli, N. Bresolin, G. Scarlato, G. Attardi, Aging-dependent functional alterations of mitochondrial DNA (mtDNA) from human fibroblasts transferred into mtDNA-less cells, Journal of Biological Chemistry 271 (1996) 15891–15897. http://dx.doi.org/10.1074/jbc.271.27.15891, 8663253.
[143] J. Hayashi, S. Ohta, Y. Kagawa, H. Kondo, H. Kaneda, H. Yonekawa, et al, Nuclear but not mitochondrial genome involvement in human age-related mitochondrial dysfunction. Functional integrity of mitochondrial DNA from aged subjects, Journal of Biological Chemistry 269 (1994) 6878–6883, 8120050.
[144] P. Seibel, Nunno C. Di, C. Kukat, I. Schäfer, Bo R. Del, A. Bordoni, et al, Cosegregation of novel 16S rRNA gene mutations with the age-associated T414G variant in human cybrids, Nucleic Acids Research 36 (2008) 5872–5881. http://dx.doi.org/10.1093/nar/gkn592, 18796524.
[145] H. Hao, L.E. Morrison, C.T. Moraes, Suppression of a mitochondrial tRNA gene mutation phenotype associated with changes in the nuclear background, Human Molecular Genetics 8 (1999) 1117–1124. http://dx.doi.org/10.1093/hmg/8.6.1117, 10332045.
[146] J.H. Deng, Y. Li, J.S. Park, J. Wu, P. Hu, J. Lechleiter, et al, Nuclear suppression of mitochondrial defects in cells without the ND6 subunit, Molecular and Cellular Biology 26 (2006) 1077–1086. http://dx.doi.org/10.1128/MCB.26.3.1077-1086.2006, 16428459.
[147] W.S. Kunz, Different metabolic properties of mitochondrial oxidative phosphorylation in different cell types—important implications for mitochondrial cytopathies, Experimental Physiology 88 (2003) 149–154. http://dx.doi.org/10.1113/eph8802512, 12525863.
[148] D.T. Johnson, R.A. Harris, S. French, P.V. Blair, J. You, K. Bemis, et al, The tissue heterogeneity of the mammalian mitochondrial proteome, American Journal of Physiology Cell Physiology 292 (2006) C689–C697.
[149] E. Saksela, Variation of the chromosome number and anomalous mitoses in HeLa cells, Acta Pathologica et Microbiologica Scandinavica 154(Suppl.) (1962) 156–159, 14496227.
[150] P.W. Andrews, I. Damjanov, D. Simon, G.S. Banting, C. Carlin, N.C. Dracopoli, et al, Pluripotent embryonal carcinoma clones derived from the human teratocarcinoma cell line Tera-2. Differentiation in vivo and in vitro, Laboratory Investigation; a Journal of Technical Methods and Pathology 50 (1984) 147–162, 6694356.
[151] G.J. Kim, S.Y. Park, H. Kim, Y.H. Chun, S.H. Park, Chromosomal aberrations in neuroblastoma cell lines identified by cross species color banding and chromosome painting, Cancer Genetics and Cytogenetics 129 (2001) 10–16. http://dx.doi.org/10.1016/S0165-4608(01)00418-6, 11520559.
[152] P.T. Nelson, D. Kondziolka, L. Wechsler, S. Goldstein, J. Gebel, S. DeCesare, et al, Clonal human (hNT) neuron grafts for stroke therapy: neuropathology in a patient 27 months after implantation, American Journal of Pathology 160 (2002) 1201–1206. http://dx.doi.org/10.1016/S0002-9440(10)62546-1, 11943704.
[153] N. Cohen, D.R. Betts, G. Rechavi, N. Amariglio, L. Trakhtenbrot, Clonal expansion and not cell interconversion is the basis for the neuroblast and non neuronal types of the SK−N−SH neuroblastoma cell line, Cancer Genetics and Cytogenetics 143 (2003) 8–84.
[154] R. Moreno-Loshuertos, R. Acin-Perez, P. Fernandez-Silva, N. Movilla, A. Perez-Martos, S.R. de Cordoba, et al, Differences in reactive oxygen species production explain the phenotypes associated with common mouse mitochondrial DNA variants, Nature Genetics 38 (2006) 1261–1268. http://dx.doi.org/10.1038/ng1897, 17013393.
[155] P.L. Roubertoux, F. Sluyter, M. Carlier, B. Marcet, F. Maarouf-Veray, C. Cherif, et al, Mitochondrial DNA modifies cognition in interaction with the nuclear genome and age in mice, Nature Genetics 35 (2003) 65–69. http://dx.doi.org/10.1038/ng1230, 12923532.
[156] X. Yu, U. Gimsa, L. Wester-Rosenlöf, E. Kanitz, W. Otten, M. Kunz, et al, Dissecting the effects of mtDNA variations on complex traits using mouse conplastic strains, Genome Research 19 (2009) 159–165, 19037013.
[157] X. Yu, L. Wester-Rosenlöf, U. Gimsa, S.A. Holzhueter, A. Marques, L. Jonas, et al, The mtDNA nt7778 G/T polymorphism affects autoimmune diseases and reproductive performance in the mouse, Human Molecular Genetics 18 (2009) 4689–4698. http://dx.doi.org/10.1093/hmg/ddp432, 19759059.
[158] M. Pravenec, M. Hyakukoku, J. Houstek, V. Zidek, V. Landa, P. Mlejnek, et al, Direct linkage of mitochondrial genome variation to risk factors for type 2 diabetes in conplastic strains, Genome Research 17 (2007) 1319–1326. http://dx.doi.org/10.1101/gr.6548207, 17693571.
[159] J.L. Mott, D. Zhang, P.L. Farrar, S.W. Chang, H.P. Zassenhaus, Low frequencies of mitochondrial DNA mutations cause cardiac disease in the mouse, Annals of the New York Academy of Sciences 893 (1999) 353–357, 10672265.
[160] A. Trifunovic, A. Wredenberg, M. Falkenberg, J.N. Spelbrink, A.T. Rovio, C.E. Bruder, et al, Premature ageing in mice expressing defective mitochondrial DNA polymerase, Nature 429 (2004) 417–423. http://dx.doi.org/10.1038/nature02517, 15164064.
[161] G.C. Kujoth, A. Hiona, T.D. Pugh, S. Someya, K. Panzer, S.E. Wohlgemuth, et al, Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging, Science 309 (2005) 481–484. http://dx.doi.org/10.1126/science.1112125, 16020738.
[162] C.A. Pinkert, I.A. Trounce, Production of transmitochondrial mice, Methods 26 (2002) 348–357. http://dx.doi.org/10.1016/S1046-2023(02)00041-5, 12054926.
[163] M. McKenzie, I.A. Trounce, C.A. Cassar, C.A. Pinkert, Production of homoplasmic xenomitochondrial mice, Proceedings of the National Academy of Sciences of the United States of America 101 (2004) 1685–1690. http://dx.doi.org/10.1073/pnas.0303184101, 14745024.
[164] S.M. Khan, R.M. Smigrodzki, R. Swerdlow, Cell and animal models of mtDNA biology: progress and prospects, American Journal of Physiology. Cell Physiology (2007), [Epub ahead of print] 16899549.
[165] Y. Wang, Y. Pan, A. Price, L.J. Martin, Generation and characterization of transgenic mice expressing mitochondrial targeted red fluorescent protein selectively in neurons: Modeling mitochondriopathy in excitotoxicity and amyotrophic lateral sclerosis, Molecular Neurodegeneration 2 (2011) 75, 22047141.
[166] J.L. Fetterman, B.R. Zelickson, L.W. Johnson, D.R. Moellering, D.G. Westbrook, M. Pompilius, et al, Mitochondrial genetic background modulates bioenergetics and susceptibility to acute cardiac volume overload, Biochemical Journal 455 (2013) 157–167. http://dx.doi.org/10.1042/BJ20130029, 23924350.