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Review Article

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

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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.

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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

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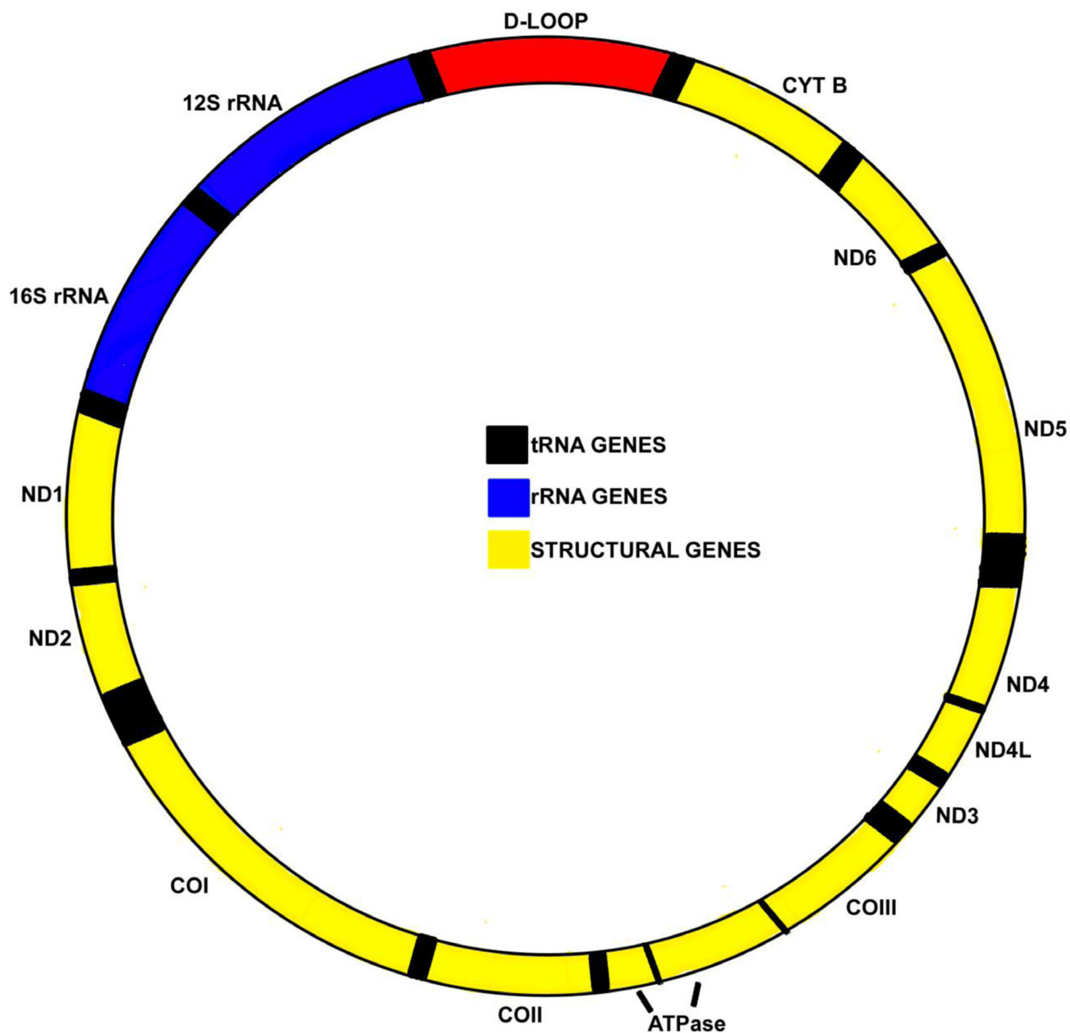

Fig. 1. The mitochondrial genome. This figure depicts the circular mitochondrial genome. Structural genes are shown in yellow. rRNA genes are shown in blue, while tRNA genes are shown in black. The regulatory region, D-loop, is shown in red. CO = cytochrome oxidase, ND = NADH dehydrogenase.

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].

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