Review
Organization, dynamics and transmission of mitochondrial DNA:
Focus on vertebrate nucleoids

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Abstract

Eukaryotic cells contain numerous copies of the mitochondrial genome (from 50 to 100 copies in the budding yeast to some thousands in humans) that localize to numerous intramitochondrial nucleoprotein complexes called nucleoids. The transmission of mitochondrial DNA differs significantly from that of nuclear genomes and depends on the number, molecular composition and dynamic properties of nucleoids and on the organization and dynamics of the mitochondrial compartment. While the localization, dynamics and protein composition of mitochondrial DNA nucleoids begin to be described, we are far from knowing all mechanisms and molecules mediating and/or regulating these processes. Here, we review our current knowledge on vertebrate nucleoids and discuss similarities and differences to nucleoids of other eukaryots.

Keywords: Mitochondrial DNA; Mitochondrial nucleoid; Mitochondrial genetics

1. Introduction

In the early 1950s, yeast geneticists reported cases where the transmission of some mitochondrial characters did not obey Mendelian rules and followed a “cytoplasmic inheritance pattern”. The carrier of this non-nuclear information was called ρ (Greek rho) and remained unknown until the mid 1960s, when the existence of mitochondrial DNA (mtDNA) molecules was formally demonstrated (reviewed in [1–3]). The mtDNA encodes few protein subunits of the mitochondrial respiratory complexes (usually 9–13) as well as tRNA and rRNA molecules for their intramitochondrial translation (reviewed in [4]). In order to understand the mechanisms governing the transmission and inheritance of mtDNA it is necessary to know the overall dynamics of mitochondria as well as the intramitochondrial organization and dynamics of mitochondrial genomes.

It was early recognized that mtDNA does not distribute homogeneously within the mitochondrial compartment, but concentrates in structures that represent the dynamic and inheritable units of mtDNA and were called chondriolites, nucleoids or mitochondrial nuclei (for a review of early studies see [5]). Although their properties and dynamics begin to be unraveled, their molecular and structural organization remain largely unknown. Given the facility of genetic manipulations in the budding yeast, it is in this organism that studies on mtDNA maintenance, organization and dynamics have achieved most significant progress. Findings on the maintenance and organization of yeast mtDNA have been extensively reviewed elsewhere [6–9] and this review will concentrate on the mtDNA of vertebrates. We will briefly present the diversity of mitochondrial genetic systems in eukaryots, summarize open questions regarding the transmission of mtDNA in mammals and review our current knowledge on mtDNA organization and dynamics. Where appropriate, we will discuss the similarities and differences between vertebrates and other eukaryots, especially the yeast S. cerevisiae.

2. The diversity of mitochondrial DNA

Despite the overall conservation of its restricted genetic function, mtDNA shows an extraordinary diversity between
eukaryots. The size of the mitochondrial genome ranges from 6 kb in some apicomplexans (like the malaria parasite *Plasmodium* sp.) to 200–400 kb in plants [4]. The unicellular flagellates of the order Kinetoplastida (e.g. *Trypanosoma* sp.) contain the most structurally complex mtDNA of all eukaryots: few dozens of maxicircles (20–40 kb) and thousands of minicircles (0.5–10 kb) are organized in a single compact structure called kinetoplast [10]. In metazoan animals, the genome is contained in thousands of identical circular mtDNA molecules with a size of 15–20 kb [11]. The mtDNA of fungi (from 19 kb in *Schizosaccharomyces pombe* to 75 kb in *Saccharomyces cerevisiae*) also maps as a circle. However, only a minor amount of mtDNA is circular and the majority of the 50–100 mtDNA molecules within a cell exist in the form of polydisperse tandem arrays of linear molecules [2,12,13]. In addition, *S. cerevisiae* differs from many yeast species (like the fission yeast *S. pombe*) and from all metazoans, in that it is a facultative anaerobe that tolerates the mutation and/or loss of mtDNA. It can spontaneously mutate into “cytoplasmic petite” mutants with deleted mtDNA (ρ−) or no mtDNA at all (ρ0) that can grow in the presence of fermentable substrates [14]. This loss of functional mtDNA can be induced (or its frequency increased) by the mutation of genes involved in mtDNA-maintenance and mitochondrial dynamics [7–9] but also in numerous, apparently unrelated, mitochondrial functions (reviewed in [6]). In contrast, mammalian cells do not spontaneously loose mtDNA and cells devoid of mtDNA can only be obtained after long-term treatment with DNA-binding agents that inhibit mtDNA polymerase [15,16] or upon knockout-out of mtTFA/TFAM [17], a protein playing a crucial role in mtDNA-organization (see below). In some human mitochondrial diseases, mutations of genes required for mtDNA stability and/or maintenance lead to the accumulation of mutations or deletions [18–20] and/or to severe depletion of the mtDNA [21,22], but not to its complete loss. Model organisms like yeast offer impressive advantages for genetic manipulation and/or maintenance and mitochondrial dynamics [7–9] but also in different mechanisms: a dramatic reduction of the number of mtDNA molecules in the development of germ cells, a clonal amplification of a small and selected part of mtDNA molecules during maturation of the primary oocyte (during which the number of mtDNA molecules increases one hundred fold) and/or an unequal repartition of mtDNA molecules between the inner mass cells and the trophoblast that further gives the extra embryonic tissues [29].

Study of heteroplasmic mice carrying two different mtDNA genotypes has later shown that segregation of the mtDNA genotypes probably occurs in oogonia, the number of mtDNA molecules of which is reduced to ∼200 [30]. Moreover, this study has demonstrated that the transmission of mtDNA heteroplasmy to the offspring is predominantly determined by random genetic drift and can be modelled as a binomial sampling process. Indeed, the mean level of heteroplasmy among the offspring of a single heteroplasmic mouse is approximately equal to that of the mother. However, the heteroplasmy level can be dramatically different between members of the offspring [30]. Similar conclusions were drawn from the genetic analysis of human pedigrees with a deleterious heteroplasmic mtDNA mutation [31].

The fate of mtDNA during female germ line development is an important aspect in mtDNA inheritance. During that process, both mitochondria and mtDNA are amplified but the volume of the organelles and the amount of mitochondrial rRNA and mRNA increase ten times more than the mtDNA copy number leading to a dramatic decrease of the number of mtDNA molecules per mitochondrion [29,32]. Further segregation of mitochondria may occur during cytoplasmic partition of oocyte in early embryogenesis, separation of the endoderm, mesoderm, and ectoderm, and migration and division of primordial germ cells in the undifferentiated gonad. This mitochondria segregation could explain segregation of mtDNA genotypes between
tissues as was observed in heteroplasmic cows [29] and is also encountered in human patients [33,34].

The existence of further and/or different segregation mechanisms is shown by the fact that segregation of mtDNA genotypes in heteroplasmic mice is also observed after birth [35]. Indeed, although these mice presented at birth with homogenous heteroplasmy in all their organs, four tissues demonstrated significantly different proportion of the two genotypes with increasing age: one mtDNA genotype increased its proportion in blood and spleen whereas the other one increased in liver and kidney [35]. The mechanisms driving this mtDNA selection/purification remain unknown [36–38] and could involve the turn over of the mtDNA molecules, that of mitochondrial organelles or even that of cells.

The reported studies with cows and mice were performed on pedigrees with neutral mtDNA polymorphisms, the transmission of which may significantly differ from that of deleterious mtDNA mutations. Indeed, in contrast to neutral polymorphisms, severe mtDNA mutations responsible for diseases in a heteroplasmic state, almost never return to homoplasmy [28,39]. Furthermore, mtDNA deletions are very rarely transmitted to the offspring [40]. However, random genetic drift through a genetic bottleneck early during development also occurs in humans with deleterious heteroplasmic mtDNA mutation as shown by the different mutation loads of 82 primary oocytes from a woman carrying a mtDNA mutation responsible for MELAS (Mitochondrial Encephalomyopathy with Lactic Acidosis and Stroke-like episodes) [31]. The possibility of efficient segregation between healthy and pathogenic mtDNA genotype is shown by the large shifts in the proportion of deleterious mtDNA that may be observed in very few generations, although the return to homoplasmy for the pathogenic genotype appears prevented [28,39]. This may be related to the fact that deleterious mtDNA-mutations do not provoke symptoms below a specific threshold proportion but that, above this threshold, small increments of the mutation proportion and are associated with significant worsening of symptoms [41]. Therefore, it is possible that a shift towards homoplasm is too harmful for cell survival and hampers the transmission to viable cells and/or embryos. It is important to stress that the nature of the symptoms, the threshold proportion and the shift in heteroplasmy observed upon transmission vary significantly between mutations [41,42].

To date, our knowledge on pathogenic mtDNA inheritance allows only global statistical prediction of the risk of mutant mtDNA transmission to a child whose mother harbours a heteroplasmic mtDNA mutation [39]. Outside extreme values, it cannot predict the risk of disease based on the mutation load of the oocyte [42]. This is due to our ignorance of the subsequent mechanisms of segregation but also of those governing the threshold of clinical expression in the different organs. To be able to decipher the mechanisms governing mtDNA transmission and segregation during development and after birth, it is necessary to know how mitochondrial DNA is physically organized within mitochondria and what factors determine the number, localization and dynamics of the putative segregation units (for discussion, see [43,44]).

4. Intramitochondrial distribution and organization of mitochondrial DNA

The early electron microscopy studies by M.M.K. Nass and S. Nass demonstrated the presence of electron-transparent areas in the mitochondria of chick embryos. These areas resembled those occupied by the bacterial nucleosome and contained mtDNA-fibers [45,46]. Later studies confirmed the presence of such structures, termed nucleoids, in the mitochondria from different animal cells and tissues. Nucleoids were most prominent in undifferentiated cells and in cells engaged in rapid proliferation [47]. Electron microscopy analysis of mtDNA molecules released by osmotic shock revealed 2 to 6 circular mtDNA-molecules that appeared bound to the membranes of mitochondrial remnants [48]. Visualization of mtDNA-molecules released by detergent-mediated solubilization of mitochondria revealed the association of mtDNA to protein structures of probable membrane derivation [49]. Analysis of serial sections revealed two to three (up to six) nucleoids per organelle, as well as membrane association of mtDNA [50]. Recent electron microscopy studies confirmed the presence of mtDNA in membrane-associated structures of ∼65 nm diameter by immunogold labeling with DNA-specific antibodies [51]. It is remarkable that the number of mtDNA-molecules per mitochondrion found in these early electron microscopy studies (2–6: [48,50]) is similar to that found 40 years later in mitochondrial particles isolated by flow cytometry (1–11: [52]) or in the nucleoids of cultured human cells (1–15: [53]; 2–8: [54]; 6–10: [51]).

Electron microscopy studies in the yeast S. cerevisiae revealed nucleoids with a similar distribution and appearance than in vertebrates: electron-transparent mtDNA-containing areas that were regularly spaced along mitochondrial filaments [55]. In contrast, the plasmodia of the true slime mold Physarum polycephalum contain spherical or ovoid mitochondria that concentrate all mtDNA in one central electron dense and elongated structure that was originally named mitochondrial nucleus [5]. In Trypanosoma sp. and other flagellates of Kinetoplastidae, the single mitochondrion concentrates all mtDNA molecules in an electron-dense structure known as kinetoplast [10]. The peculiar aspect of mitochondrial nuclei and kinetoplasts point to significant structural and molecular differences to the nucleoids of vertebrates and fungi.

In fluorescence microscopy, the DNA-binding dye DAPI is routinely used to visualize the intracellular and intramitochondrial distribution of mtDNA in yeast cells, slime molds and trypanosomes (see [5,10,55]), depicting 10–40 nucleoids in yeast cells [55]. In DAPI-treated mammalian cells, some studies revealed an unexpectedly homogeneous and/or diffuse distribution of mtDNA [56,57], while others depicted discrete punctate structures scattered throughout mitochondria [53,58,59]. These controversial results, together with the difficulties encountered by some laboratories to obtain sufficiently intense DAPI signals, led to the development of alternative labeling methods based on the use of other DNA-binding dyes (ethidium, sybr-green, syto13 or pico-green) in living cells [19,51,59,60] or the incorporation of the thymidine
analogue BrdU, fluorescence in situ hybridization or DNA specific antibodies in fixed cells [51,54,59,61,62]. Most of these studies revealed the distribution of mtDNA to hundreds of punctate nucleoids (~300–800) regularly scattered throughout the mitochondrial compartment (Fig. 1), with some cell lines depicting lower nucleoid numbers (~50–100: [60]). Some authors (but not all: [54]) reported a preferential localization of nucleoids to the tips of mitochondria [59,62]. Quantitative analysis of nucleoids by different methods showed that these structures contain 2–15 mtDNA molecules each [51,53,54] and a comparison between human skin fibroblasts and immortal human cell lines (see also [63]) revealed slight differences in the number of mtDNA-molecules per nucleoid [54].

Altogether, these studies showed that the number of mtDNA-molecules and nucleoids is higher in humans than in the budding yeast [2,55]. It is not yet clear how the mtDNA copy number is regulated in animal cells [64]. The existence of mitochondrial nucleoids containing a definite number of mtDNA molecules may modify the way we search for answers to this open question. We may start to look for factors that regulate the number of nucleoids per cell and/or the number of mtDNA molecules per nucleoid. The existence of nucleoids containing various mtDNA-molecules also leads to the question of nucleoid-composition in heteroplasmic cells: do different mtDNA molecules localize to heteroplastic nucleoids or do they distribute to different homoplasmic nucleoids? Fluorescence in situ hybridization has been used to visualize nucleoids [59] and to distinguish wild-type mtDNA and mtDNA carrying large deletions [65,66], but these tools have not been yet applied to investigate heteroplasmy within mitochondria and/or nucleoids. The development of tools for the efficient discrimination and visualization of mtDNA-variants by microscopy is required to understand the (in)stability of heteroplasmy and the segregation of mtDNA-mutations [43,44] between generations, during embryo development and tissue differentiation (see above) as well as in cultured cells (see [67]).

5. Nucleoid composition and structure

Among the expected nucleoid components are proteins involved in the replication [68], transcription [69], repair [70] and perhaps recombination [71,72] of mtDNA. However, we also have to consider the existence of molecules ensuring their packaging, their association to the inner membrane, their scattered intramitochondrial distribution and/or their dynamics. So far, three main strategies have led to the identification of nucleoid-components and nucleoid-associated proteins: the purification and identification of mtDNA-binding proteins, the isolation and analysis of mtDNA-nucleoids and dedicated genetic screens in yeast. Here, we will review these approaches and present the molecules identified by these means (see Table 1 for a summary).

5.1. Major mtDNA-binding proteins

The search for mitochondrial proteins capable of binding DNA led to the identification of yeast Abf2p [73], which was initially called HM [74]. Yeast cells lacking functional Abf2p retain functional mtDNA (if maintained in non-fermentable media), but display diffuse and enlarged nucleoids [75]. The Abf2p protein carries two domains (HMG boxes) characteristic of a family of DNA-binding proteins (HMG-proteins) that are unrelated to nuclear histones and resemble HU-proteins involved in bacterial genome condensation [76,77]. Close homologs of yeast Abf2p have been identified in mammals (mtTFA/TFAM: [78]), in Xenopus (mtTFA: [79]), Drosophila (mtTFA: [80]) and in Physarum polycephalum (Glom: [81]). The mammalian mtTFA-protein is involved in mtDNA packaging [82] and contains an additional domain involved in transcription [78,83]. The Glom protein of Physarum contains, in addition to the HMG-boxes, a highly basic lysine-rich region comparable to that of proteins packaging mtDNA in kinetoplasts (KAPs: kinetoplast associated proteins). KAPs are highly

Fig. 1. Human mitochondrial DNA localizes to hundreds of punctate nucleoids that distribute throughout the mitochondrial compartment and are enriched in mtTFA. Human skin fibroblasts were decorated with antibodies against DNA (A, B), against the mitochondrial protein mitofilin (A) and/or against the mtDNA-packaging protein mtTFA (B). Insets depict enlargements of the boxed areas. Bars: 20 μm. (A) Mitochondrial filaments are homogeneously labeled with the mitofilin antibody and mtDNA is restricted to punctate nucleoids. (B) The mtTFA protein is enriched in mitochondrial nucleoids.
basic DNA-binding proteins that lack HMG-boxes and have some similarity to nuclear histone H1 (see [10] and references therein). It is interesting to note that the mtDNA packaging proteins of eukaryots (Abf2p/Gloom/KAPs) and nuclear HMG-proteins can complement mutant *Escherichia coli* lacking HU-protein [81,84,85]. In summary, the mtTFA/Abi2p proteins of vertebrates and yeast are highly abundant mitochondrial proteins that play a central role in packaging and compacting of mtDNA, are present in a molar excess over mtDNA (see for example: [75,86]) and represent major components of purified nucleoids (see below). The other major mtDNA binding protein, mitochondrial single-stranded DNA binding protein (mtSSB), is similarly abundant to mtTFA/TFAM [86]. It was the single detectable polypeptide retained in mtDNA–protein complexes isolated under highly stringent conditions and was shown to bind single stranded DNA and to coat single strands of replicative intermediates (see [87] and references therein). It has homologs in *D. melanogaster* and yeast and its DNA binding characteristics and structure are closely related to those of *E. coli* SSB [88–91]. It is required for mtDNA-replication in vitro and for mtDNA-replication and development in *D. melanogaster* [68,92]. A third mitochondrial protein able to bind mtDNA is the human LON protease, its precise role in the function of mtDNA-molecules or nucleoids remains unknown [93,94].

In contrast to other putative components of vertebrate nucleoids (see below and Table 1), human mtTFA/TFAM and mtSSB belong to the small group of proteins that show a restricted intramitochondrial distribution and localize almost exclusively to mtDNA-nucleoids (Fig. 1; [54,62]) and are co-enriched with mtDNA in protocols aiming the purification of mtDNA-nucleoids [62,95]. Note however that mtTFA was restricted to mtDNA-nucleoids in primary skin fibroblasts but displayed a wider intramitochondrial distribution (with clear nucleoid-enrichment) in immortal cell lines [54].

5.2. Purification and analysis of mitochondrial nucleoids

As described above, several studies have reported the association of mtDNA and/or of mtDNA-associated molecules to membranes (see e.g. [70] and references therein). Therefore, and irrespectively of the species and the source of mitochondria, the protocols for the isolation of mtDNA-nucleoids are all based on the solubilization of cells or mitochondria with detergents followed by the enrichment of mtDNA-containing structures by centrifugation in density gradients [96–99]. In addition, some authors reversibly cross-link mtDNA to proteins by treatment with formaldehyde [96,100]. It is interesting to note that various approaches fail to achieve complete solubilization of nucleoids with detergents (both in vertebrates and in yeast: [62,75]), which may point to interactions of nucleoids with non-membranous structures. Ideally, the conditions chosen for cross-linking, solubilization and purification should allow the retention of “true” components and lead to the loss of “unspecific” contaminants. However, it is impossible to establish such “ideal” conditions beforehand and the distinction between components and contaminants requires localization and functional characterization of newly identified molecules.

Early studies revealed the enrichment of 16–20 proteins in preparations of mtDNA–protein complexes from bovine heart [101], rat liver [102] and Xenopus oocytes [99]. Nucleoid preparations from *S. cerevisiae* [103] and *P. polycephalum* [97] contained polypeptide patterns of similar complexity and kinetoplast preparations revealed only a small set of associated proteins [96]. Nucleoid preparations from vertebrates were shown to contain mtSSB and mtTFA/TFAM [87,104], but the identity of most other nucleoids-associated proteins remained unknown.

Spelbrink and co-workers established protocols for the enrichment of nucleoids from cultured human cells and used Western blot analysis to reveal partial co-purification of TFAM, mtSSB, POLG and POLB with mtDNA [62]. Fluorescence microscopy revealed that some putative nucleoid-components (GFP-tagged Twinkle, mtSSB, mtTFA/TFAM) localized preferentially to mtDNA-nucleoids, while others (POLG) distribute rather homogeneously throughout mitochondria [19,61,62].

Recently, Bogenhagen and co-workers adapted established protocols [99] for the identification of mtDNA-associated proteins in the mitochondria of *Xenopus laevis* oocytes [95]. They found that highly purified nucleoid fractions contained proteins involved in mtDNA-maintenance and function (Table 1: group A) and a series of mitochondrial proteins apparently unrelated to mtDNA (Table 1: group B): adenine nucleotide

<p>| Table 1: Nucleoid-components and nucleoid associated proteins in vertebrates |
|---------------------------------|----------------|---------------|
| Group A: Proteins involved in mtDNA maintenance, replication and/or transcription |</p>
<table>
<thead>
<tr>
<th>Reference</th>
<th>Co-purification with mtDNA</th>
<th>Intramitochondrial distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>POLG A</td>
<td>[61,62]</td>
<td>partial</td>
</tr>
<tr>
<td>POLG B</td>
<td>[62]</td>
<td>partial</td>
</tr>
<tr>
<td>Twinkle</td>
<td>[62]</td>
<td>partial</td>
</tr>
<tr>
<td>mtSSB</td>
<td>[87,95]</td>
<td>partial</td>
</tr>
<tr>
<td>mtTFA/TFAM</td>
<td>[54,62,95]</td>
<td>yes</td>
</tr>
<tr>
<td>POLRMT</td>
<td>[69]</td>
<td>N.D.</td>
</tr>
<tr>
<td>mtTFB (Xenopus)</td>
<td>[79]</td>
<td>N.D.</td>
</tr>
<tr>
<td>TFB1M (human)</td>
<td>[135]</td>
<td>N.D.</td>
</tr>
<tr>
<td>TFB2M (human)</td>
<td>[135]</td>
<td>mitochondria*</td>
</tr>
<tr>
<td>RNase MRP</td>
<td>[136]</td>
<td>N.D.</td>
</tr>
<tr>
<td>Group B: Proteins with yet unknown nucleoid-function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LON protease</td>
<td>[94]</td>
<td>N.D.</td>
</tr>
<tr>
<td>BRC1</td>
<td>[137]</td>
<td>N.D.</td>
</tr>
<tr>
<td>ANT1</td>
<td>[95]</td>
<td>yes</td>
</tr>
<tr>
<td>PDC-E2</td>
<td>[95]</td>
<td>yes</td>
</tr>
<tr>
<td>BCKD-E2</td>
<td>[95]</td>
<td>yes</td>
</tr>
<tr>
<td>PHB2</td>
<td>[95]</td>
<td>yes</td>
</tr>
</tbody>
</table>

translocator 1 (ANT1), the lipoyl-containing E2 subunits of pyruvate decarboxylase (PDC-E2) and prohibitin 2 (PHB2). It is possible that the tight binding of mtDNA to these membrane (or membrane-associated) proteins contributes to the anchoring of nucleoids to the inner mitochondrial membrane. However, the nature and specificity of these interactions remains obscure. These abundant proteins probably distribute throughout the entire mitochondrion, and it is therefore improbable that they are determinant for the scattered distribution of nucleoids. Further experiments are required to establish the role of these proteins in nucleoid organization and function.

The analysis of nucleoid-fractions from the yeast *S. cerevisiae* also led to the identification of proteins for which the only known function is related to mtDNA (that were homologous to mtDNA-interacting proteins of vertebrates: Table 1, group A) and of proteins for which the previously known function was unrelated to mtDNA [100,105]. The proteins of the latter category were involved in (1) protein import and mitochondrial biogenesis, (2) the citric acid cycle and upstream reactions and (3) amino acid metabolism [105]. Interestingly, some of the latter yeast proteins (Lpd1, Pda1, Pdb1, Kgd1) were components of homologous protein complexes (PDH/PDC, BCKAD) and one of them (Kgd2) was the homolog of a vertebrate protein (BCKAD-E2) [95,105]. Various functional studies have shown that some of the yeast proteins are bi-functional, contribute to nucleoid-organization and function and couple metabolic regulation to mitochondrial DNA maintenance (see [9,105] and references therein).

### 5.3. Genetic screens in yeast

Genetic screens in yeast led to the identification of a series of outer membrane proteins (mdm10p, mdm12p, mmm1p) that form complexes required for the maintenance of mitochondrial morphology and for the linkage of mitochondria with the cytoskeleton [106–109]. They localize to dot-like structures on the mitochondrial surface that are adjacent to mitochondrial nucleoids [110–112] and their mutation and/or deletion lead to the rapid loss of mtDNA and/or to defects in nucleoid morphology and transmission [110]. They appear to cooperate with two inner membrane proteins (mdm31p, mdm32p) required for the maintenance of mitochondrial morphology and of mtDNA [113]. These outer and inner membrane proteins, or their yet unknown partners, are ideal candidates for the linkage of nucleoids to membranes and to the cytoskeleton, but physical interactions between outer membrane proteins, inner membrane proteins and/or mtDNA have not yet been demonstrated. Models of a molecular system enabling interactions across both mitochondrial membranes have been proposed [9], but their molecular architecture remains elusive (see e.g. [114]) and none of these molecules were identified in nucleoid preparations [100,105]). To date, the analysis of vertebrate genomes has not allowed the identification of putative homologs of these proteins. Homolog proteins may be absent from vertebrate mitochondria or escape detection due to too low sequence similarity. This points to further differences in the organization and dynamics of yeast and vertebrate mtDNA.

The identification of new nucleoid components and the functional characterization of mtDNA-associated proteins represents a substantial progress in our understanding of nucleoid composition. Advances in the identification and characterization of further nucleoid-components may unravel other similarities and/or differences between the nucleoids of yeasts and vertebrates. In addition, future work may well lead to the identification of new molecules ensuring fundamental nucleoid properties, such as their association to the inner membrane, their scattered intramitochondrial distribution and/or their (limited) mobility.

### 6. Dynamics of mtDNA nucleoids

Before the direct visualization of mtDNA, the mobility of mtDNA-molecules and/or nucleoids was indirectly inferred from the functional complementation between wild-type and/or mutant genomes, which requires the intermitochondrial exchange of mtDNA and/or their products (RNA and proteins). Complementation was established within heteroplasmic cells containing varying mutant proportions [115,116] and after fusion between cells carrying different mutations: several reports by the group of J. Hayashi showed that mitochondrial genomes can complement each other 10–14 days after cell fusion [117–119]. Using other mtDNA-mutations and cell lines, the group of G. Attardi reported that such intermitochondrial complementation was a very rare event [120,121]. The reasons for these divergences have not been solved [122], but the fact that mice with high proportions of mutant mtDNA retain functional mitochondria argues for the efficient exchange of functional mtDNA and mtDNA-derived molecules (RNA, proteins) between mitochondria [123]. The overall conclusion we draw from such complementation studies is that the intramitochondrial mobility of mtDNA may be reduced, but is sufficient for functional complementation between fusing mitochondria.

The development of mitochondrial fusion assays based on the mixing of fluorescent matrix proteins unambiguously demonstrated that mitochondria represent a single cellular compartment that exchanges matrix proteins by fusion [124–126]. F. Legros and co-workers used this fusion assay to demonstrate that nucleoids are also exchanged between mitochondria. Upon fusion of ρ+ and ρ0 cells, nucleoids diffused into ρ0 mitochondria with kinetics similar to those of fluorescent matrix proteins [54]. Hayashi and co-workers had reported similar results earlier, but the images depicted a diffuse intramitochondrial DAPI-stain [56] that did not reflect the nucleoid structure and distribution described by most other authors (see above). Upon fusion between ρ+ cells, however, nucleoids displayed a lower mobility and the nucleoids derived from one cell population were still absent from some regions of the mitochondrial network after 12 h [54]. This suggests that mtDNA-nucleoids have a lower intramitochondrial mobility than the matrix fluorescent proteins diffusing throughout the entire mitochondrial compartment within 8–12 h [124]. In vivo time-lapse imaging of nucleoids shows that that they are mobile structures that fuse and divide. These reactions can be associated, or not, to mitochondrial mobility, fusion and fission.
Quantitative analysis of their dynamics showed that, in accordance to the observations obtained with fusion assays [54], nucleoids have a reduced mobility and tend to maintain their relative intramitochondrial position [51]. It is interesting to note that nucleoid-mobility appears to be limited in a similar manner in *S. cerevisiae*. Upon mating of normal haploid yeast cells, mtDNA remains immobile within the fused mitochondrial compartment of the zygote [127]. However, upon mating of ρ+ and ρ0 cells, mtDNA diffuses freely from ρ+ to ρ0 mitochondria [128].

Iborra and co-workers [51] showed that nucleoids are in close proximity to microtubules and to KIF5B, a kinesin homolog involved in the interaction of mitochondria with microtubules [129,130]. This suggests the possibility that the intramitochondrial positioning and the dynamics of nucleoids are governed by interactions with the microtubule network. As discussed above, the molecules and structures responsible for such a link across the mitochondrial double membrane remain largely unknown (for proposed models see e.g. [9,51]).

The limited mobility and intermixing of human nucleoids [51,54] may contribute to the segregation of mtDNA molecules upon cytokinesis, especially in rapidly growing cells. This point may be clarified in the future, when it will be possible to observe mtDNA variants and nucleoid dynamics in heteroplasmic cells. These experiments will allow us to know whether mtDNA-molecules are exchanged between nucleoids, whether mitochondrial genomes are “mixed” by nucleoid fusion. All these issues are central to understand (in)stability of mtDNA-heteroplasmy and the segregation of mtDNA-mutations. As stated above, some of these experiments will only be possible after the development of tools enabling efficient discrimination and localization of different mtDNA molecules.

In the budding yeast, the transmission of mitochondria to buds and the retention of mitochondria in the mother cells are actively regulated processes mediated by the actin-cytoskeleton (reviewed in [23,131]). In the fission yeast, mitochondrial positioning is driven by association with dynamic microtubules and mitotic spindle poles [132]. In vertebrates, it is generally assumed that the fragmentation of mitochondria during mitosis ensures their equal transmission to daughter cells upon cytokinesis, but in fact, we know very little about organelle inheritance and transmission in vertebrates (see for example [133,134]). Investigating the behavior of mitochondria and mtDNA during mitosis and cytokinesis should provide important information on the transmission of mitochondria and on the inheritance/segregation of mtDNA. It is known that the amount of mtDNA is strictly controlled during embryonic development [30,32] and it will be interesting to follow the number and dynamics of nucleoids and during this process.

7. Conclusions

The organization and dynamics of mtDNA nucleoids within the mitochondria of cultured mammalian cells begin to be unraveled. However, we are far away from understanding these processes in respect to heteroplasmy and in more complex systems, like a developing embryo or an entire organism. Proteins involved in mtDNA-packaging and function have been identified, but it is well possible that novel molecules mediating and regulating nucleoid distribution and dynamics are identified in the future.

8. Note added in proof


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