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Review

# Mammalian mitochondrial nucleoids: Organizing an independently minded genome

Ian J. Holt [a,*], Jiuya He [a], Chih-Chieh Mao [a], Jerome D. Boyd-Kirkup [a], Peter Martinsson [b], Hiroshi Sembongi [a], Aurelio Reyes [a], Johannes N. Spelbrink [b]

[a] MRC-Dunn Human Nutrition Unit, Wellcome Trust-MRC Building, Hills Road, Cambridge CB2 OXY, UK
[b] Institute of Medical Technology and Tampere University Hospital, FI-33014 University of Tampere, Finland

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**Abstract**

Mitochondrial DNA is arranged in nucleoprotein complexes, or nucleoids. Nucleoid proteins include not only factors involved in replication and transcription but also structural proteins required for mitochondrial DNA maintenance. Although several nucleoid proteins have been identified and characterized in yeast over the course of the past decade, little was known of mammalian mitochondrial nucleoids until recently. Two publications in the past year have expanded considerably the pool of putative mammalian mitochondrial nucleoid proteins; and analysis of one of the candidates, ATAD3p, suggests that mitochondrial nucleoid formation and division are orchestrated, not random, events.


*Keywords:* Mammalian; Nucleoid; Mitochondrial D-loop

## 1. Introduction

Mitochondria contain their own DNA (mtDNA); at 16,569 bp it accounts for only 0.0005% of the human genome, a seemingly trivial amount. However, there is not much waste, so its 37 genes make up ~0.1% of the total number of human genes. Then if one considers DNA mass, the number rises to ~1% in many tissues, as mtDNA is polyploid, whereas nuclear DNA is diploid in somatic cells and haploid in gametes. Mitochondrial DNA provides the cell with several key components of the respiratory chain and ATP synthase, including the core of the membrane embedded arm of complex I, and the catalytic subunits of cytochrome *c* oxidase. Neither respiration nor ATP synthase function in the absence of mtDNA, in which case all the energy demands of an animal cell must be met by glycolysis. To widespread surprise, growth of animal cells without mtDNA, and therefore with no capacity to respire, can be achieved in the laboratory, as first demonstrated with chicken cells, and later with cultured human cells (Desjardins et al., 1985; King and Attardi, 1989).

There is however no question that mtDNA is essential to life for complex multicellular organisms, such as humans. Almost two decades ago defects in mtDNA were linked to human pathologies (Holt et al., 1988), and new causes of mtDNA disease continue to be identified (Spinazzola et al., 2006). These diseases are associated with a diverse array of clinical signs and symptoms, hampering diagnosis and confounding efforts to accurately estimate the incidence of mtDNA disease. More generally mutations in mtDNA have been linked to common neurodegenerative diseases, most convincingly Parkinson's disease (Bender et al., 2006; Kraytsberg et al., 2006); and shown to cause an ageing phenotype (Trifunovic et al., 2004). Therefore, mtDNA is a molecule worthy of attention.

Although there was preliminary evidence of mammalian mtDNA being associated with membranes as long ago as 1978 (Albring et al., 1977), many researchers and writers

* Corresponding author. Tel.: +44 12 23 25 28 40; fax: +44 12 23 25 28 45.
*E-mail address:* ih@mrc-dunn.cam.ac.uk (I.J. Holt).

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of textbooks depict mtDNA as naked and floating free in the matrix, the inner most compartment of the mitochondrion. Later studies in yeast demonstrated that mtDNA is, like DNA elsewhere, arranged in nucleoprotein complexes, or nucleoids (Bateman et al., 2002; Chen et al., 2005; Kaufman et al., 2003, 2000; MacAlpine et al., 2000). Mammalian mtDNA also forms discrete foci and these are substantially fewer than the copies of mtDNA; based on two studies the average number of mtDNA molecules per nucleoid is around six, in cultured human cells (Iborra et al., 2004; Legros et al., 2004). A thorough review of yeast and mammalian mitochondrial nucleoids was published in 2005 (Chen and Butow, 2005); here we focus on subsequent studies of mammalian mitochondrial nucleoids. First, however, it is important to review the rudiments of yeast mitochondrial nucleoids.

## 2. Protein composition of yeast mitochondrial nucleoids

Mitochondrial nucleoids will inevitably include proteins involved in replication and expression, and many of these (e.g. polymerases) are readily identifiable based on their homology to related proteins of other systems. Factors required for mtDNA maintenance, organisation and segregation are more difficult to predict. Isolation of formaldehyde cross-linked mitochondrial nucleoprotein (Kaufman et al., 2000) revealed several proteins previously implicated in mtDNA metabolism, including Abf2, which is a member of the high mobility group (HMG) family of DNA binding proteins. Abf2 is required for the maintenance of wild-type mtDNA in yeast; it is analogous to the prokaryotic protein HU, which is a component of bacterial nucleoids. Abf2 is reported to be sufficiently abundant in yeast to coat mtDNA and is assumed to be the primary mtDNA packaging protein, although it is now clear there is some redundancy (Chen et al., 2005). Rim1p, the yeast mitochondrial single-strand DNA binding protein (mtSSB) also cross-linked to mtDNA, as did Mgm101 (Kaufman et al., 2000), a protein previously linked to DNA replication and repair (Meeusen et al., 1999). The inventory of formaldehyde cross-linked proteins also contained several proteins that were far from obvious yeast mitochondrial nucleoid components, such as aconitase, Ilv5 and two subunits of α-keto glutarate dehydrogenase. Aconitase is a free radical-sensitive component of the TCA cycle, which can prevent mtDNA loss, in budding yeast lacking Abf2 (Chen et al., 2005). Ilv5 is so named because of its involvement in branched chain amino acid biosynthesis (i – ile, l – leu and v – val). A site-directed mutagenesis study of Ilv5 produced two distinct phenotypes; either the amino-acid biosynthetic function was preserved and mtDNA loss occurred, or else mtDNA was maintained and amino acid synthesis was compromised (Bateman et al., 2002). The two classes of mutation fell neatly into different parts of the gene, so it appears that the gene encodes a chimera: two proteins with unrelated functions in a single polypeptide chain. Although fascinating, this result realised the worst fears of the mitochondrial nucleoid hunters: no candidate however implausible could be rejected out of hand, as it might, like Ilv5, be multi-functional. The association of the chaperone Hsp60 with yeast mtDNA was another surprise (Kaufman et al., 2000), although it had earlier been shown to have single-strand DNA binding activity and to stimulate a DNA polymerase (Smiley et al., 1992). Considerable evidence has now accumulated to suggest Hsp60 is an important component of yeast mitochondrial nucleoids. Hsp60 alone was depleted when nucleoprotein was treated with single-strand nuclease (Kaufman et al., 2000); the protein influences the activity of a presumed origin of replication, and a temperature sensitive mutant of Hsp60 displayed abnormal nucleoid structure, based on deconvolution microscopy, and perturbed segregation, when grown at the non-permissive temperature (Kaufman et al., 2003).

## 3. Protein composition of mitochondrial nucleoids of higher eukaryotes, circa 2005

Transcription factor A (Tfam) is the animal homologue of Abf2; it is required for mitochondrial transcription (Fisher and Clayton, 1988) and mtDNA maintenance (Larsson et al., 1998). According to one report it is, like Abf2, sufficiently plentiful to coat mtDNA entirely, and so has been proposed to be the mtDNA packaging protein (Alam et al., 2003). Although it was not formally tested until 2003 (Garrido et al., 2003), it was the first recognized mammalian mitochondrial nucleoid protein. Twinkle, a mitochondrial DNA helicase discovered in 2001, was the first mammalian protein shown to co-localize with mitochondrial nucleoids by confocal microscopy (Spelbrink et al., 2001). Other replication factors (mitochondrial DNA polymerase and mtSSB) have subsequently been shown to co-fractionate with mammalian mtDNA (Garrido et al., 2003).

Daniel Bogenhagen's group began to tackle the problem of identifying mitochondrial nucleoid components by purifying nucleoprotein from frog oocyte mitochondria (Bogenhagen et al., 2003). This was an excellent starting material as oocytes have an extraordinarily high mtDNA copy number ($1 \times 10^5$ mtDNAs, or approximately one-third of the DNA mass of the egg). Encouragingly the preparation contained Tfam and mtSSB, however the authenticity of the other proteins is uncertain; to date no follow up study has been carried out to test whether they are genuine components of animal mitochondrial nucleoids, although two of them were later found in human mitochondrial nucleoprotein preparations (see below), which adds credibility. The presence of the adenine nucleotide translocator (ANT) was certainly provocative, as mutations in human ANT1 have been linked to mitochondrial disease (Kaukonen et al., 2000); however, ANT is the single most abundant protein of the mitochondrial inner membrane and so its presence as a contaminant cannot be dismissed lightly.