THE ORGANIZATION AND INHERITANCE OF THE MITOCHONDRIAL GENOME

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Abstract | Mitochondrial DNA (mtDNA) encodes essential components of the cellular energy-producing apparatus, and lesions in mtDNA and mitochondrial dysfunction contribute to numerous human diseases. Understanding mtDNA organization and inheritance is therefore an important goal. Recent studies have revealed that mitochondria use diverse metabolic enzymes to organize and protect mtDNA, drive the segregation of the organellar genome, and couple the inheritance of mtDNA with cellular metabolism. In addition, components of a membrane-associated mtDNA segregation apparatus that might link mtDNA transmission to mitochondrial movements are beginning to be identified. These findings provide new insights into the mechanisms of mtDNA maintenance and inheritance.

Mitochondria supply most of the cell's energy, which is produced as ATP by the oxidative phosphorylation apparatus of the mitochondrial inner membrane. The biogenesis of the ATP-synthesizing machinery requires contributions from two physically separated genomes: one in the nucleus and the other in mitochondria. In humans, more than 80 proteins participate directly in oxidative phosphorylation, of which 13 are encoded by mtDNA. The distribution of oxidative phosphorylation components that are encoded by the nuclear and mitochondrial genomes varies between species. However, in all species the essential contribution of the mitochondrial genome demands that mtDNA is faithfully inherited to ensure that respiratory function is maintained during growth and development.

Although 4 decades have elapsed since the discovery of mtDNA[1,2], its organization and inheritance remain poorly understood. mtDNA is packaged into protein–DNA complexes that are called, by analogy to the bacterial chromosome, mitochondrial nucleoids (mt-nucleoids). Overall, ~30 proteins from different species have been identified as potential components of mt-nucleoids (TABLE 1). Some have known functions in mtDNA transactions, and might therefore be expected to be present in mt-nucleoids. These proteins include the Abf2 and TFAM (transcription factor A, mitochondrial) family of mtDNA-packaging factors and the mitochondrial ssDNA-binding proteins. However, surprisingly, recent proteomic analyses of yeast[3,4] and *Xenopus laevis* mt-nucleoids[5] revealed a group of proteins for which the known functions are ostensibly unrelated to mtDNA transactions. Therefore, mitochondria might have evolved unique strategies for the organization and inheritance of their genomes by using proteins that also function in mitochondrial metabolism, potentially coupling these important processes.

Another rapidly developing field of mitochondrial biology focuses on understanding the segregation of mt-nucleoids during cell division. In addition to genes that are directly involved in mtDNA recombination, several other genes have recently been discovered that control mt-nucleoid distribution and stability. Interestingly, these include genes that control mitochondrial fusion, morphogenesis and movement. These findings indicate the existence of a dedicated mt-nucleoid segregation apparatus that coordinates nucleoid segregation with mitochondrial dynamics and cell division[6–9].

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Table 1 | **mtDNA-associated proteins in yeast, mammals and *Xenopus laevis***

Protein*	Primary function	mtDNA stability in mutant
Yeast		
Abf2[‡§‖]	mtDNA packaging	ρ^{o} or ρ^{-}
Aco1[§]	Citric acid cycle	ρ^{o}
Arg5,6[¶]	Arginine biosynthesis	Stable
Ald4[§]	Ethanol metabolism	Stable
Atp1[§]	ATP synthesis	ρ^{o}-lethal
Cha1[§¶]	Catabolism of hydroxy amino acids	Stable
Idh1[§]	Citric acid cycle	Moderate instability
Idp1[§]	Oxidative decarboxylation of isocitrate	Stable
Ilv5[§]	Biosynthesis of Val, Ile and Leu	Moderate instability
Ilv6[§]	Biosynthesis of Val, Ile and Leu	Stable
Kgd1[‡§]	Citric acid cycle	Moderate instability
Kgd2[‡§]	Citric acid cycle	Moderate instability
Lpd1[‡§]	Citric acid cycle, catabolism of branched-chain amino acids	Moderate instability
Lsc1[§]	Citric acid cycle	Stable
Mgm101[§‖]	mtDNA maintenance or repair	Unstable ρ^{+} and *ori*-lacking ρ^{-}
Mip1[§‖]	mtDNA replication	ρ^{o}
Mnp1[‡]	Putative mitochondrial ribosomal protein	Stable
mtHsp60[§]	Mitochondrial chaperonin	Unstable *ori*-containing ρ^{-}
mtHsp10[§]	Mitochondrial chaperonin	Unknown
mtHsp70[§]	Protein import	Unknown
Pda1[§]	Oxidation of pyruvate	Moderate mtDNA instability
Pdb1[§]	Oxidation of pyruvate	Moderate mtDNA instability
Rim1[§]	mtDNA replication	ρ^{o}
Rpo41[§]	mtDNA transcription	ρ^{o} or ρ^{-}
Sls1[§]	Coordination of transcription and translation	ρ^{o} or ρ^{-}
Yhm2[§]	Mitochondrial carrier	Stable
Mammals		
TFAM[‡#]	mtDNA transcription and packaging	mtDNA depletion
Twinkle[‖]	mtDNA replication	Multiple mtDNA deletions
mtSSB[‡#]	mtDNA replication	Unknown
Polymerase γ[#]	mtDNA replication	Multiple mtDNA deletions
BRCA1[#]	Tumour suppressor	Unknown
PRSS15[**]	Protein degradation	Unknown
Xenopus laevis		
mtTFA[‡]	mtDNA transcription and packaging	Unknown
mtSSB[‡]	mtDNA replication	Unknown
PDC-E2[‡]	Oxidation of pyruvate	Unknown
BCKAD-E2[‡]	Catabolism of branched-chain amino acids	Unknown
Prohibitin 2[‡]	Protein folding	Unknown
ANT1[‡]	ADP–ATP exchange on inner membrane	Unknown

*Mitochondrial DNA (mtDNA)-associated proteins were identified by various procedures as follows: [‡]density gradient centrifugation of detergent-solubilized mitochondria[5,12,49,111,112]; [§]formaldehyde crosslinking followed by density gradient centrifugation[3,4]; [‖]direct co-localization with mtDNA by GFP tagging[9,13,14,102]; [¶]protein array-based screens for mtDNA-binding factors[113]; [#]immunocytochemistry[64,114]; and [**]co-immunoprecipitation with known nucleoid proteins[115]. ANT1, adenine nucleotide translocator 1; BCKAD-E2, branched-chain α-ketoacid dehydrogenase, E2 subunit; BRCA1, breast cancer 1; Hsp, heat-shock protein; mtTFA, mitochondrial transcription factor A; mtSSB, a mitochondrial ssDNA-binding protein; *ori*, origin of replication; PDC-E2, pyruvate dehydrogenase, E2 subunit; PRSS15, serine protease 15 (also known as LON); TFAM, transcription factor A, mitochondrial; ρ^{o}, respiratory-deficient petite cells that lack mtDNA; ρ^{-}, respiratory-deficient cells that contain mtDNA that is deleted for large segments of the wild-type mitochondrial genome.

Table 2 | **An interspecies comparison of mitochondrial nucleoids**

Species	Cytological appearance	Size	Number per cell	Number of mitochondrial genomes per nucleoid	Size of mitochondrial genome	Visualization tools	References
Saccharomyces cerevisiae	Globular foci	~0.2–0.4 μm in aerobic and ~0.6–0.9 μm in anaerobic cells (diameter)	~40–60 in aerobic and ~7.6 in anaerobic cells	~1–2 in aerobic and ~20 in anaerobic cells	75–80 kb	DAPI, GFP tagging	12,65,102,116
Physarum polycephalum	Rod shape	Up to ~1.5 μm in length	~15	~40–80	63 kb	DAPI, ethidium and thionine staining, light or electron microscopy	117,118
Crithidia fasciculata	Disk shape	~1.0 μm × ~0.35 μm	1	Several thousand mini circles and a few dozen maxi circles	0.5–10 kb for mini circles and 20–40 kb for maxi circles	DAPI and ethidium staining, immunofluorescence and GFP tagging, light or electron microscopy	119,120
Humans	Globular foci	~0.068 μm (diameter)	466–806 in cell lines	~2–10	16.5 kb	Ethidium and PicoGreen staining, immunocytochemical staining with DNA-specific antibodies, bromodeoxyuridine labelling, GFP tagging	14,64,121–125

DAPI, 4′,6-diamidino-2-phenylindole.

Here we review our current understanding of the organization and inheritance of mitochondrial nucleoids. Because of its amenability to genetic analysis, the yeast *Saccharomyces cerevisiae* has provided the most detailed information on these processes, we therefore focus mainly on studies in this species. Findings from other species are discussed where appropriate.

Packaging of mtDNA

Mitochondrial nucleoids have been visualized using various methods, revealing interspecies differences in the size, morphology and number of nucleoids (TABLE 2). For example, staining yeast mtDNA with 4′,6 diamidino-2-phenylindole (DAPI) shows nucleoids as 'strings of beads' that line up with the mitochondrial reticulum[10–12] (FIG. 1a). Mitochondrial nucleoids can also be detected by visualizing nucleoid-specific proteins that are tagged with GFP (FIG. 1b)[9,13,14]. These structures are the packaged and condensed form of mtDNA. The linear monomer of the ~75-kb mitochondrial genome in yeast is ~25 μm in length, and is compacted into a globular nucleoid structure with an average diameter of ~0.3 μm in aerobic cells. This contains one to two genome equivalents of mtDNA within a mitochondrial reticulum that has an average radius of ~0.5 μm. In mammals, mt-nucleoids have a diameter of just 0.068 μm; these nucleoids contain several molecules of the circular mitochondrial genome, which is 16.5-kb long.

Abf2 and TFAM — key players in mtDNA packaging. The core packaging elements of mt-nucleoids are mainly non-histone, HIGH MOBILITY GROUP (HMG) PROTEINS that show homology to the DNA-binding HMG proteins of nuclear chromatin[15]. These positively charged mtDNA-binding proteins contain two HMG boxes and are evolutionarily conserved from yeast to humans[16–26]. The best-studied HMG protein in mt-nucleoids is yeast Abf2. This protein exists in solution as an 18.6-kDa monomer and is present at an estimated ratio of 1 molecule for every 15–30 bp of mtDNA[26,27], which is sufficient to coat the entire mitochondrial genome. ATOMIC FORCE MICROSCOPY shows that Abf2 introduces sharp bends of ~78° into the DNA backbone, which seems to be sufficient to compact DNA[28]. Abf2 can introduce superhelical turns into relaxed circular DNA[24,26], but this supercoiling might not be essential for mtDNA packaging[29]. Abf2 generally binds non-specifically to dsDNA, although binding seems to be excluded from simple DNA sequences, such as poly(dA) tracts, which occur in many regulatory and intergenic spacer regions in yeast mtDNA[26,27].

Yeast cells that lack Abf2 have an unstable mitochondrial genome. When cells are grown on media that contain glucose — which allows cells to obtain energy through fermentation, avoiding the need for respiration — respiratory-deficient PETITE MUTANTS are produced[26]. However, mtDNA can be maintained when *abf2Δ* mutants are grown on media that contain a non-fermentable carbon source, and in this case mtDNA is hypersensitive to damage from endogenously produced free radicals and from mutagens such as ethidium bromide[3,30]. These observations support roles for Abf2 in mtDNA packaging and protection.

The mammalian Abf2 homologue, TFAM, is also thought to be involved in packaging the mitochondrial genome. TFAM bends DNA *in vitro* and can complement the mtDNA-instability phenotype of the yeast *abf2Δ* mutation[20,31,32]. TFAM can therefore be considered a non-specific DNA-binding protein. Depending

HIGH MOBILITY GROUP (HMG) PROTEINS
A family of non-histone proteins that contain DNA-binding HMG-box domains.

ATOMIC FORCE MICROSCOPY
A method that is used to image materials at the atomic level.

PETITE MUTANTS
Respiratory-deficient mutants. These either contain a highly repeated, random fragment of the wild-type mitochondrial genome (ρ^-) or completely lack mtDNA (ρ^o).

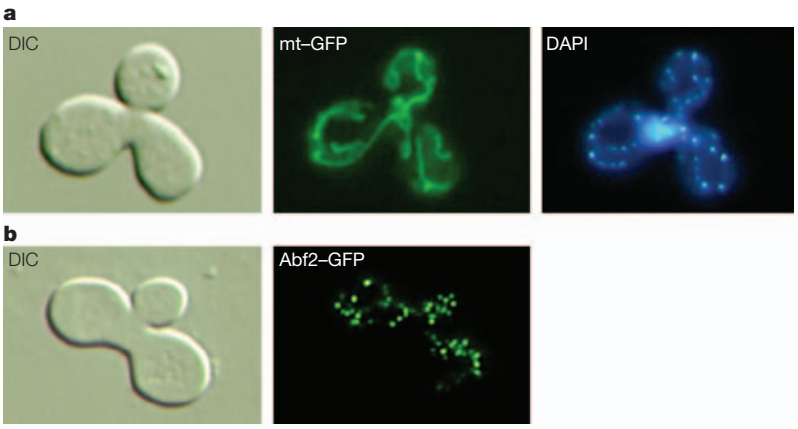

Figure 1 | **Cytological visualization of yeast mitochondrial nucleoids. a** | The co-visualization of mitochondria and mitochondrial DNA (mtDNA) in a budding yeast zygote. Cells are visualized by differential interference contrast (DIC) microscopy (left panel). Mitochondria in the same cells were tagged with a GFP construct in which residues 1–52 of citrate synthase (a mitochondrial enzyme) were fused to the amino terminus of GFP (middle panel). mtDNA in these cells was also stained with 4′,6-diamidino-2-phenylindole (DAPI) (right panel). Note the 'string-of-beads' appearance of the mtDNA. **b** | A visualization of yeast mitochondrial nucleoids by direct GFP tagging of the nucleoid protein, Abf2. As before, the left panel shows DIC imaging and the right panel shows GFP fluorescence. These images are reproduced, with permission, from REF. 9 © (1998) Rockefeller University Press.

on the cell type, the amount of TFAM that is associated with mtDNA might vary. It is estimated to be present at a ratio of 1 molecule for every 10–20 bp of mtDNA in HeLa cells[33,34], but estimates of the amount of TFAM that is associated with mtDNA in other cell types are much lower[35,36]. The *X. laevis* TFAM counterpart, mttfa-A, has also been identified as an abundant component of purified mt-nucleoids[5], indicating that it has a similar function.

TFAM has other functions in addition to its proposed role in mtDNA packaging. It was initially identified as a transcription factor that facilitates the assembly and promoter recognition of the mitochondrial transcriptional machinery[16,20,31,37]. Through FOOTPRINT ANALYSIS, TFAM has been found at both the light and heavy strand promoter regions (which are origins of asymmetrical replication) of mammalian mtDNA[38,39]. TFAM therefore also shows some DNA-binding specificity that is related to its transcriptional function, as well as non-specific DNA-binding properties. There is also evidence that TFAM might function in repairing mtDNA. Expression of TFAM is elevated in cell lines that are resistant to the DNA-damaging agent cisplatin[40], and TFAM is reported to bind with increased affinity to mtDNA that has been damaged either oxidatively or by cisplatin[41].

Moderate increases in *ABF2* expression (2–2.5 fold) lead to comparable increases in mtDNA copy number in yeast[42]. Abf2 produces this effect either by regulating mtDNA replication or by providing increased protection against degradative turnover, thereby augmenting the copy number. Correlations between TFAM levels and mtDNA copy number have also been reported in mice[43,44] and in a chicken lymphoma cell line[45]. As TFAM is also required for mtDNA transcription[31], these effects on mtDNA copy number might be a consequence of transcriptional control. However, overexpression of human TFAM in mice in a genetic background that does not alter expression from mtDNA still results in increased mtDNA copy number[33]. Furthermore, the level of expression of the *Drosophila melanogaster* TFAM homologue is correlated with mtDNA copy number, but not with mtDNA transcription[46,47]. Finally, the 25-amino-acid, C-terminal tail of TFAM that is required for its transcriptional function is absent from yeast Abf2, and the yeast protein does not stimulate mitochondrial transcription *in vitro*[48]. These results support the suggestion that the roles of TFAM and its homologues in transcription and mtDNA packaging are separable.

Is Abf2 sufficient for mtDNA packaging in yeast? Abf2 shows phased binding to DNA[27] and there is evidence that the physiological concentration of this protein might only allow for a limited compacting capacity[29]. This indicates that mtDNA packaging is more elaborate, requiring other proteins to maintain nucleoid morphology, gene expression and genome maintenance. Indeed, the presence of mt-nucleoids in cells that lack Abf2, although they are altered in morphology[49], indicates that other packaging factors are present. This suggestion has been strengthened by the identification of genes that interact with *ABF2* and have products that are present in mt-nucleoids. Two examples are the metabolic genes *ILV5* and *ACO1* (aconitase 1) (see below). Both of these genes are required for mtDNA maintenance, and their overexpression suppresses the mtDNA instability phenotype of *abf2Δ* mutants[3,4,50,51].

Ilv5 — a prototypical bifunctional mt-nucleoid protein. Ilv5 is a mitochondrial enzyme that functions in branched-chain amino-acid biosynthesis[52]. Direct evidence that Ilv5 is a bifunctional protein came from the identification of two classes of Ilv5 mutants, which independently affect either the biosynthetic activity or the mtDNA maintenance function of the protein[53]. Furthermore, conditions that activate *ILV5* expression, such as amino-acid starvation, result in an increase in the number of mtDNA nucleoids without any increase in the overall amount of mtDNA[54]. This 'parsing' of mtDNA into nucleoids is dependent on Ilv5.

As revealed from the analysis of ρ^--petite mtDNAs, the parsing of mtDNA into nucleoids is a two-step process. The first step results in an increase in the number of mtDNA molecules, owing to REDUCTIONAL RECOMBINATION across the tandem repeats of ρ^- mtDNA or, in the case of ρ^+ mtDNA, to the separation of interlinked genomes. The second step parses the individual mtDNA molecules into smaller nucleoids, increasing nucleoid number without any increase in the amount of mtDNA. This second step requires Ilv5. In its absence, reductional recombination still occurs, but there is no increase in the number of nucleoids. Altogether, these results indicate that Ilv5 functions in assembling mtDNA into mt-nucleoid structures.

FOOTPRINT ANALYSIS
A technique for identifying sites where proteins bind to DNA at a single-nucleotide resolution.

REDUCTIONAL RECOMBINATION
Recombination within the highly repeated sequences of ρ^--petite genomes, which produces shorter mtDNA molecules with a reduced number of repeat units.

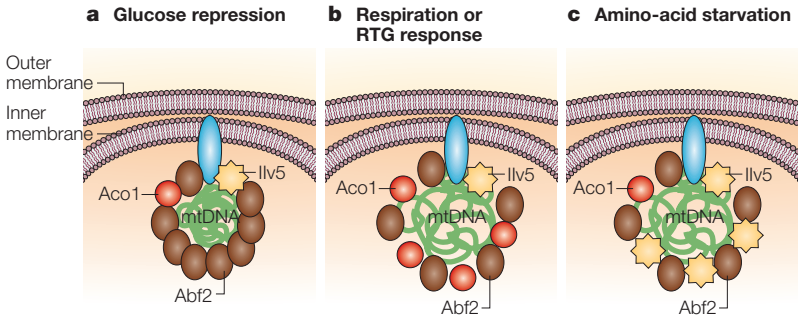

Figure 2 | **A model for the metabolic remodelling of mitochondrial nucleoids in yeast. a** | In conditions in which respiration is repressed by the presence of glucose, mitochondrial DNA (mtDNA) is tightly packaged by the protein Abf2, limiting mtDNA transactions. **b** | When cells are shifted to respiratory conditions, or when mitochondrial dysfunction activates the retrograde (RTG) pathway, *ACO1* (aconitase 1) expression is increased, resulting in more Aco1 in mitochondrial nucleoids (mt-nucleoids). **c** | Similarly, in conditions of amino-acid starvation, the expression of *ILV5* is activated by the general amino-acid control pathway and Ilv5 accumulates in mt-nucleoids. The increased levels of Aco1 and Ilv5 that are shown in **b** and **c** substitute for Abf2 in packaging mtDNA into a metabolically favourable conformation and/or protect mtDNA in the remodelled conformation. The blue oval represents a putative protein, or complex of proteins, that connects mt-nucleoids to the inner mitochondrial membrane.

The bifunctional mitochondrial aconitase, Aco1. The yeast mitochondrial aconitase Aco1 is a KREBS-CYCLE enzyme that converts citrate into isocitrate. Under all growth conditions tested, Aco1 is essential for mtDNA stability[3,50], including that of ρ^- mtDNAs (X.J.C., X. Wang and R.A.B., unpublished observations). This protein was shown to be bifunctional in an analysis of mutations of three cysteine residues that coordinate the assembly of the Aco1 iron–sulphur cluster[3], which is essential for catalytic activity[55]. As a result of these mutations, Krebs-cycle activity and the growth of cells are blocked under conditions that require respiration, but mtDNA stability is unaffected[3]. This is reminiscent of the bifunctionality of some aconitase isoforms in mammals and bacteria. A cytosolic mammalian isoform of aconitase, also known as iron-responsive element-binding protein, binds to 5′ or 3′ UTRs of specific mRNAs when its iron–sulphur cluster is disassembled in response to iron depletion and oxidative stress[56]. This binding modulates the translation and stability of mRNAs that are involved in iron homeostasis. It has also been reported that aconitase from mammalian mitochondria and *Bacillus subtilis* can bind mRNA[57,58]. Whether a similar 'iron-sulphur switch' controls the conversion between the enzymatic and mtDNA-maintenance forms of Aco1 is unknown. However, as suggested by Shadel[59], such a regulatory mode could allow Aco1 to function as a stress sensor for the protection of mtDNA. In this model, the disassembly of the Aco1 iron–sulphur cluster, induced by free radicals, could result in the reallocation of Aco1 from the Krebs cycle to mt-nucleoids, stabilizing mtDNA under oxidative stress conditions.

Wild-type mtDNA is unstable in *abf2Δ* cells that are grown on media containing glucose — which represses mitochondrial function — but can be stably maintained on media that stimulate respiration[3]. This is mainly due to the robust expression of *ACO1* in respiring cells[60]. Ectopic expression of *ACO1* from a constitutive promoter to a level which is comparable to that induced by respiratory growth is sufficient to suppress the loss of mtDNA from *abf2Δ* cells that are grown in the presence of glucose. Under respiratory conditions, the extra Aco1 is expected to stimulate the Krebs cycle and energy metabolism, but is thought to simultaneously substitute for Abf2 in reorganizing mtDNA. This might also provide metabolically coupled protection of mtDNA against oxidative damage during respiratory growth.

A model for the metabolic remodelling of mt-nucleoids. The expression of both *ILV5* and *ACO1* is metabolically regulated. *ILV5* is activated by the general amino-acid control pathway in response to amino-acid starvation[52]. Expression of *ACO1* is stimulated by the haem activator protein (HAP) complex and retrograde (RTG) signalling pathways[61,62], which are upregulated by respiratory growth or mitochondrial dysfunction, respectively. Therefore, the genetic interactions of *ILV5* and *ACO1* with *ABF2* indicate that the structural organization of mt-nucleoids could be subject to metabolic remodelling (FIG. 2). This is supported by the observation that activation of *ILV5* expression by amino-acid starvation increases mtDNA transmission through an increase in the number of nucleoids[54]. Simultaneous inactivation of both the HAP and RTG systems leads to mtDNA instability in an *ACO1*-dependent manner[3]. Conversely, activation of either HAP or RTG suppresses mtDNA instability in *abf2Δ* mutant cells.

These findings indicate that the molecular composition of nucleoids might change owing to the fluctuating levels of these bifunctional proteins in response to physiological cues. In glucose-rich media, for example, where the general amino-acid control and HAP pathways and respiratory activity are repressed, Abf2 might be the predominant factor in packaging mtDNA into a compact structure that has limited accessibility to the mitochondrial transcription apparatus and other mtDNA-transaction systems. This tightly packaged conformation might also be beneficial to the mitochondrial genome for reducing exposure to adverse microenvironments. In response to increased biosynthetic or respiratory activities, mtDNA might be remodelled into a more active conformation that is favourable for gene expression, which would depend on increased amounts of Ilv5 and Aco1. These two proteins might globally or locally substitute for Abf2 and reorganize mtDNA into a metabolically favourable conformation. They might also specifically protect metabolically active mt-nucleoids so that mtDNA is still protected in the less tightly packed conformation. The observation that overexpression of *ILV5* and *ACO1* suppresses the hypersensitivity of mtDNA to attack by the mutagen ethidium bromide in an *abf2Δ* background might be a reflection of such a protective role (REF. 3; X.J.C., X. Wang and R.A.B., unpublished observations). Comparative analysis of nucleoid composition and structure in cells with different metabolic states will allow the testing of this model.

KREBS CYCLE
Also known as the tricarboxylic acid (or TCA) cycle. A metabolic pathway in mitochondria that breaks down the products of carbohydrate, fat and protein metabolism into carbon dioxide and water to generate energy. It also provides precursors for other compounds, such as certain amino acids.

Mitochondrial nucleoid division

The division of mt-nucleoids has been observed in the slime mould *Physarum polycephalum* and in human cell lines. In *P. polycephalum*, division is synchronized with mitochondrial fission[63], whereas in human cells it occurs in the absence of mitochondrial division[64]. The molecular mechanisms that drive the division of mt-nucleoids are unclear, but recent studies indicate that heat shock protein 60 (Hsp60), another bifunctional protein, might be involved[65].

Hsp60 is an essential CHAPERONIN that is required for the correct folding of many proteins that are imported into mitochondria[66]. It is functionally and structurally homologous to the bacterial chaperonin GroEL and is organized into torroidal structures that are composed of two seven-member rings. Hsp60 also binds with high specificity *in vitro* to mtDNA *ori* sequences[4], which are putative origins of mtDNA replication, and this binding is preferential for the template strand of *ori* mtDNA. mtDNAs that contain intact *ori* sequences are unstable in *hsp60* temperature-sensitive (*hsp60^{ts}*) mutant backgrounds, in which the chaperonin activity of Hsp60 is close to that of the wild type. However, mtDNAs that lack *ori* sequences or have mutations in these sequences are as stable in the *hsp60^{ts}* background as in wild-type cells[65]. The mtDNA instability in *hsp60^{ts}* mutants might arise because *ori*-dependent segregation complexes fail to assemble correctly owing to a compromised Hsp60 function at *ori* sequences.

How does Hsp60 function in stabilizing mtDNA? Using high-resolution DECONVOLUTION MICROSCOPY, Kaufman *et al.*[65] found that nucleoids in *hsp60^{ts}* mutant cells are fewer in number than in wild-type cells and have an abnormal appearance, often having elongated, barbell-like or serpentine structures. This indicates that nucleoid division is delayed after mtDNA replication, which would impair nucleoid transmission in these mutants. The interaction between Hsp60 and mtDNA *ori* sequences could therefore be an important determinant of nucleoid division. Although further studies will be necessary to understand the relationship between Hsp60, *ori* sequences, nucleoid division and nucleoid transmission, these findings indicate that nucleoid division is a regulated process that is important for mtDNA transmission.

Mitochondrial nucleoid segregation

At the G_1–S transition of the yeast cell cycle, a subset of mitochondrial structures are selected and actively moved into the bud that will form the new daughter cell[67,68]. Although the segregation of mt-nucleoids into daughter cells is dependent on the movement of mitochondrial structures, these two events are not necessarily coupled.

Much of our current understanding of the mechanism of mt-nucleoid segregation has benefited from the use of the yeast zygote system. In yeast the mitochondrial genome is inherited from both parents after the formation of zygotes from two strains of opposite mating type. As the zygotes have defined positions for bud emergence during the first post-zygotic cell division, the relative movements of mitochondria and mt-nucleoids can therefore be followed cytologically with reference to this unique topographical landmark. In addition, within a relatively few divisions, diploid buds that are formed from heteroplasmic zygotes become homoplasmic. These unique features provide a robust experimental system for following how individual parental mtDNAs are segregated into the progeny. This allows easy tracking of the drift from the heteroplasmic to homoplasmic states of mtDNAs during cell division.

Early genetic studies in yeast indicated that most parental mtDNAs do not mix in zygotes[69,70], and that only a small fraction of the total mtDNA pool is transferred post-zygotically to diploid buds[71]. Together with more recent investigations[6,8], these results provide us

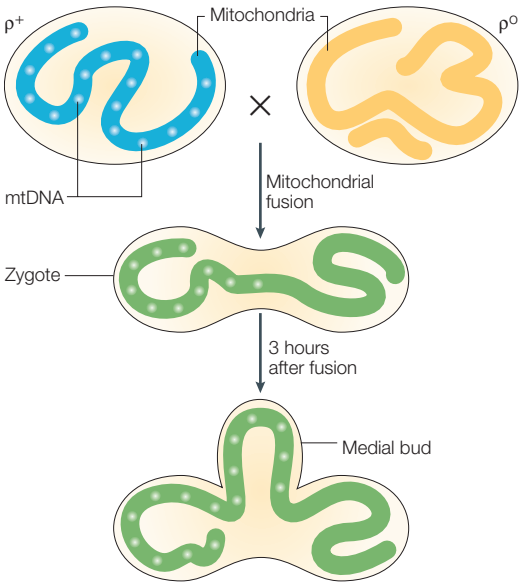

Figure 3 | **Understanding the mechanism of mitochondrial nucleoid segregation.** An experimental system that can be used to track the sorting of mitochondrial protein and DNA (mtDNA) after mating between two strains of the yeast *Saccharomyces cerevisiae*. The two strains are ρ⁺ (which carry normal mtDNA) and ρ° (which lack mtDNA). Haploid ρ⁺ cells are transformed with a vector from which a GFP fusion protein is expressed. This protein is targeted to the mitochondrial matrix or to the inner or outer mitochondrial membranes, and is expressed under the control of the inducible galactosidase (*GAL*) promoter so that it is induced in cells grown on galactose. After growth of the transformed ρ⁺ cells on a galactose medium, these cells are mated in a glucose-containing medium to non-transformed ρ° cells. Zygotes are examined by differential interference contrast and fluorescence microscopy at various time points after mating, and mtDNA is stained with 4′,6-diamidino-2-phenylindole (DAPI). The mitochondrial GFP fusion protein (indicated by the green colour) can be seen to spread evenly throughout the zygote and into the medial diploid bud within ~3 hours of mating. However, in most zygotes (>80%) mtDNA is preferentially segregated to the medial diploid bud, indicating that mtDNA movements are controlled independently of mitochondrial proteins. These experiments also indicate the existence of an mtDNA segregation apparatus.

CHAPERONIN
A protein complex that is required for correct protein folding.

DECONVOLUTION MICROSCOPY
Microscopy using computer image-processing techniques to reconstruct cross-sectional images from several focal planes, which yields high-resolution images.

with important information on the relative sorting of mitochondrial proteins and mt-nucleoids. Soon after mating parental mitochondria fuse, which allows the mixing of mitochondrial constituents within a reticulum that extends throughout the zygote and into the diploid buds. Surprisingly, in crosses in which only one of the two haploid parents contained mtDNA, mt-nucleoids in most zygotes were preferentially sorted to the first medial bud[9] (FIG. 3). These studies indicated that mt-nucleoid segregation is a directed process that is driven by a putative nucleoid-segregation apparatus. Before discussing the molecular nature of this apparatus, we first consider how mtDNA structure itself can influence mt-nucleoid organization and inheritance.

mtDNA recombination and segregation. mtDNA structure can influence both the number and size of nucleoids. The wide variation among species in mtDNA size, shape, replication mechanisms, unique sequence elements and the presence or absence of a highly active recombination system indicates that the details of nucleoid assembly and dynamics will also vary. In *S. cerevisiae*, a fraction of mtDNA is present as concatemers[72], which can arise by ROLLING-CIRCLE REPLICATION or homologous recombination. Fangman and co-workers[73] showed that the level of Mgt1 (also known as the mitochondrial cruciform-cutting endonuclease, Cce1), which cuts cruciform structures such as recombination junctions, has an important effect on how mtDNAs segregate both mitotically and in crosses. Their studies focused on mtDNA in ρ^--petite mutants, which generally have fewer mt-nucleoids than ρ^+ cells, despite having about the same amount of mtDNA.

As expected, overexpression or deletion of *MGT1* affects the number of recombination junctions in mtDNA. Interestingly, manipulation of *MGT1* also affects mt-nucleoid number, the mitotic stability of mtDNA and its transmission properties in crosses. In *mgt1Δ* cells, more mtDNA is connected by recombination junctions, fewer nucleoids are observed and the efficiency of mitotic and zygotic propagation of mtDNA from parent cells is reduced; overexpression of *MGT1* had the opposite effect. Therefore, the rate of resolution of recombination junctions determines the number of, and consequently, the transmission of mt-nucleoids.

A further relationship between mtDNA structure, recombination and inheritance has been indicated from the analysis of another yeast gene, *MHR1*. This gene encodes a mitochondrial matrix protein that promotes the ATP-independent pairing of homologous DNAs *in vitro*[74]. Shibata and co-workers[74,75] observed a high rate of formation of petites in a temperature-sensitive *mhr1* mutant, and showed that this mtDNA instability is exacerbated when the *mhr1* mutation is combined with an *MGT1* deletion. Furthermore, in mating experiments, the rate at which post-zygotic HOMOPLASMY was achieved was reduced approximately 2-fold from *mhr1/mhr1* zygotes and accelerated 2.5-fold from zygotes overexpressing *MHR1* (REF. 76). These effects were not observed from similar manipulations of the expression of either *MGT1* or *ABF2*.

MHR1 therefore seems to be the primary determinant of homoplasmy in vegetatively growing cells. The generation of homoplasmic cells from heteroplasmic ones is usually an efficient process, regardless of how the HETEROPLASMY originated — whether from a mutation in vegetatively growing cells or from matings between haploid cells of different mtDNA genotypes. Based on the apparent enrichment of genome-length, monomeric mtDNA molecules in buds versus the prevalence of concatemers in mother cells, Ling and Shibata[76] suggested that Mhr1 has a role in mtDNA concatemer formation by promoting a type of rolling-circle replication that was first proposed by Clark-Walker and co-workers[77]. The model proposed by Ling and Shibata[76] states that homoplasmy could be achieved in relatively few divisions by the transmission of a small number of randomly selected concatemers, which are converted by unknown mechanisms to genome-length monomers during the partitioning of mtDNA from the mother to the daughter cells.

Direct measurements of the rates of segregation of mitochondrial genomes have been made by McAlpine *et al.*[78] using FLUORESCENCE *IN SITU* HYBRIDIZATION (FISH). Heteroplasmic zygotes were created in which one of the two parental mitochondrial genomes was known from genetic studies to have a marked segregational or replicative advantage over the other in crosses. Although these experiments focused on biased rather than purely random outputs of mtDNAs, about eight post-zygotic divisions were nevertheless required for the appearance of near-homoplasmic diploid cells. A further interesting finding was that few if any nucleoids contained both parental mitochondrial genomes. As it is known that genotypically distinct mitochondrial genomes interact in such mating experiments (for example, through recombination), questions arise about genome selection in mt-nucleoids and the processes that allow genotypically distinct mtDNAs in separate nucleoids to interact.

Links with mitochondrial dynamics and morphology. Mitochondria are dynamic structures: they can readily fuse and divide, and undergo morphological transitions, which are regulated by various nuclear genes[68,79,80]. Intriguing links between mitochondrial dynamics and mtDNA inheritance come from the observation that interference with fusion, but not fission, compromises mt-nucleoid stability[81].

Among the yeast genes that function in mitochondrial fusion are those that encode the outer membrane proteins Fzo1, which is a GTPase, and Ugo1, which contains a proposed single-membrane-spanning domain[82]. The *D. melanogaster* homologue of Fzo1, *fuzzy onion*[83], is known to be required for mitochondrial fusion in spermatogenesis. Fzo1 and Ugo1 form a complex with Mgm1, a dynamin-related GTPase that is located in the intermembrane space. This complex, which is a key component of the mitochondrial fusion apparatus[84–86], is in contact with the inner mitochondrial membrane. The process of fusion requires the coordinated fusion of the outer and inner

ROLLING-CIRCLE REPLICATION
A form of DNA replication in which a circular DNA molecule produces linear daughter molecules.

HOMOPLASMY
The state of the mitochondrial genetic system in which all copies of the mitochondrial genome within a cell are identical.

HETEROPLASMY
The state of the mitochondrial genetic system in which a cell contains mitochondrial genomes that are genetically different.

FLUORESCENCE *IN SITU* HYBRIDIZATION
A microscopic technique that uses fluorescently tagged DNA probes to detect the cytological localization of specific DNAs by *in situ* hybridization.

mitochondrial membranes. Current models propose that remodelling these membranes during the fusion process is achieved through contacts between outer and inner membranes that are mediated by the linking of the GTPases Fzo1 and Mgm1 by Ugo1 (REFS 84,86). Mutations in the components of this apparatus not only lead to aberrant mitochondrial morphology, but also to a loss of mtDNA stability.

How does disruption of mitochondrial fusion affect mtDNA stability? When fusion is compromised, the continuing fission process gives rise to fragmented mitochondrial structures, some of which might no longer contain mtDNA. Inheritance of those mitochondria would lead to the production of ρ° petites. Indeed, in cultured *Nicotiana tabaccum* cells, mitochondrial fission products often lack mt-nucleoids[87]. Frequent fusions are probably required to overcome the heterogeneous distribution of mtDNA nucleoids and the eventual segregation into progeny cells of those mitochondria that lack mtDNA. Alternatively, defects in the assembly of a functional mtDNA segregation apparatus (see below) could underlie the compromised mt-nucleoid inheritance in mitochondrial fusion mutants. If this is the case, other genes that are involved in mitochondrial morphogenesis, such as those that have roles in general membrane remodelling and mitochondrial volume control, might also affect the stability and inheritance of mt-nucleoids. This might explain the correlation between increased mtDNA instability and mitochondrial morphology in the *mdm38* mutant, which is defective for an inner membrane K^+/H^+ antiporter that is necessary for mitochondrial volume control[88], and in cells treated with nigericin, which alters mitochondrial ion homeostasis[89].

Membrane tethering and the mt-nucleoid segregation apparatus. What is the molecular mechanism that drives the segregation of mt-nucleoids during cell division? Early biochemical data support the view that, similar to the bacterial chromosome, mtDNA is membrane-associated, therefore providing a platform for mtDNA segregation[90]. The molecular constituents that might tether mt-nucleoids to the membrane are now being revealed through the genetic analysis of several yeast mitochondrial membrane proteins that affect mitochondrial structure and motility, as well as mt-nucleoid organization and stability[91–94].

The mitochondrial membrane proteins Mmm1, Mdm10 and Mdm12 are present in a complex that connects mitochondria to the actin cytoskeleton[7,95]. Mutations of the encoded genes not only result in aberrant mitochondrial morphology and alterations in mitochondrial motility, but also lead to changes in nucleoid structure and loss of mtDNA[96,97]. Mdm10 is also involved in the assembly of the PRE-PROTEIN TRANSLOCASE of the outer mitochondrial membrane[98], highlighting the possibility that this protein has many functions, any of which could lead to mitochondrial morphology defects when absent.

PRE-PROTEIN TRANSLOCASE
A complex of proteins that function in the import of nuclear-encoded mitochondrial proteins that were synthesized on cytoplasmic ribosomes.

An important insight into the link between the structure of mitochondria, their distribution within cells and nucleoid stability came from a study of Mmm1 by Jensen and co-workers[91,93], who showed that this protein forms punctate structures, a subset of which were localized in apposition to mt-nucleoids. In contrast to Mdm10 and Mdm12, which are located in the outer membrane, Mmm1 is thought to span both the outer and inner mitochondrial membranes, although the N-terminal region that extends to the matrix is not essential for mtDNA maintenance[99]. Interactions of Mmm1 with other proteins might link mt-nucleoids to the outer membrane and cytoskeleton, which together would provide a way to tether mt-nucleoids to components that control mitochondrial distribution and morphology.

Dimmer *et al.*[100] recently showed that two inner mitochondrial membrane proteins, Mdm31 and Mdm32, also have dual roles in nucleoid stability and mitochondrial morphology. Importantly, the absence of either protein is lethal in combination with a deletion of any one of *MMM1*, *MDM10* and *MDM12*, as well as with a deletion of *MMM2*, which encodes another mitochondrial distribution protein that is located in the outer mitochondrial membrane[101]. No synthetic lethality was observed among these four genes. Although direct biochemical evidence is lacking, these findings raise the possibility that the connection between nucleoids and the Mdm10–Mdm12–Mmm1 complex is mediated through Mdm31 and Mdm32. Further support for this comes from the finding that, in the absence of either Mdm31 or Mdm32, mtDNA-nucleoid morphology is disordered and apposition of Mmm1 to mt-nucleoids mostly disappears, whereas the abundance of Mdm10, Mdm12, Mmm1 and Mmm2 is unchanged. Because these proteins do not seem to be evolutionarily conserved, and the mitochondrial movements are not linked to the actin cytoskeleton in higher eukaryotes, other mechanisms might operate in these organisms to ensure the coordinated transmission of mt-nucleoids with mitochondrial inheritance.

The significance of the apposition of mt-nucleoids to the outer membrane proteins remains to be clarified, as does the biochemical nature of this association. Remarkably, some mt-nucleoid proteins, such as Mgm101 (REF. 102) and certain mutant forms of Ilv5, seem to occupy the same sites as mt-nucleoids in cells that lack mtDNA (J. Batman and R.A.B., unpublished observations). These findings support the existence of a structure that is stably maintained regardless of the respiratory state of the cell. Identifying the determinants that specify the localization of these proteins might provide important clues to how nucleoids associate with the inner mitochondrial membrane.

FIGURE 4 summarizes the current state of knowledge about the yeast mt-nucleoid segregation apparatus. According to this general model, contacts between the inner and outer mitochondrial membrane might have to be maintained to preserve the association of nucleoids with the segregation apparatus and with mitochondrial tethering devices. So, the latter would not only contribute to mitochondrial movements, but also to nucleoid

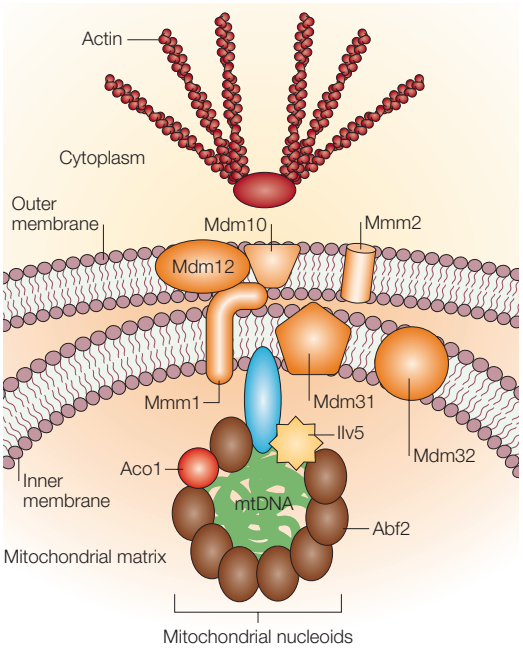

Figure 4 | **A model of the yeast mitochondrial nucleoid segregation apparatus.** The core components of the segregation apparatus consist of the Mdm10–Mdm12–Mmm1 (mitochondrial membrane proteins) complex, which might also interact with Mmm2. This core complex forms the contact site between the inner and outer mitochondrial membranes. It is located primarily in the outer membrane, with the possible extension of Mmm1 into the inner membrane and its possible protrusion into the mitochondrial matrix. The inner membrane proteins, Mdm31 and Mdm32, are also proposed to be part of this double membrane-spanning complex, on the basis of genetic interactions with Mdm10, Mdm12, Mmm1 and Mmm2 (REF. 100). This supramolecular complex mediates the attachment of mitochondria to actin cables in the cytosol and tethers mitochondrial nucleoids (mt-nucleoids) on the matrix side through as yet unknown proteins. This provides a mechanism for linking mt-nucleoids to the cytoskeleton so that mt-nucleoids move with mitochondria into the progeny cell during cell division[7]. How the Mdm10–Mdm12–Mmm1 complex, Mmm2, and the inner membrane proteins Mdm31 and Mdm32 interact has yet to be clarified.

movements and therefore to mtDNA inheritance. The underlying model is that mt-nucleoid interaction with the segregation apparatus — perhaps in the form of the mitochondrial REPLISOME[102] — is a crucial feature in nucleoid organization and maintenance. Disruption of the connection between the inner and outer mitochondrial membranes would also disrupt the link to nucleoids, leading to their disorganization and instability.

Concluding remarks

Cytological, biochemical and genetic studies have provided insights into the organization and composition of mt-nucleoids. One concept that is emerging from these investigations is that mitochondria have recruited various proteins of mitochondrial metabolism and biogenesis to mt-nucleoids. This would allow mtDNA packaging, nucleoid division and nucleoid inheritance to be coupled to these processes. Much remains to be learned about how these bifunctional proteins, together with the core nucleoid components such as Abf2 or TFAM, participate in mtDNA organization and inheritance. The role of many other metabolic proteins that are associated with mtDNA (TABLE 1) remains to be determined. The full physiological significance of the organizational remodelling of mt-nucleoids with respect to mtDNA expression and the oxidative phosphorylation capacity of the cell also await further exploration. Another challenge will be to develop a molecular-level understanding of the mt-nucleoid segregation apparatus and how it functions in mtDNA transmission. This will not only require a detailed view of the composition and organization of mt-nucleoids, but also insights into how these structures functionally interact with mitochondria, and possibly with other cellular components.

Many advances in understanding the organization and segregation of mt-nucleoids have benefited from the amenability of the genetic system in yeast. It will be interesting to determine what aspects of the molecular mechanisms that underlie these fundamental processes are conserved in humans. Furthermore, understanding how mt-nucleoids are organized and segregated could also be of clinical importance. Mutations in mtDNA are responsible for a range of neuromuscular disorders[103,104] and are suspected to underlie cellular ageing[105]. Recent studies have demonstrated that increased mtDNA mutations can directly cause premature ageing in mouse models[106]. How these mtDNA mutations arise is a pressing issue in mitochondrial disease and ageing. Mutations in several mt-nucleoid proteins — including the Twinkle helicase, PolgA and the Ant1 isoform of the adenine nucleotide translocase — cause inherited forms of adult-onset neuromuscular diseases[14,107,108]. This indicates that functional loss of other mt-nucleoid proteins, especially those vulnerable to oxidative damage, such as aconitase, might contribute to sporadic or ageing-related mtDNA mutations that are due to compromised mtDNA metabolism and organization.

Perturbation of mt-nucleoid segregation might also directly contribute to the pathogenesis of mitochondrial diseases. In many cases the severity of mitochondrial disorders is determined by the ratio of wild-type to mutant mtDNA molecules in the cells of the affected tissue[103,109]. The ratio of mtDNAs of different genotypes within heteroplasmic cells is determined by how these mtDNAs segregate during development. Bottlenecks in mtDNA segregation during development can lead to dramatic shifts in the extent of heteroplasmy in specific tissues when a fetus inherits mixed mtDNAs from its mother[110]. Defects in the organization and inheritance of mt-nucleoids could directly alter these bottlenecks. Elucidating the molecular mechanisms that underlie mt-nucleoid segregation should help to understand the pathogenesis of these diseases.

REPLISOME
A DNA-replicating structure that is located at the replication fork, which consists of DNA-replication enzymes and associated proteins.

1. Nass, M. M. K. & Nass, S. Intramitochondrial fibres with DNA characteristics. I. Fixation and elctron staining reactions. *J. Cell Biol.* **19**, 593–611 (1963).
2. Schatz, G., Haslbrunner, E. & Tuppy, H. Deoxyribonucleic acid associated with yeast mitochondria. *Biochem. Biophys. Res. Commun.* **15**, 127–132 (1964).
3. Chen, X. J., Wang, X., Kaufman, B. A. & Butow, R. A. Aconitase couples metabolic regulation to mitochondrial DNA maintenance. *Science* **307**, 714–717 (2005).
This paper shows that the mitochondrial aconitase is an mt-nucleoid component that is essential for mtDNA maintenance, and its overexpression can suppress mtDNA loss from cells that lack Abf2. It also proposes that the bifunctional metabolic enzymes in mt-nucleoids might remodel nucleoid organization in response to physiological cues.
4. Kaufman, B. A. *et al. In organello* formaldehyde crosslinking of proteins to mtDNA: identification of bifunctional proteins. *Proc. Natl Acad. Sci. USA* **97**, 7772–7777 (2000).
This work revealed the association of numerous metabolic proteins with mtDNA by *in organello* formaldehyde crosslinking.
5. Bogenhagen, D. F., Wang, Y., Shen, E. L. & Kobayashi, R. Protein components of mitochondrial DNA nucleoids in higher eukaryotes. *Mol. Cell. Proteomics* **2**, 1205–1216 (2003).
The paper describes the identification of several metabolic proteins that are associated with mtDNA in *X. laevis* oocyte mitochondria and is the most comprehensive analysis of mt-nucleoids in a metazoan species.
6. Azpiroz, R. & Butow, R. A. Patterns of mitochondrial sorting in yeast zygotes. *Mol. Biol. Cell* **4**, 21–36 (1993).
Using pedigree studies of yeast zygotic cells and their first buds, these authors showed that mtDNA movement is controlled separately from the movement of bulk mitochondrial matrix constituents.
7. Boldogh, I. R. *et al.* A protein complex containing Mdm10p, Mdm12p, and Mmm1p links mitochondrial membranes and DNA to the cytoskeleton-based segregation machinery. *Mol. Biol. Cell* **14**, 4618–4627 (2003).
8. Nunnari, J. *et al.* Mitochondrial transmission during mating in *Saccharomyces cerevisiae* is determined by mitochondrial fusion and fission and the intramitochondrial segregation of mitochondrial DNA. *Mol. Biol. Cell* **8**, 1233–1242 (1997).
9. Okamoto, K., Perlman, P. S. & Butow, R. A. The sorting of mitochondrial DNA and mitochondrial proteins in zygotes: preferential transmission of mitochondrial DNA to the medial bud. *J. Cell Biol.* **142**, 613–623 (1998).
10. Williamson, D. H. & Fennell, D. J. The use of fluorescent DNA-binding agent for detecting and separating yeast mitochondrial DNA. *Methods Cell Biol.* **12**, 335–351 (1975).
11. Williamson, D. H. & Fennell, D. J. Visualization of yeast mitochondrial DNA with the fluorescent stain 'DAPI'. *Methods Enzymol.* **56**, 728–733 (1979).
12. Miyakawa, I., Sando, N., Kawano, K., Nakamura, S. & Kuroiwa, T. Isolation of morphologically intact mitochondrial nucleoids from the yeast, *Saccharomyces cerevisiae*. *J. Cell Sci.* **88**, 431–439 (1987).
13. Meeusen, S. *et al.* Mgm101p is a novel component of the mitochondrial nucleoid that binds DNA and is required for the repair of oxidatively damaged mitochondrial DNA. *J. Cell Biol.* **145**, 291–304 (1999).
14. Spelbrink, J. N. *et al.* Human mitochondrial DNA deletions associated with mutations in the gene encoding Twinkle, a phage T7 gene 4-like protein localized in mitochondria. *Nature Genet.* **28**, 223–231 (2001).
The first demonstration that human mt-nucleoids can be specifically visualized by GFP tagging of the mitochondrial Twinkle helicase.
15. Landsman, D. & Bustin, M. A signature for the HMG-1 box DNA-binding proteins. *BioEssays* **15**, 1–8 (1993).
16. Antoshechkin, I. & Bogenhagen, D. F. Distinct roles for two purified factors in transcription of *Xenopus* mitochondrial DNA. *Mol. Cell. Biol.* **15**, 7032–7042 (1995).
17. Antoshechkin, I., Bogenhagen, D. F. & Mastrangelo, I. A. The HMG-box mitochondrial transcription factor xl-mtTFA binds DNA as a tetramer to activate bidirectional transcription. *EMBO J.* **16**, 3198–3206 (1997).
18. Dequard-Chablat, M. & Allandt, C. Two copies of *mthmg1*, encoding a novel mitochondrial HMG-like protein, delay accumulation of mitochondrial DNA deletions in *Podospora anserina*. *Eukaryot. Cell* **1**, 503–513 (2002).
19. Larsson, N. G., Garman, J. D., Oldfors, A., Barsh, G. S. & Clayton, D. A. A single mouse gene encodes the mitochondrial transcription factor A and a testis-specific nuclear HMG-box protein. *Nature Genet* **13**, 296–302 (1996).
20. Parisi, M. A. & Clayton, D. A. Similarity of human mitochondrial trancription factor 1 to high mobility group proteins. *Science* **252**, 965–969 (1991).
21. Pierro, P., Capaccio, L. & Gadaleta, G. The 25 kDa protein recognizing the rat curved region upstream of the origin of the L-strand replication is the rat homologue of the human mitochondrial transcription factor A. *FEBS Lett.* **457**, 307–310 (1999).
22. Sasaki, N. *et al.* Glom is a novel mitochondrial DNA packaging protein in *Physarum polycephalum* and causes intense chromatin condensation without suppressing DNA functions. *Mol. Biol. Cell* **14**, 4758–4769 (2003).
23. Takata, K. *et al.* Drosophila mitochondrial transcription factor A: characterization of its cDNA and expression pattern during development. *Biochem. Biophys. Res. Commun.* **287**, 474–483 (2001).
24. Caron, F., Jacq, C. & Rouviere-Yaniv, J. Characterization of a histone-like protein extracted from yeast mitochondria. *Proc. Natl Acad. Sci. USA* **76**, 4265–4269 (1979).
25. Certa, U., Colavito-Shepanski, M. & Grunstein, M. Yeast may not contain histone H1: the only known 'histone H1-like' protein in *Saccharomyces cerevisiae* is a mitochondrial protein. *Nucleic Acids Res.* **12**, 7975–7985 (1984).
26. Diffley, J. F. & Stillman, B. A close relative of the nuclear, chromosomal high-mobility group protein HMG1 in yeast mitochondria. *Proc. Natl Acad. Sci. USA.* **88**, 7864–7868 (1991).
The study identified the yeast *ABF2* gene and showed that it is required for mtDNA maintenance on media that contain glucose as the carbon source.
27. Diffley, J. F. X. & Stillman, B. DNA binding properties of an HMG1-related protein from yeast mitochondria. *J. Biol. Chem.* **267**, 3368–3374 (1992).
28. Friddle, R. W. *et al.* Mechanism of DNA compaction by yeast mitochondrial protein Abf2p. *Biophys. J.* **86**, 1632–1639 (2004).
29. Brewer, L. R. *et al.* Packaging of single DNA molecules by the yeast mitochondrial protein Abf2p. *Biophys. J.* **85**, 2519–2524 (2003).
30. O'Rourke, T. W., Doudican, N. A., Mackereth, M. D., Doetsch, P. W. & Shadel, G. S. Mitochondrial dysfunction due to oxidative mitochondrial DNA damage is reduced through cooperative actions of diverse proteins. *Mol. Cell. Biol.* **22**, 4086–4093 (2002).
31. Fisher, R. P. & Clayton, D. A. Purification and characterization of human mitochondrial transcription factor 1. *Mol. Cell. Biol.* **8**, 3496–3509 (1988).
32. Parisi, M. A., Xu, B. J. & Clayton, D. A. A human mitochondrial transcriptional activator can functionally replace a yeast mitochondrial HMG-box protein both *in vivo* and *in vitro*. *Mol. Cell. Biol.* **13**, 1951–1961 (1993).
The authors showed that the human TFAM can functionally substitute for the yeast Abf2 in the maintenance of mtDNA.
33. Ekstrand, M. I. *et al.* Mitochondrial transcription factor A regulates mtDNA copy number in mammals. *Hum. Mol. Genet.* **13**, 935–944 (2004).
34. Takamatsu, C. *et al.* Regulation of mitochondrial D-loops by transcription factor A and single-stranded DNA-binding protein. *EMBO Rep.* **3**, 451–456 (2002).
35. Fisher, R. P., Lisowsky, T., Breen, G. A. & Clayton, D. A. A rapid, efficient method for purifying DNA-binding proteins. Denaturation–renaturation chromatography of human and yeast mitochondrial extracts. *J. Biol. Chem.* **266**, 9153–9160 (1991).
36. Maniura-Weber, K., Goffart, S., Garstka, H. L., Montoya, J. & Wiesner, R. J. Transient overexpression of mitochondrial transcription factor A (TFAM) is sufficient to stimulate mitochondrial DNA transcription, but not sufficient to increase mtDNA copy number in cultured cells. *Nucleic Acids Res.* **32**, 6015–6027 (2004).
37. McCulloch, V. & Shadel, G. S. Human mitochondrial transcription factor B1 interacts with the C-terminal activation region of h-mtTFA and stimulates transcription independently of its RNA methyltransferase activity. *Mol. Cell. Biol.* **23**, 5816–5824 (2003).
38. Fisher, R. P., Topper, J. N. & Clayton, D. A. Promoter selection in human mitochondria involves binding of a transcription factor to orientation-independent upstream regulatory elements. *Cell* **50**, 247–258 (1987).
39. Ghivizzani, S. C., Madsen, C. S., Nelen, M. R., Ammini, C. V. & Hauswirth, W. W. *In organello* footprint analysis of human mitochondrial DNA: human mitochondrial transcription factor A interactins at the origin of replication. *Mol. Cell. Biol.* **14**, 7717–7730 (1994).
40. Yoshida, Y. *et al.* P53 physically interacts with mitochondrial transcription factor A and differentially regulates binding to damaged DNA. *Cancer Res.* **63**, 3729–3734 (2003).
41. Yoshida, Y. *et al.* Human mitochondrial transcription factor A binds preferentially to oxidatively damaged DNA. *Biochem. Biophys. Res. Commun.* **295**, 945–951 (2002).
42. Zelenaya-Troitskaya, O., Newman, S. M., Okamoto, K., Perlman, P. S. & Butow, R. A. Functions of the HMG box protein, Abf2p, in mitochondrial DNA segregation, recombination and copy number in *Saccharomyces cerevisiae*. *Genetics* **148**, 1763–1776 (1998).
43. Hansson, A. *et al.* A switch in metabolism precedes increased mitochondrial biogenesis in respiratory chain-deficient mouse hearts. *Proc. Natl Acad. Sci. USA* **101**, 3136–3141 (2004).
44. Larsson, N. G. *et al.* Mitochondrial transcription factor A is necessary for mtDNA maintenance and embryogenesis in mice. *Nature Genet.* **18**, 231–236 (1998).
In this study, the use of a mouse model showed that the disruption of mammalian TFAM leads to mtDNA depletion and embryonic death.
45. Matsushima, Y. *et al.* Functional domains of chicken mitochondrial transcription factor A for the maintenance of mitochondrial DNA copy number in lymphoma cell line DT40. *J. Biol. Chem.* **278**, 31149–31158 (2003).
46. Goto, A., Matsushima, Y., Kadowaki, T. & Kitagawa, Y. *Drosophila* mitochondrial transcription factor A (d-TFAM) is dispensable for the transcription of mitochondrial DNA in Kc167 cells. *Biochem. J.* **354**, 243–248 (2001).
47. Matsushima, Y., Garesse, R. & Kaguni, L. S. *Drosophila* mitochondrial transcription factor B2 regulates mitochondrial DNA copy number and transcription in schneider cells. *J. Biol. Chem.* **279**, 26900–26905 (2004).
48. Dairaghi, D., Shadel, G. & Clayton, D. Addition of a 29 residue carboxyl-terminal tail converts a simple HMG box-containing protein into a transcriptional activator. *J. Mol. Biol.* **249**, 11–28 (1995).
This study showed that a 29-residue carboxyl-terminal tail of human TFAM is important for specific DNA binding and is essential for transcriptional activation. This sequence is absent in the yeast Abf2, which is not required for mitochondrial transcription.
49. Newman, S. M., Zelenaya-Troitskaya, O., Perlman, P. S. & Butow, R. A. Analysis of mitochondrial DNA nucleoids in wild-type and a mutant strain of *Saccharomyces cerevisiae* that lacks the mitochondrial HMG-box protein, Abf2p. *Nucleic Acids Res.* **24**, 386–393 (1996).
50. McCammon, M. T., Epstein, C. B., Przybyla-Zawislak, B., McAlister-Henn, L. & Butow, R. A. Global transcription analysis of Krebs tricarboxylic acid cycle mutants reveals an alternating pattern of gene expression and effects on hypoxic and oxidative genes. *Mol. Biol. Cell* **14**, 958–972 (2003).
51. Zelenaya-Troitskaya, O., Perlman, P. S. & Butow, R. A. *ILV5* encodes a bifunctional mitochondrial protein involved in branched chain amino acid biosynthesis and maintenance of mitochondrial DNA. *EMBO J.* **14**, 3268–3276 (1995).
52. Petersen, J. G. L. & Holmberg, S. The *ILV5* gene of *Saccharomyces cerevisiae* is highly expressed. *Nucleic Acids. Res.* **14**, 9631–9651 (1986).
53. Bateman, J. M., Perlman, P. S. & Butow, R. A. Mutational bisection of the mitochondrial DNA stability and amino acid biosynthetic functions of Ilv5p of budding yeast. *Genetics* **161**, 1043–1052 (2002).
54. MacAlpine, D. M., Perlman, P. S. & Butow, R. A. The number of individual mitochondrial DNA molecules and mitochondrial DNA nucleoids in yeast are co-regulated by the general amino acid control pathway. *EMBO J.* **19**, 767–775 (2000).
55. Hirling, H., Henderson, B. R. & Kuhn, L. C. Mutational analysis of the [4Fe–4S]-cluster converting iron regulatory factor from its RNA-binding form to cytoplasmic aconitase. *EMBO J.* **13**, 453–461 (1994).
56. Hentze, M. W., Muckenthaler, M. U. & Andrews, N. C. Balancing acts: molecular control of mammalian iron metabolism. *Cell* **117**, 285–297 (2004).
57. Alen, C. & Sonenshein, A. L. *Bacillus subtilis* aconitase is an RNA-binding protein. *Proc. Natl Acad. Sci. USA* **96**, 10412–10417 (1999).
58. Nanda, S. K. & Leibowitz, J. L. Mitochondrial aconitase binds to the 3′ untranslated region of the mouse hepatitis virus genome. *J. Virol.* **75**, 3352–3362 (2001).
59. Shadel, G. S. Mitochondrial DNA, aconitase 'wraps' it up. *TIBS* **30**, 294–296 (2005).
60. DeRisi, J. L., Iyer, V. R. & Brown, P. O. Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* **278**, 680–686 (1997).

61. Butow, R. A. & Avadhani, N. G. Mitochondrial signaling: the retrograde response. *Mol. Cell* **14**, 1–15 (2004).
62. Liu, Z. & Butow, R. A. A transcriptional switch in the expression of yeast tricarboxylic acid cycle genes in response to a reduction or loss of respiratory function. *Mol. Cell. Biol.* **19**, 6720–6728 (1999).
63. Kuroiwa, T., Kawano, S. & Hizume, M. Studies of mitochondrial structure and function in *Physarum polycephalum*. V. Behavior of mitochondrial nucleoids throughout mitochondrial division cycle. *J. Cell Biol.* **72**, 687–694 (1977).
64. Garrido, N. *et al.* Composition and dynamics of human mitochondrial nucleoids. *Mol. Biol. Cell* **14**, 1583–1596 (2003).
The authors provide a detailed description of human mt-nucleoids and a direct observation of nucleoid division.
65. Kaufman, B. A., Kolesar, J. E., Perlman, P. S. & Butow, R. A. A function for the mitochondrial chaperonin Hsp60 in the structure and transmission of mitochondrial DNA nucleoids in *Saccharomyces cerevisiae*. *J. Cell Biol.* **163**, 457–461 (2003).
66. Cheng, M. Y. *et al.* Mitochondrial heat shock protein hsp60 is essential for assembly of proteins imported into yeast mitochondria. *Nature* **337**, 620–625 (1989).
67. Boldogh, I., Fehrenbacher, K., Yang, H. C. & Pon, L. A. Mitochondrial movement and inheritance in budding yeast. *Gene* 18 July 2005 (doi:10.1016/j.gene.2005.03.049).
68. Yaffe, M. B. The machinery of mitochondrial inheritance and behavior. *Science* **283**, 1493–1497 (1999).
69. Strausberg, R. L. & Perlman, P. S. The effect of zygotic bud position on the transmission of mitochondrial genes in *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* **163**, 131–144 (1978).
70. Zinn, A. R., Pohlman, J. K., Perlman, P. S. & Butow, R. A. Kinetic and segregational analysis of mitochondrial DNA recombination in yeast. *Plasmid* **17**, 248–256 (1987).
71. Dujon, B. in *The Molecular Biology of the Yeast* Saccharomyces (eds Strathern, J. N., Jones, E. W. & Broach, J. R.) 505–635 (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1981).
72. Bendich, A. J. Structural analysis of mitochondrial DNA molecules from fungi and plants using moving pictures and pulsed-field gel electrophoresis. *J. Mol. Biol.* **255**, 564–588 (1996).
73. Lockshon, D. *et al.* A role for recombination junctions in the segregation of mitochondrial DNA in yeast. *Cell* **81**, 947–955 (1995).
The authors show that mutations in the recombination-junction-resolving enzyme Mgt1 result in the reduction of mt-nucleoid number and consequently impair the inheritance of mtDNA.
74. Ling, F. & Shibata, T. Recombination-dependent mtDNA partitioning: *in vivo* role of Mhr1p to promote pairing of homologous DNA. *EMBO J.* **21**, 4730–4740 (2002).
75. Ling, F., Makishima, F., Morishima, N. & Shibata, T. A nuclear mutation defective in mitochondrial recombination in yeast. *EMBO J.* **14**, 4090–4101 (1995).
76. Ling, F. & Shibata, T. Mhr1p-dependent concatemeric mitochondrial DNA formation for generating yeast mitochondrial homoplasmic cells. *Mol. Biol. Cell* **15**, 310–322 (2004).
77. Maleszka, M. R., Skelly, P. J. & Clark-Walker, G. D. Rolling circle replicaton of DNA in yeast mitochondria. *EMBO J.* **10**, 3923–3929 (1991).
78. MacAlpine, D. M., Kolesar, J., Okamoto, K., Butow, R. A. & Perlman, P. S. Replication and preferential inheritance of hypersuppressive petite mitochondrial DNA. *EMBO J.* **20**, 1807–1817 (2001).
79. Shaw, J. M. & Nunnari, J. Mitochondrial dynamics and division in budding yeast. *Trends Cell. Biol.* **12**, 178–184 (2002).
80. Westermann, B. & Prokisch, H. Mitochondrial dynamics in filamentous fungi. *Fungal Genet. Biol.* **36**, 91–97 (2002).
81. Scott, S. V., Cassidy-Stone, A., Meeusen, S. L. & Nunnari, J. Staying in aerobic shape: how the structural integrity of mitochondria and mitochondrial DNA is maintained. *Curr. Opin. Cell Biol.* **15**, 482–488 (2003).
82. Sesaki, H. & Jensen, R. E. *UGO1* encodes an outer membrane protein required for mitochondrial fusion. *J. Cell Biol.* **152**, 1123–1134 (2001).
83. Hales, K. G. & Fuller, M. T. Developmentally regulated mitochondrial fusion mediated by a conserved, novel, predicted GTPase. *Cell* **90**, 121–129 (1997).
84. Sesaki, H. & Jensen, R. E. Ugo1p links the Fzo1p and Mgm1p GTPases for mitochondrial fusion. *J. Biol. Chem.* **279**, 28298–28303 (2004).
85. Sesaki, H., Southard, S. M., Yaffe, M. P. & Jensen, R. E. Mgm1p, a dynamin-related GTPase, is essential for fusion of the mitochondrial outer membrane. *Mol. Biol. Cell* **14**, 2342–2356 (2003).
86. Wong, E. D. *et al.* The intramitochondrial dynamin-related GTPase, Mgm1p, is a component of a protein complex that mediates mitochondrial fusion. *J. Cell Biol.* **160**, 303–311 (2003).
87. Arimura, S., Yamamoto, J., Aida, G. P., Nakazono, M. & Tsutsumi, N. Frequent fusion and fission of plant mitochondria with unequal nucleoid distribution. *Proc. Natl Acad. Sci. USA* **101**, 7805–7808 (2004).
88. Nowikovsky, K. *et al.* The *LETM1/YOL027* gene family encodes a factor of the mitochondrial K^+ homeostasis with a potential role in the Wolf–Hirschhorn syndrome. *J. Biol. Chem.* **279**, 30307–30315 (2004).
89. Kucejova, B., Kucej, M., Petrezselyova, S., Abelovska, L. & Tomaska, L. A screen for nigericin-resistant yeast mutants revealed genes controlling mitochondrial volume and mitochondrial cation homeostasis. *Genetics* 14 July 2005 (doi:10.1534/genetics.105.046540).
90. Albring, M., Griffith, J. & Attardi, G. Association of a protein structure of probable membrane derivation with HeLa cell mitochondrial DNA near its origin of replicaton. *Proc. Natl Acad. Sci. USA* **74**, 1348–1352 (1977).
91. Burgess, S. M., Delannoy, M. & Jensen, R. E. *MMM1* encodes a mitochondrial outer membrane protein essential for establishing and maintaining the structure of yeast mitochondria. *J. Cell Biol.* **126**, 1375–1391 (1994).
92. Berger, K. H., Sogo, L. F. & Yaffe, M. P. Mdm12p, a component required for mitochondrial inheritance that is conserved between budding and fission yeast. *J. Cell Biol.* **136**, 545–553 (1997).
93. Hobbs, A. E., Srinivasan, M., McCaffery, J. M. & Jensen, R. E. Mmm1p, a mitochondrial outer membrane protein, is connected to mitochondrial DNA (mtDNA) nucleoids and required for mtDNA stability. *J. Cell Biol.* **152**, 401–410 (2001).
94. Sogo, L. F. & Yaffe, M. P. Regulation of mitochondrial morphology and inheritance by Mdm10p, a protein of the mitochondrial outer membrane. *J. Cell Biol.* **126**, 1361–1373 (1994).
95. Boldogh, I., Vojtov, N., Karmon, S. & Pon, L. A. Interaction between mitochondria and the actin cytoskeleton in budding yeast requires two integral mitochondrial outer membrane proteins, Mmm1p and Mdm10p. *J. Cell Biol.* **141**, 1371–1381 (1998).
96. Berger, K. H. & Yaffe, M. P. Mitochondrial DNA inheritance in *Saccharomyces cerevisiae*. *Trends Microbiol.* **8**, 508–513 (2000).
97. Hanekamp, T. *et al.* Maintenance of mitochondrial morphology is linked to maintenance of the mitochondrial genome in *Saccharomyces cerevisiae*. *Genetics* **162**, 1147–1156 (2002).
98. Meisinger, C. *et al.* The mitochondrial morphology protein Mdm10 functions in assembly of the preprotein translocase of the outer membrane. *Dev. Cell* **7**, 61–71 (2004).
99. Kondo-Okamoto, N., Shaw, J. M. & Okamoto, K. Mmm1p spans both the outer and inner mitochondrial membranes and contains distinct domains for targeting and foci formation. *J. Biol. Chem.* **278**, 48997–49005 (2003).
100. Dimmer, K. S., Jakobs, S., Vogel, F., Altmann, K. & Westermann, B. Mdm31 and Mdm32 are inner membrane proteins required for maintenance of mitochondrial shape and stability of mitochondrial DNA nucleoids in yeast. *J. Cell Biol.* **168**, 103–115 (2005).
101. Youngman, M. J., Hobbs, A. E., Burgess, S. M., Srinivasan, M. & Jensen, R. E. Mmm2p, a mitochondrial outer membrane protein required for yeast mitochondrial shape and maintenance of mtDNA nucleoids. *J. Cell Biol.* **164**, 677–688 (2004).
102. Meeusen, S. & Nunnari, J. Evidence for a two membrane-spanning autonomous mitochondrial DNA replisome. *J. Cell Biol.* **163**, 503–510 (2003).
The authors observed that mtDNA replication exclusively takes place in discrete membrane-spanning structures — mitochondrial replisomes. These structures are proposed to provide a mechanism for linking mtDNA replication and inheritance.
103. Taylor, R. W. & Turnbull, D. M. Mitochondrial DNA mutations in human disease. *Nature Rev. Genet.* **6**, 389–402 (2005).
104. Wallace, D. C. Mitochondrial diseases in man and mouse. *Science* **283**, 1482–1488 (1999).
105. Chomyn, A. & Attardi, G. mtDNA mutations in aging and apoptosis. *Biochem. Biophys. Res. Commun.* **304**, 519–529 (2003).
106. Trifunovic, A. *et al.* Premature ageing in mice expressing defective mitochondrial DNA polymerase. *Nature* **429**, 417–423 (2004).
107. Kaukonen, J. *et al.* Role of adenine nucleotide translocator 1 in mtDNA maintenance. *Science* **289**, 782–785 (2000).
108. Van Goethem, G., Dermaut, B., Lofgren, A., Martin, J. J. & Van Broeckhoven, C. Mutation of *POLG* is associated with progressive external ophthalmoplegia characterized by mtDNA deletions. *Nature Genet.* **28**, 211–212 (2001).
109. DiMauro, S. & Schon, E. A. Mitochondrial DNA mutations in human disease. *Am. J. Med. Genet.* **106**, 18–26 (2001).
110. Shoubridge, E. A. Mitochondrial DNA segregation in the developing embryo. *Hum. Reprod.* **15** (Suppl. 2), 229–234 (2000).
111. Cho, J. H., Ha, S. J., Kao, L. R., Megraw, T. L. & Chae, C.-B. A novel DNA-binding protein bound to the mitochondrial inner membrane restores the null mutation of mitochondrial histone Abf2p in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **18**, 5712–5723 (1998).
112. Sato, H. & Miyakawa, I. A 22 kDa protein specific for yeast mitochondrial nucleoids is an unidentified putative ribosomal protein encoded in open reading frame YGL068W. *Protoplasma* **223**, 175–182 (2004).
113. Hall, D. A. *et al.* Regulation of gene expression by a metabolic enzyme. *Science* **306**, 482–484 (2004).
114. Cohen, G., Rapatz, W. & Ruis, H. Sequence of the *Saccharomyces cerevisiae CTA1* gene and amino acid sequence of catalase A derived from it. *Eur. J. Biochem.* **176**, 150–163 (1988).
115. Liu, T. *et al.* DNA and RNA binding by the mitochondrial lon protease is regulated by nucleotide and protein substrate. *J. Biol. Chem.* **279**, 13902–13910 (2004).
116. Shiiba, D., Fumoto, S. I., Miyakawa, I. & Sando, N. Isolation of giant mitochondrial nucleoids from the yeast *Saccharomyces cerevisiae*. *Protoplasma* **198**, 177–185 (1997).
117. Kuroiwa, T. Mitochondrial nuclei. *Int. Rev. Cytol.* **75**, 1–59 (1982).
118. Moriyama, Y. & Kawano, S. Rapid, selective digestion of mitochondrial DNA in accordance with the *matA* hierarchy of multiallelic mating types in the mitochondrial inheritance of *Physarum polycephalum*. *Genetics* **164**, 963–975 (2003).
119. Klingbeil, M. M. & Englund, P. T. Closing the gaps in kinetoplast DNA network replication. *Proc. Natl Acad. Sci. USA* **101**, 4333–4334 (2004).
120. Lukes, J. *et al.* Kinetoplast DNA network: evolution of an improbable structure. *Eukaryot. Cell* **1**, 495–502 (2002).
121. Ashley, N., Harris, D. & Poulton, J. Detection of mitochondrial DNA depletion in living human cells using PicoGreen staining. *Exp. Cell Res.* **303**, 432–446 (2005).
122. Iborra, F. J., Kimura, P. R. & Cook, P. R. The functional organization of mitochondrial genomes in human cells. *BMC Biol.* **2**, 9 (2004).
123. Legros, F., Malka, F., Frachon, P., Lombes, A. & Rojo, M. Organization and dynamics of human mitochondrial DNA. *J. Cell Sci.* **117**, 2653–2662 (2004).
124. Magnusson, J., Orth, M., Lestienne, P. & Taanman, J. W. Replication of mitochondrial DNA occurs throughout the mitochondria of cultured human cells. *Exp. Cell Res.* **289**, 133–142 (2003).
125. Margineantu, D. H. *et al.* Cell cycle dependent morphology changes and associated mitochondrial DNA redistribution in mitochondria of human cell lines. *Mitochondrion* **1**, 425–435 (2002).

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Competing interests statement

The authors declare no competing financial interests.

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