Review

Mitochondrial inheritance in budding yeasts: towards an integrated understanding

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Recent advances in yeast mitogenomics have significantly contributed to our understanding of the diversity of organization, structure and topology in the mitochondrial genome of budding yeasts. In parallel, new insights on mitochondrial DNA (mtDNA) inheritance in the model organism *Saccharomyces cerevisiae* highlighted an integrated scenario where recombination, replication and segregation of mtDNA are intricately linked to mitochondrial nucleoid (mt-nucleoid) structure and organelle sorting. In addition to this, recent discoveries of bifunctional roles of some mitochondrial proteins have interesting implications on mito-nuclear genome interactions and the relationship between mtDNA inheritance, yeast fitness and speciation. This review summarizes the current knowledge on yeast mitogenomics, mtDNA inheritance with regard to mt-nucleoid structure and organelle dynamics, and mito-nuclear genome interactions.

Yeast mitogenomics: a network of information

Mitochondria are a central platform for diverse cellular functions including respiration, meabolite biosynthesis, ion homeostasis and apoptosis. Mitochondrial DNA (mtDNA), which encodes subunits of the oxidative phosphorylation (OXPHOS) complexes, is pivotal for ensuring functional mitochondria and has an active role in determining phenotypic diversity and fitness in animals and budding yeasts owing to sophisticated interactions between the mitochondrial and chromosomal genomes [1–3]. Understanding of the mechanism driving mtDNA transmission has far-reaching implications in different fields. Being under strong selection, mtDNA haplotypes (mitotypes) variations (i.e. heteroplasmy) cause human degenerative syndromes [4], senescence in fungi [5] and male sterility in plants [6].

The class of Hemiascomycetes or budding yeasts includes some of the most important yeasts for basic, applied and medical research, features that have made Hemiascomycetes the focus of extensive mitogenomics research (Box 1). The first efforts in yeast mitogenomics date back to 1980. In 1995, the mitochondrial genome of *Wickerhamomyces canadensis* was successfully sequenced [7]. Since then, a burgeoning number of different yeast mitochondrial genomes have been submitted to databases (see the GOBASE at http://gobase.bcm.umontreal.ca). These ever-growing data contributed to an unprecedented understanding of yeast mtDNA organization and revealed numerous patterns of variation in genomic architecture, coexisting with the conserved role of the mitochondria as ATP-producing organelles. Furthermore, the availability of mtDNA sequences from representatives of different Hemiascomycetes clades allowed the examination of divergence in mitochondrial genomes from an evolutionary perspective [8]. From a functional point of view, another important aspect highlighted by yeast mitogenomics is how mtDNA structural organization affects its transmission. Recent discoveries on mtDNA replication and segregation in *Saccharomyces cerevisiae* provided an integrated picture of mtDNA inheritance based on three strictly linked structural levels: genomic, mitochondrial nucleoid (mt-nucleoid) and organelle [9,10]. Here, we review the current status of yeast mitogenomics and discuss new insights on mtDNA inheritance.

Charting mitochondrial genome variability in budding yeasts

The explosion of yeast mitogenomics has revealed a wide divergence in mtDNA organization and topology across budding yeasts. In contrast to closely spaced and mostly intronless nuclear genes, yeast mtDNA genes are intron-rich and separated by long noncoding stretches, which contribute to a large diversity in genome size (Table 1). Intergenic regions, as well as group I and group II introns, variable domains of ribosomal RNA (rRNA) genes and some protein coding genes can host repetitive elements, such as alternating and nonalternating AT stretches and short GC-rich clusters, which favor recombinations involved in mitochondrial genome plasticity [11]. In *S. cerevisiae* long AT spacers formed approximately 50% of mtDNA, whereas 10–80 bp long GC clusters are lower than 5% and can be folded in a variety of stem-to-loop structures acting as mobile elements [12]. Occurring in almost all yeast species, GC-rich clusters are indeed absent from *Candida glabrata* mtDNA, reinforcing the view that GC-rich clusters are most likely mobile elements [13]. Other intergenic sequences, the *ori* regions, are specific only for *Saccharomyces sensu stricto* yeasts. In *S. cerevisiae* they are approximately 300 bp long and contain three conserved GC-rich sequences, AT clusters, and a nonanucleotide promoter for the *RPO41*-encoded mitochondrial RNA polymerase [11]. The wild-type (ρ^+) mtDNA has seven or

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Box 1. *S. cerevisiae*, the heart of mitochondriology

S. cerevisiae is a leading model organism for mitochondrial studies owing to its accessibility in terms of molecular genetics and experimental manipulation. *S. cerevisiae* is currently the only eukaryotic organism in which large-scale analyses of the genome, transcriptome and proteome have been carried out efficiently. The availability of the yeast gene deletion libraries nowadays allows systematic screening to assign functions to almost all of nonessential yeast genes. Three genomic databases, the *Saccharomyces* Genome Database, the Yeast Proteome Database and MIPS (Munich Information Center for Protein Sequences), have organized available information about yeast genes and proteins onto the genome framework. Moreover, several features make *S. cerevisiae* particularly attractive for studying mitochondria inheritance. In most higher eukaryotes, the mtDNA transmission is difficult to study by strain crossing owing to uniparental inheritance. In contrast, mtDNA inheritance is biparental in crossing between *S. cerevisiae* strains harboring wild-type (ρ^+) mtDNA, even if the heteroplasmic state is not stably maintained. Furthermore, being a facultative aerobe, *S. cerevisiae* can survive the complete loss or alterations of its mtDNA, providing different mitochondrial mutants which show distinct kinds of mtDNA segregation patterns in crossing with ρ^+ strains (Table I). Intergenic deletion mutants lack various sets of intergenic sequences, such as *ori* regions, whereas ant^R and ant^S mutants harbor mtDNAs with point mutations or small deletions conferring resistance or sensitivity to specific drugs, respectively. Petite strains (ρ^-) are respiratory-deficient mutants whose ρ^+ mtDNAs are extensively deleted, with the remaining sequences amplified and organized as oligomeric repeats (head-to-tail, palindromic head-to-head, and tail-to-tail) [38]. Petite strains that have an amplified active *ori5* region have been extensively studied (referred to as *ori5* ρ^-). The term petite comes from the characteristic reduced colony size of ρ^- strains on solid media with limiting amounts fermentable carbon source. The petites fall into four classes on the basis of their behavior in crosses with ρ^+ strains. If there is no mtDNA at all, the mutants are called ρ^0. ρ^0 give rise to only ρ^+ progeny. For ρ^- mutants, a fraction of the progeny inherit ρ^- mtDNA and these petites are called suppressive (S) petites. Those petite mutants whose mtDNA is not transmitted to progeny of crosses with ρ^+ strains are called neutral (N). In the case of hypersuppressive (HS) petites, >95% of progeny exclusively contain the ρ^- genome [38]. Petite mutants are different from *pet* mutants, another class of respiratory-deficient mutant strains carrying mutations in the nuclear genes involved in mitochondrial functions and from respiration-deficient mutant harboring point mutation in mtDNA (*mit*$^-$).

Table I. Overview of mitochondrial mutants exploited to study different mtDNA transmission patterns

mtDNA segregation[a]	Parental strains (input)	Progenies (output)
Biparental	A ρ^+×B ρ^+	A ρ^+, B ρ^+, AB ρ^+ recombinant
	ant^S×ant^R	ant^S, ant^R, recombinant
	A ant^R×B ant^R	A ant^R, B ant^R, AB ant^R recombinant
Biased	ρ^+×S ρ^-	> S ρ^- progeny
	ρ^+×intergenic deletion mutant	> ρ^+ progeny
	intergenic deletion mutant	50% ρ^+, 25% intergenic deletion
	A×intergenic deletion mutant B	mutant A, 25% intergenic deletion mutant B
Uniparental	ρ^+×N ρ^- or ρ^0	ρ^+ progeny
	ρ^+×HS ρ^-	>> 95% HS ρ^-

[a]Three kinds of segregation are observed: biparental, uniparental and biased. In biparental transmission, three mtDNA molecules can be inherited in progeny, similar to parental and recombinant mtDNAs. Biased transmission indicates a preferential transmission of one parental mitotype related to the other one. Uniparental transmission indicates the inheritance of only one parental mitotype.

eight *ori* sequences, but some of them are inactive, owing to the presence of short insertion in the *RPO41* promoter.

A core of gene-coding regions is highly conserved in budding yeasts, specifying for eight vital mitochondrial proteins, that is subunits of the ATP synthase complex (*atp6*, *atp8* and *atp9*), apocytochrome *b* (*cytb* or *cob*), subunits of the cytochrome *c* oxidase complex (*cox1*, *cox2* and *cox3*) and a ribosomal protein (*var1* or homologous *rps3*). Moreover mtDNAs encode large (*rnl*) and small (*rns*) rRNA genes, RNA component of the RNAse P (*rnpB*) and a set of tRNA genes (Table 1). However, gene content varies among yeasts. Similar to Metazoa and Protists, mtDNAs of aerobic yeasts harbor *nad* genes encoding for seven NADH:ubiquinone oxidoreductase (complex I) subunits. Comparative analyses revealed that group I introns, coding for endonucleases, reverse transcriptases and mRNA maturases and intron-related open reading frames of unknown function have a patchy distribution among Hemiascomycetes [12], similar to the scattered distribution observed for *rnpB* and *rps3* genes (Table 1).

Slight differences also occur in the translation code. Generally, most of the yeasts use the so-called 'yeast mitochondrial code' where the UGA triplet is read as tryptophan instead of as a stop codon as occurs in the universal genetic code; ATA sequence translates as methionine rather than isoleucine; and the four codons of the CUN family specify threonine instead of leucine (see [14] for a review). However, some species, such as *Yarrowia lipolytica* [15], *Candida* spp. [16–18], *Debaryomyces hanseni* [14] and *Dekkera/Bruxellensis* spp. [19] show only the UGA codon specifying tryptophan as a deviation from the universal translation code.

The gene order varies considerably within Hemiascomycetes, reflecting rearrangements taking place during the yeast evolutionary route. Many of these gene order reshufflings could be mediated by inversion or movement of short intergenic repeats and mobile introns [19]. The most conserved syntenic region is *cox1–atp8–atp6*, which forms a transcription unit in *S. cerevisiae*, *Naumovia castellii* and *Kazachstania servazzii* [20]. This cluster is cotranscribed with *nad4* in *Y. lipolytica* [15] or also includes the *atp9–cox2* genes in some small mitochondrial genomes, such as *C. glabrata*, *Lachancea thermotolerans* [21], and *N. castellii* [20]. *nad4L–nad5* genes formed an uninterrupted pair in all ascomycetes having complex I, as well as in basidiomycetes and zygomycetes [22].

A large variability was also noted for the genome topology. In early studies, mtDNA was believed to be exclusively circular in yeast as in most eukaryotes [23]. Subsequent research revealed that mitochondria of some yeasts with a circular-mapping mtDNA contain only a tiny fraction of circular molecules [24]. In *S. cerevisiae* nearly all mtDNAs

Table 1. Phylogenetic position, fermentative lifestyle and mitochondrial genomic features from representative Hemiascomycetes species

Species[a]	WGD	Crabtree effect	Petite phenotype	*rps3*	*nad* genes	*ori*	GC (%)	IR (%)	Protein genes (%)[b]	tRNA	Introns		Accession No.	mtDNA size (kb); topology	Ref.
											Group I	Group II			
Saccharomyces cerevisiae	+	+	+	■	□	■	17.1	62.0	7.8	24	9	4	AJ011856	85.8; PL	[12]
Saccharomyces pastorianus	+	+	+	■	□	■	19.1	64.9	10.0	24	□	1	EU852811	70.6; CM	[72]
Kazachstania servazzii	+	+	+	■	□	□	23.2	43.0	21.3	23	5	□	AJ430679	30.8; CM	[20]
Naumovia castelli	+	+	+	■	□	□	20.4	39.7	27.5	23	3	□	AF437291	25.7; CM	[20]
Candida glabrata	+	+	+	■	□	□	17.6	14.9	26.9	23	3	□	AJ511533	20.1; CM	[13]
Vanderwaltozyma polyspora	+	nr	–	■	□	□	15.2	33.4	29.3	23	□	□	AM698041	21.7; L	[73]
Lachancea thermotolerans	–	+	–	■	□	□	24.8	26.6	29.9	24	3	□	AJ634268	23.5; CM	[21]
Kluyveromyces lactis	–	–	–	■	□	□	26.1	55.0	16.8	22	4	□	AY654900	40.3; CM	[74]
Eremothecium gossypii	–	+	–	■	□	□	18.8	51.4	27.6	23	□	□	AE016821	23,5; CM	[30]
Wickerhamomyces canadensis	–	nr	nr	■	■	□	18.1	17.1	51.3	25	2	□	D31785	27.7; CM	[7]
Brettanomyces custersianus	–	–	–	■	■	□	21	22.3	42.8	25	4	□	GQ354525	30.0; CM	[19]
Dekkera bruxellensis	–	+	+	■	■	□	15.2	60.5	10.2	25	4	□	GQ354526	76.4; CM	[19]
Debaryomyces hansenii	–	–	–	□	■	□	27.0	12.2	36.8	25	6	□	DQ508940	29.5; CM	[28]
Candida parapsilosis	–	–	–	□	■	□	24.3	7.7	36.6	24	7	□	AY423711	30.9; L	[16]
Candida metapsilosis	–	–	–	□	■	■	25.1	12.3	46.5	24	1	□	AY962591	23.1; L	[17]
Candida orthopsilosis	–	–	–	□	■	■	25.0	14.1	45.4	24	2	□	AY962590	22.5; C	[17]
Candida albicans	–	–	+	□	■	□	32.2	36.1	31.0	30	nr	nr	AF285261	40.4; CM	[18]
Hanseniaspora uvarum	–	–	–	□	□	□	29.3	5.1	28.0	23	1	□	DQ058142	11.1[c]; L	[26]
Candida zemplinina	–	–	–	■	□	□	21.3	8.6	27.3	25	5	1(IIB)	AY445918	23.1; CM	[75]
Yarrowia lipolytica	–	–	–	■	■	□	22.7	16.1	51.1	27	17	□	AJ307410	47.9; CM	[15]

Abbreviations: WGD, whole-genome duplication; *rps3*, gene encoding ribosomal protein S3 or its homologous *var1* in *S. cerevisiae*; *nad*, genes *nad1 nad6 nad2 nad3 nad4 nad5* and *nad4L*; *ori*, *ori* regions; IR, intergenic region; CM, circular map of the mtDNA obtained by genetic and physical approaches; L, linear mtDNA; C, circular mtDNA; PL, polydisperse linear mtDNA; nr, not reported.

Symbols: +, post-WGD; –, pre-WGD; filled squares, gene is present; open squares, gene is absent.

[a]Species arranged in order according to their phylogenetic distance from *Saccharomyces cerevisiae*, following the tree topology reported by [19].

[b]Intronic sequences within protein-coding genes and intronic genes have been excluded in calculating protein gene %.

[c]The inverted terminal repeats of *Hanseniaspora uvarum* mtDNA have been excluded from genome size.

are organized in polydisperse linear head-to-tail tandem concatemers, which span from ~75 to 150 kb, accompanied by small amounts of circular monomers with the same size [24]. Concatemers are predominant in mother cells and nondividing cells, whereas circles are the major form of mtDNA in growing buds [25]. Unlike *S. cerevisiae*, some *Candida* species [16,17] and *Hanseniaspora uvarum* [26] harbor linear mtDNA with terminal mitochondrial telomeres (Table 1).

It is thus clear that the different budding yeasts show highly divergent genome architectures. This observed diversity brings to mind two important questions. Which are the ancient and ongoing evolutionary events that determine this variability? How could this structural organization affect mtDNA replication and transmission?

The first question points towards evolutionary framework to understanding the mitochondrial genome divergence described by comparative mitogenomics. Despite this variability, the mitochondrial genome has a monophyletic origin that has arisen from an endosymbiotic form of α-Proteobacteria more than 10^9 years ago (see [27] for a review). This ancestor genome probably contained hundreds of genes, against the 30–40 in the yeast mtDNA. Thus, the adaptation to an intracellular existence led to a reductive evolution in the gene repertoire through gene loss and gene transfer from the endosymbiont into the nuclear genome [27]. In addition, *S. cerevisiae* phagolysosomes could consume the mitochondria releasing partially degraded mtDNA. These fragments can be subsequently integrated in the nuclear DNA (nuDNA) with an estimated rate of one transfer event per 10^5 generations [27]. Integration of mitochondrial DNA fragments into nuclear chromosomes (giving rise to nuDNA sequences of mitochondrial origin) is an ongoing process that shapes nuclear genomes and has a species-specific distribution in yeasts [28].

Some budding yeasts have taken the gene reduction to the next level and can live even without a functional

Box 2. From the nucleus point of view

All the mitochondrial functions depend on coordinated expression of nuclear and mitochondrial genomes. Their integration represents one of the most remarkable symbioses in the history of life. Approximately 12–13% of the *S. cerevisiae* nuclear genes are devoted to encoding for the mitochondrial proteome [76]. The availability of both mitochondrial and nuclear genome sequences, as well as of large-scale proteomic data from *S. cerevisiae*, has allowed not only reconstructing the reductive evolution that shaped mtDNA but also focusing on the evolutionary origin of nuDNA-encoded mitochondrial proteins in yeast. Proteomic studies demonstrated that most of the proteins imported to mitochondria are newly recruited nuclear proteins with no ancestral function in the organelle. Thus, nuclear expansive gene processes have contributed to transform the endosymbionts into ATP-exporting organelles. By reconstructing the phylogenesis of 400 mitochondrial proteins, Karlberg *et al.* [77] demonstrated that only 38 proteins, involved in the mitochondrial respiratory system and translational machinery, derive from the α-proteobacterium endosymbiotic ancestor. Approximately 200 proteins mainly engaged in gene expression regulation, mRNA stability and splicing are novel 'eukaryotic' mitochondrial proteins without bacterial or archaeal orthologous. These eukaryotic genes have evidently evolved from the nuDNA by gene duplication and functional divergence. Approximately half of these duplications have been suggested to arise from WGD [29]. Finally, a small cohort of nuDNA-encoded mitochondrial proteins are of unspecified bacterial origin and might have been introduced from bacteria other than the ancestral endosymbiont through lateral gene transfer. Accordingly, Marcotte *et al.* [78] used a computational method to identify subcellular locations of proteins from the phylogenetic distribution of their homologs. On the basis of these results, the *S. cerevisiae* mitochondrial proteome is composed of 630 proteins divided into three groups: prokaryote-derived (58%), eukaryote-derived (20%) and yeast-specific (22%).

More recently, the availability of nuclear genome sequences from pre- and post-WGD species other than *S. cerevisiae* has opened up new challenges in understanding the evolution of mitochondrial genes. These were analyzed for evolution rate and codon usage patterns [35]. Post-WGD species display a higher mutation rate for mitochondrial genes compared to pre-WGD species. Moreover, nuDNA-encoded mitochondrial genes have significantly stronger codon usage bias than non-mitochondrial genes in all three studied pre-WGD species. In contrast, codon usage bias is relaxed for mitochondrial genes in post-WGD species. These observations substantiate the hypothesis that functional constraints on mitochondrial genes are less severe when fermentation plays a greater role in cellular energy production.

mitochondrial genome (petite-positive) (Box 1). These yeasts belong to the *Saccharomyces* complex, which evolved after whole-genome duplication (WGD) occurred in the ancestor around 100 million years ago when the fruit-bearing angiosperms appeared in the earth's flora [29,30]. The WGD event and the ensuing gene loss were linked to the evolution of an efficient fermentative and anaerobic lifestyle in this lineage (see [31] for a review). Although smaller scale duplications contributed to the development of a "make, accumulate and consume ethanol" strategy in *Saccharomyces* species [32], several studies support the 'WGD–fermentation hypothesis' [33]. Accordingly, post-WGD species carry out fermentation even under aerobic conditions with plentiful glucose concentration (Crabtree effect; Table 1). In contrast, pre-WGD species mostly respire under such conditions, but few species are Crabtree-positive [19,34]. Therefore, it is probable that several lineages of Hemiascomycetes independently developed aerobic fermentation as an adaptation to a low-oxygen and high-sugar environment. In these conditions, the role of mitochondria in generating energy by respiration is attenuated as confirmed by the relaxation of nuDNA-encoded mitochondrial functions observed in post-WGD yeasts [35] (Box 2).

The analysis of phylogenetic distribution of mtDNA features in pre- and post-WGD species suggested a possible correlation between some mitogenomics properties and phenotypic traits. The *nad* genes, the ribosomal protein-encoding genes and the universal translation code are ancestral traits in the protistan common ancestor of fungi [8]. Nevertheless, all the post-WGD species, which prevent glycolytic overflow by aerobic fermentation, lack *nad* genes and use the yeast mitochondrial genetic code. A mosaic situation characterizes the pre-WGD species, suggesting that the *nad* gene loss occurred several times and independently in the evolution of yeast and it is not a precondition for developing a Crabtree-positive phenotype [19]. Likewise, the scattered distribution of the *rnpB* and *rps3* genes indicates that mitochondrial gene loss occurred early in the budding yeast lineage, but is also an ongoing process [8].

Mitochondrial DNA transmission: an integrated concept

Evolutionary reconstruction of yeast mitochondrial history is only one of the new challenges revealed by the ever-increasing availability of nuclear and mitochondrial genome sequences. Another question is related to understanding how the genetic information content of mtDNA molecules flows from mother to daughter cell. This intricate process consists of two phases: the replication of mtDNA in several copies and the segregation of duplicated mtDNA into progeny [36]. Unlike nuDNA, the replication of mtDNA is not strictly linked to the cell cycle and there is no strict control of mtDNA partitioning at cytokinesis. Currently all these mechanisms are not completely understood in *S. cerevisiae* and have not yet been studied in other yeasts, but mitochondrial genomic diversity suggests species-specific replication and segregation strategies.

At the molecular level, mtDNA exists in a nested hierarchy of populations [37]. Multiple mtDNAs are packed into highly organized structures called mt-nucleoids which are transmitted as whole with the organelle. Furthermore, there are multiple mitochondria in each cell. Hence, determining the mitochondrial transmission mechanism relies on studying multiples levels of sophisticated interactions among mtDNAs, mt-nucleoids and the organelle–transport system.

The genome view

From a genomic point of view, *S. cerevisiae* is the best model for studying mitochondrial inheritance (Box 1). Its high copy number (from 50 to 200) mtDNA is biparentally inherited in mating experiments, but this heteroplasmic state is transient and homoplasmic cells originate from a heteroplasmic zygote after no more than 20 mitotic cell divisions (vegetative segregation) [38]. Homoplasmy restoration was initially explained on the basis of the random

choice of an mtDNA template for replication and segregation [38]. However, if each mtDNA replicates independently and then randomly segregates into progeny, approximately three mtDNAs should enter into each mitotic cell and this is much smaller than the actual mtDNA copy numbers [36]. This so-called 'ploidy paradox' suggests that a single mitochondrial genome is randomly chosen taking advantage of the subsequent rounds of replication to quickly generate homoplasmic cells. Further studies on hypersuppressiveness (HS) suggested that *ori* sequences are required for the HS phenotype and their number might confer a transmission advantage to a HS petite (ρ^-) mutant [39]. To support this replicative model is the fact that, similar to the replication origin of mammalian mtDNA, *ori* sequences are the origin for mtDNA replication and are primed by Rpo41p RNA polymerase.

Although some biochemical evidence supports mtDNA Rpo41p-primed replication (see [40] for a review), alternatives emerged through observations that an *ori*-devoid ρ^- genome, composed entirely of AT base pairs, can be stably maintained [41]. Furthermore, neither the replication of ρ^- mtDNAs nor the preferential mtDNA transmission of an HS ρ^- strain need Rpo41p RNA polymerase [42,43]. The direct relationship between the density of mtDNA recombination junctions and the efficiency of mtDNA transmission [44], as well as the high frequency of progeny harboring recombinant mtDNA unlike that of either parent [11], support the existence of mtDNA recombination-dependent replication (RDR) beyond a RNA-primed mechanism. Based on *S. cerevisiae* mtDNA topology, Maleszka *et al.* [24] hypothesized that mtDNA circular molecules replicate through a rolling circle (RC) replication, whereas the linear mtDNAs merely followed an amplification strategy. The RC mechanism has a recombination-dependent initiation and forms linear head-to-tail concatamers composed of several units with the same sequence and these are the most common form of mtDNA molecules in the mother cell and nondividing cells. Ling and Shibata [45,46] further proposed a segregative model where concatamers determine the quick segregation of homoplasmic cells from a heteroplasmic zygote. A few circular mtDNAs are randomly selected as templates for RC replication to form concatemers, which are selectively transmitted to daughter cells, after which the concatemers are processed into circular, unit-sized mtDNAs (Figure 1). An alternative to the RDR mechanism could be replication via Cairns-type theta intermediates where monomers are joined by crossing-over (CO) to generate multimers (Figure 1). The evidence of *ori*-mediated RNA-primed replication could support this alternative mechanism, but labeling experiments showed that concatemers are the main products of mtDNA replication, excluding multimers formation through monomers CO [45].

Even if the molecular basis of RDR remains to be explored, several studies on *trans*-acting elements involved in mtDNA transmission suggested an association between replication, recombination and segregation. The *CCE1* gene encoding mitochondrial cruciform cutting endonuclease, which cleaves Holliday junctions formed during mitochondrial CO, is an important factor in mtDNA segregation, because its deletion abolishes the transmission advantage of a HS ρ^- mutant [45]. The mt-nucleoid component Abf2p promotes intermediates of Holliday junctions in ρ^+ mtDNA *in vivo* [47] and affects the mtDNA copy number [48]. Other studies showed that Mhr1p, a mitochondrial matrix protein involved in mitochondrial ATP-independent gene conversion, induces *in vitro* pairing of single-stranded DNA (ssDNA) to its homologous double-stranded DNA (dsDNA) [45,49]. The *mhr1-1* mutant is defective in ρ^+ mtDNA recombination and partitioning. This evidence supports Mhr1p mediating the RDR initiation, by joining linearized ssDNA with a homologous circular dsDNA molecule [45].

More recently, the base excision repair enzyme Ntg1p required for repairing oxidative DNA damages *in vivo* was also found to be involved in mtDNA recombination [9]. Generally, a double-stranded break (DSB) initiates DNA recombination, after which a homologous DNA pairing protein directs the formation of a heteroduplex joint. *In vitro* experiments indicated that Ntg1p alone can introduce a DSB near the promoter end of the *ori5* region in HS *ori5* ρ^- mtDNA. Moreover, both HS and concatemer formation of HS *ori5* ρ^- mtDNA are simultaneously suppressed by an *ntg1* null mutation [9]. These results support the hypothesis that Ntg1p-mediated DSB begins RDR in *ori5*, followed by Mhr1p-mediated homologous pairing of the processed nascent DNA ends with circular mtDNA (Figure 1a). Concatamers essential for quick mtDNA vegetative segregation could be generated through a Mhr1p-dependent homologous DNA pairing between 3′-ssDNA tails and a circular mtDNA (RC replication) or 3′-dsDNA tails and an intact circular mtDNA (monomer joining by CO) (Figure 1b). The *ori* sequences might facilitate RDR and confer selective advantage for mtDNA transmission in a HS phenotype, acting as hot spots for recombination rather than as mere replication origins [23]. Although the segregative model is exhaustive in understanding the molecular basis of mtDNA homoplasmy, it considers a single mtDNA molecule as unit of inheritance, disregarding the hierarchal organization inherent in mitochondrial genomes.

The mitochondrial nucleoid view

Mitochondrial nucleoids represent the next level of mtDNA organization. They are dynamic protein–mtDNA macrocomplexes located in the mitochondrial matrix and act as unit of mtDNA inheritance [44]. *S. cerevisiae* mt-nucleoids contain 3–4 mtDNA copies and approximately 30 proteins (see [50] for a review). The major mtDNA-binding protein is the high-mobility group-like nonhistone protein Abf2, which bends the DNA backbone with a preference for GC-rich gene sequences [10], resulting in compaction of dsDNA molecules into 190 nm structures [51]. The mt-nucleoid also includes proteins from precursor protein import pathways into mitochondria, such as heat shock protein 60 (Hsp60). This chaperonin is required for the proper folding of mitochondrial proteins and associates with ssDNA regions in a strand-specific manner, as confirmed by *in vitro* ssDNA binding of active *ori* sequences [52]. Moreover *hsp60* temperature-sensitive mutants show defects in mtDNA transmission and alteration of the mt-nucleoid morphology, strengthening the regulatory role of Hsp60p in mtDNA replication and nucleoid division [53]. Interestingly the *ori*-dependent interaction of Hsp60p to mtDNA

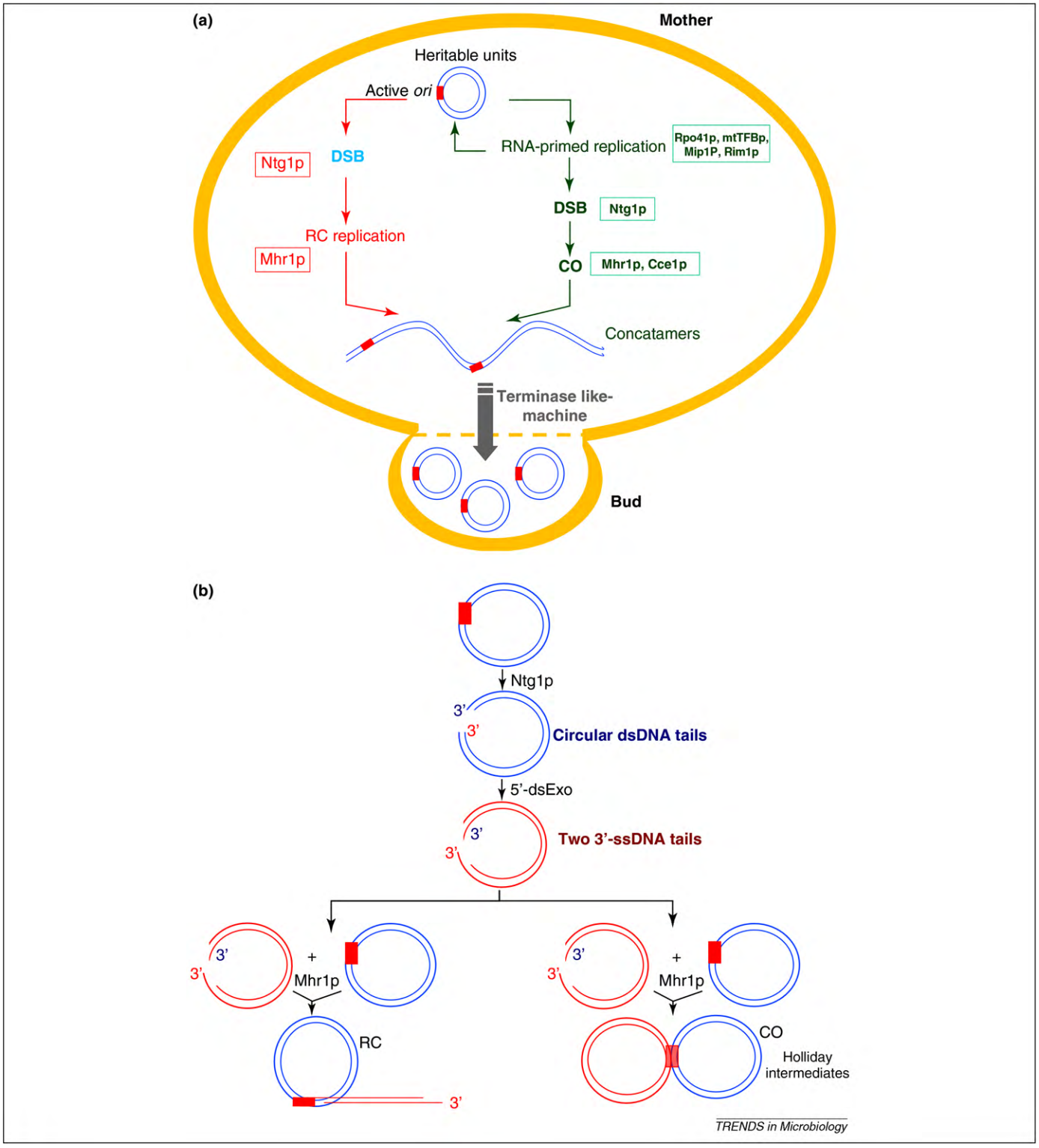

Figure 1. Steps in concatemer formation and segregation from mother to bud cell. **(a)** Concatamers can be generated from mtDNA circles by two different mechanisms of replication and recombination: homologous DNA pairing-dependent rolling circle (RC) replication (in red) and RNA-priming dependent replication followed by homologous recombination (such as crossing-over, CO) (in green). In both cases, Ntg1p introduces the double-stranded break (DSB) in an active replication origin (*ori*). In the red pathway (RC), Mhr1p mediates the initiation of RC replication to produce concatamers (linear head-to-tail array of multiple mtDNA units). In the green pathway (CO), Mhr1p mediates the CO initiation to produce heteroduplex Holliday intermediates. Then concatemers are selectively transmitted to daughter cells by a terminase-like machine and, upon the transmission, the concatemers are processed into circular, unit-sized mtDNA. Putative proteins involved in each step are boxed. **(b)** Molecular differences between RC replication and CO. A DSB generated by Ntg1p is cleaved to two 3′-ssDNA tails by a putative 5′-dsDNA exonuclease (5′-dsExo). If Mhr1p pairs only a single 3′ terminus of the two 3′-ssDNA tails to a homologous intact circular mtDNA, RC replication begins and produces concatamers. If Mhr1p pairs both 3′-ssDNA tails to a homologous sequence on the same intact circular mtDNA, a heteroduplex Holliday intermediate is formed; then another Cce1p-mediated DSB event generates CO products of circular mtDNA multimers of discrete sizes (not shown). Adapted, with permission, from Ref. [9].

might imply that *ori* regions participate in nucleoid sorting as association sites between mtDNA and the mitochondrial membrane. Because proteins related to mtDNA replication, repair and recombination also take part in mt-nucleoid structure, the mt-nucleoid might be considered as a self-replicating unit faithfully inherited during cell division [50]. Furthermore, the metabolic proteins Aco1 (the aconitase that converts citrate into isocitrate) and Iv5 (the mitochondrial enzyme involved in branched-chain amino acid biosynthesis) are mt-nucleoid components essential for both mtDNA maintenance and mt-nucleoid remodeling in response to metabolic changes [10,54].

An important aspect related to mt-nucleoid inheritance is motility. Cytological localization experiments indicate that the diffusion of mt-nucleoids within the organelle is limited [55,56]. Moreover, there is evidence of a partitioning apparatus driving the nucleoid sorting during cell segregation via extra-mitochondrial attachments to cytoskeleton [57,58]. Indeed, after crossing, fusion between parental mitochondria creates a single continuous organelle in the zygote that conducts the rapid mixing of mitochondrial protein. Fusion also places parental mitochondrial genomes into one compartment, but mtDNA from both parents does not freely diffuse through the zygotic mitochondrial reticulum [56]. In addition, nucleoid inheritance is a DNA-independent [57], non-random process affected by the site of first bud formation [55,56]. As similar molecules tend to remain together, daughter cells with three different mitochondrial genotypes become possible after mating: those that bud from the midpoint of the zygote are likely to contain mtDNA from both parents as well as a higher frequency of recombinant genotypes; those that bud from either end of the zygote frequently contain mtDNA from only one of the two parents [57]. This behavior indicates a strict cooperation between mt-nucleoids and the mitochondria in partitioning mtDNAs to the daughter cell in an active and ordered manner. Accordingly, genes that control mitochondrial fusion, morphogenesis and movement are also involved in mt-nucleoid segregation [55–59].

The organelle view

The third level of hierarchy is specific to mitochondria. In yeast, one to ten mitochondria form a subcortical dynamic reticulum maintained by fission and fusion events. Together with this structure, mt-nucleoids were also transmitted. Because mitochondria cannot be generated *de novo*, daughter cells receive these organelles from parents prior to completion of cytokinesis. Time-lapse imaging of mitochondria revealed that mitochondria segregate generally in four steps: (i) polarization of mitochondria towards the site of bud emergence in the G1 phase; (ii) linear, polarized movement of some mitochondria from mother cell to developing buds (anterograde movement) and of other mitochondria towards the tip of the mother cell that is distal to the bud (retrograde movement) during S phase; (iii) transient immobilization of the organelle at the opposite ends of dividing; and (iv) release of mitochondria from retention zones in the mother cell and bud, and redistribution in the dividing cells during mitosis [59].

The total inventory of proteins having a role in organelle inheritance is far from complete. Many proteins are necessary to spatially coordinate organelle transport at the mitochondrial surface and partitioning of mt-nucleoids occurring in the matrix across the outer and inner membranes. Likewise, complex strategies are involved to temporally coordinate mitochondrial partitioning to cell cycle events and perpetuate the mtDNA population during cell proliferation (see [60] for a review). Multiple lines of evidence show that mt-nucleoid inheritance is mediated by the organelle attachment to actin cables through mitochondrial outer membrane proteins Mmm1, Mdm10 and Mdm12 [58]. Deletion of any one of these subunits resulted in loss of mtDNA or defects in mt-nucleoid maintenance. Therefore the Mmm1p-Mdm10p-Mdm12p complex is considered the mitochondrial counterpart of the kinetochore, the so-called mitochore that links the mt-nucleoid to the cytoskeleton.

The mitochore also participates in specialized mechanisms driving anterograde and retrograde mitochondrial motility, determining nucleoid transfer from mother to daughter cells [58]. The Arp2/3p complex controls the anterograde movement, stimulating the polymerization of actin filaments at the interface between actin cables and mitochondria [61]. In this respect, the mitochore continuously mediates the reversible, cyclic binding of mitochondria to actin cables, which are used as tracks for the organelle movement. Otherwise, retrograde movement occurs by passive transport of mitochondria towards the distal tip of the mother cell directed by the retrograde translocation of elongating actin used as 'conveyor belts' [62]. Retention of mitochondria at the bud tip requires an actin-independent mechanism involving the class V myosin Myo2p and the Rab-GTPase Ypt11p [63], whereas retention in the mother cell is an actin-dependent process mediated by Num1 and Dnm1 proteins [64]. However, many other organelle-inheritance driving proteins have not been classified and require further research. We can expect to find a huge number of them in forthcoming research, because sophisticated regulatory networks are needed to spatially and temporary coordinate organelle transport in the cell.

From the cell to species

The multilevel organization of mtDNA spans single molecules, macro-complexes, the organelle, and up to individual cells. The understanding of the mechanisms acting at each level and their interactions remains the biggest challenge not only for a mechanistic view of mitochondrial genome inheritance but also for the implications that the inheritance of different mitotypes might have on the cellular phenotype. As coordination between mitochondrial and nuclear genes is required for assuring mitochondrial functions, even subtle differences in mito-nuclear genotypes might lead to unintended functional changes on which natural or artificial selection can act on [1,3]. This integrated nuclear-mitochondrial system strictly depends on mito-nuclear crosstalk. Intergenomic signaling is a kind of mito-nuclear crosstalk found to downregulate nuDNA-encoded *COX* genes in the absence of mtDNA [65]. More recently, it was found that mtDNA, acting independently of the OXPHOS system, regulates the expression of other nuclear-encoded mitochondrial proteins [66]. Even if it is

Box 3. The case of interspecific hybrids

Mitochondrial transmission was also extensively studied using outbreeding among *Saccharomyces sensu stricto* species. Unlike the nuclear genome, which mixes two parental haplotypes, the mitochondrial genome is unilaterally inherited both in natural and artificial interspecific hybrids. Although the molecular mechanism driving uniparental segregation is unclear, mtDNA analysis from natural *S. cerevisiae*×*Saccharomyces bayanus* brewing hybrids revealed that the *S. cerevisiae*-like mtDNA is never inherited, suggesting a selective advantage to acquiring the *S. bayanus*-like mtDNA in a brewing environment. Natural *S. cerevisiae*×*Saccharomyces kudriavzevii* hybrids isolated from wine all inherited the *S. kudriavzevii*-like mtDNA. The hybrid CID1 isolated from cider showed a different origin for the mtDNA with regard to nuclear parental genomes (see [79] for a review).

Marinoni *et al.* [80] reported that *S. cerevisiae*-like mtDNA outcompetes the *S. bayanus*-like mtDNA and is preferentially transmitted to the progeny in artificial *S. cerevisiae*×*S. bayanus* hybrids. The preferential transmission of *S. cerevisiae*-like mtDNA in these hybrids was also observed by Lee *et al.* [69] and was correlated to different numbers of *ori* regions (eight in *S. cerevisiae* versus four in *S. bayanus*). However another study on 25 artificial *S. cerevisiae*×*Saccharomyces uvarum* hybrids also showed that *S. uvarum*-like mtDNA can be inherited [2]. Moreover, sibling hybrids from repeated crosses between the same parental pair inherit the same mitotype. These findings indicate that mitochondrial transmission might be strain-specific and dependent on the combination of parental mitotypes that compete in the context of a common nuclear background. Which of the two mtDNA molecules become fixed in hybrid progeny might hinge on the nuclear genotype and mito-nuclear interactions [2].

not clear how the mitochondrial genome can affect nuclear gene expression independent of respiration, it is possible that some mtDNA-encoded products are bifunctional and participate both in intergenomic signalling and respiration, similar to Aco1p or Atp6p, which are both involved in mtDNA stability as well as in the tricarboxylic acid cycle and in ATP synthesis, respectively [54,67]. This bifunctionality implies that mtDNA variation might result in significant metabolic and fitness outcomes. Epistatic interactions between nuclear and mitochondrial genomes on yeast fitness were inferred at the intraspecific level by observing how assorted combinations of mtDNA and nuDNA resulted in different growth abilities [1]. Other evidence was obtained through studying *S. cerevisiae* and *Saccharomyces uvarum* outcrossing (Box 3), where different mitotypes affect the fermentative and respiratory performance [2]. Hybrids bearing the *S. cerevisiae* mtDNA genome show an increased respiratory activity with regard to hybrids bearing *S. uvarum* mtDNA molecules.

From a coevolutionary perspective, one challenging question is whether nuclear-encoded proteins function equally well with different mitotypes and whether the resulting fitness determines reproductive barriers. Cybrids constructed from different *Saccharomyces* species suggest that mito-nuclear incompatibility is common among yeasts [68]. Recently, experiments based on chromosomal replacement lines demonstrated that incompatibility of nuclear and mitochondrial genomes could contribute to hybrid sterility [69]. Replacing the *S. bayanus AEP2* allele with the one from *S. cerevisiae* restored respiration and sporulation in sterile hybrids harboring *S. cerevisiae* mtDNA. Thus, incompatibility between nuDNA and mtDNA could result in reduction of OXPHOS performance and fitness and could be part of a mechanism leading to the formation of reproductive barriers. Coadaptation could be driven by mtDNA because mtDNA-encoded proteins show a higher substitution rate than their coevolving, nuDNA-encoded counterparts [70]. The dynamic nature of the mitochondrial genome probably needs a fast coevolution of nuDNA-encoded mitochondrial proteins, shaping the pattern of sequence divergence among *Saccharomyces* species [69]. The relaxation of nuclear-encoded mitochondrial proteins was demonstrated in post-WGD species [35]. Owing to its high variability and rearrangement-based plasticity, mtDNA has been proposed as a promising candidate for driving population divergence in budding yeasts [71].

Concluding remarks

Comparative mitogenomics and functional studies have recently revolutionized the knowledge about mtDNA organization, structure and inheritance, but the goal of understanding the mechanism of mtDNA transmission is getting closer. Studies on mtDNA segregation in *S. cerevisiae* suggest that mtDNA transmission is strictly related to mt-nucleoid sorting and whole-organelle inheritance and an integrated view is required at each hierarchical level to dissect the complexity of mitochondrial partitioning. Several mitochondrial proteins are multifunctional and take part in the OXPHOS pathway, as well as in mt-nucleoid organization, mtDNA transmission and mito-nuclear crosstalk, confirming this complex scenario. Future studies on global regulatory mechanisms underlying mtDNA inheritance should reveal the relationships between mitotypes, fitness and speciation in Hemiascomycetes.

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