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Schizosaccharomyces pombe as a fundamental model for research on mitochondrial gene expression: Progress, achievements and outlooks

Nhu Dinh  | Nathalie Bonnefoy 

Institute for Integrative Biology of the Cell (I2BC), Université Paris-Saclay, CEA, CNRS, 91198 Gif-sur-Yvette cedex, France

Correspondence

Nathalie Bonnefoy, I2BC, Université Paris-Saclay, CEA, CNRS, 91198 Gif-sur-Yvette cedex, France.
Email: nathalie.bonnefoy@i2bc.paris-saclay.fr

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Abstract

Schizosaccharomyces pombe (fission yeast) is an attractive model for mitochondrial research. The organism resembles human cells in terms of mitochondrial inheritance, mitochondrial transport, sugar metabolism, mitogenome structure and dependence of viability on the mitogenome (the *petite*-negative phenotype). Transcriptions of these genomes produce only a few polycistronic transcripts, which then undergo processing as per the tRNA punctuation model. In general, the machinery for mitochondrial gene expression is structurally and functionally conserved between fission yeast and humans. Furthermore, molecular research on *S. pombe* is supported by a considerable number of experimental techniques and database resources. Owing to these advantages, fission yeast has significantly contributed to biomedical and fundamental research. Here, we review the current state of knowledge regarding *S. pombe* mitochondrial gene expression, and emphasise the pertinence of fission yeast as both a model and tool, especially for studies on mitochondrial translation.

KEYWORDS

gene expression, gene fusions, mitochondrial biogenesis, mitoribosome, OXPHOS complexes, PPR proteins, *Schizosaccharomyces pombe*

Abbreviations: 5' RACE, 5' rapid amplification of cDNA ends; CoQ, coenzyme Q; Cryo-EM, cryo-electron microscopy; D-loop, displacement loop; dNTPs, deoxynucleotides triphosphate; EMSA, electrophoretic mobility shift assay; ETC, electron transport chain; GDP, guanosine diphosphate; GEF, G nucleotide exchange factor; GTP, guanosine triphosphate; *H. sapiens*, *Homo sapiens*; HMG, high mobility group; IMM, inner mitochondrial membrane; IP, immunoprecipitation; *K. lactis*, *Kluyveromyces lactis*; LSU, ribosomal large subunit; MIOREX, mitochondrial organisation of gene expression; mtDNA, mitochondrial DNA; mtRNA, mitochondrial RNA; nDNA, nuclear DNA; nt, nucleotide; OXPHOS, oxidative phosphorylation; Poly, DNA polymerase gamma; Pol ζ , DNA polymerase zeta; PPR, penta-tricopeptide repeat; RNase P, ribonuclease P; RNase Z^L, ribonuclease Z long; *S. cerevisiae*, *Saccharomyces cerevisiae*; *S. cryophilus*, *Schizosaccharomyces cryophilus*; *S. japonicus*, *Schizosaccharomyces japonicus*; *S. octosporus*, *Schizosaccharomyces octosporus*; *S. osmophilus*, *Schizosaccharomyces osmophilus*; *S. pombe*, *Schizosaccharomyces pombe*; ssDNA, single-stranded DNA; SSU, ribosomal small subunit; TAP, tandem affinity purification; UTR, untranslated region.

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1 | INTRODUCTION

Over the past 40 years, mitochondrial research has often employed *Schizosaccharomyces pombe* (fission yeast) as a complementary model to *Saccharomyces cerevisiae* (budding yeast), owing to several advantages. Firstly, molecular studies of *S. pombe* are supported by a well-maintained genetic and protein database (PomBase),¹ coupled with a single-gene deletion library (Bioneer),² a system to investigate bypassable gene essentiality,³ and more recently, a genetic engineering toolkit (POMBOX).⁴ Secondly, similar to budding yeast, fission yeast is experimentally tractable, possessing a short generation time (~3 h), and typically proliferating in a haploid state. However, *S. pombe* better resembles the mitochondrial physiology of *Homo sapiens* (Table 1).⁵ For example, mitochondria are uniparentally inherited, and moved along microtubules in both organisms.^{6,7} Regarding the fission yeast, mitochondrial transport utilises the tethering protein Mmb1 and the polymer dynamics, rather than the tubulin-associated protein motors as per human cells.⁷ Whilst, *S. cerevisiae* shows a biparental pattern of mitochondrial inheritance, and depends on actin filaments for mitochondrial trafficking.^{6,7}

In general, neither *S. pombe* nor human cells could proliferate in culture efficiently by galactose fermentation (Table 1). The energy metabolism of *S. pombe* adheres to the Crabtree effect, which is the glucose-dependent suppression of respiration in favour of fermentation for rapid

growth.⁸ When galactose is the sole carbon source, fission yeast could import the substrate using Ght2, and afterwards engage Gal1, Gal7 and Gal10 in the conversion of galactose to glucose-6-phosphate (the Leloir pathway).^{9,10} However, the general sugar transporter shows a relatively low affinity for galactose, and the galactose-assimilating genes are constitutively repressed. As a result, glucose-6-phosphate is produced from galactose in limited quantity, and must be both fermented and respired to generate sufficient energy for cellular proliferation. In comparison, despite similarly abiding by the Crabtree effect, *S. cerevisiae* is capable of efficient galactose fermentation.^{8–10}

Fission yeast also parallels human cells concerning the mitogenome (mitochondrial DNA, mtDNA) size, structure and dependence (Table 1). Both *S. pombe* and human mtDNAs are compact, and possess short intergenic regions, and few or no introns.^{11,12} Transcription of these genomes produce only a few polycistronic transcripts, in which rRNAs and mRNAs are virtually always punctuated by tRNAs, and contain short to no untranslated regions (UTRs). Furthermore, *S. pombe* and humans are generally *petite*-negative, requiring the mtDNAs for viability (though the contrary could be obtained in certain genetic backgrounds or under specific laboratory conditions).^{13–15} In contrast, the *S. cerevisiae* mitogenome is four- to five-fold larger, riddled with non-coding sequences, and could be lost under certain conditions (the *petite*-positive phenotype).^{16,17} A recent transcriptomics study on this species revealed the presence of

TABLE 1 Comparison of mitochondrial-related features between yeasts and humans.

	<i>S. cerevisiae</i>	<i>S. pombe</i>	<i>H. sapiens</i>
Meiotic inheritance	Biparental	Uniparental	Uniparental
Mitochondrial transport	Actin filaments	Microtubules	Microtubules
Efficient galactose fermentation	Yes	No	No
mtDNA			
Size	~86 kb	~19 kb	~17 kb
Origins of replication	8	Unknown	2
Transcription units	14	3	2
Introns	≤ 13	≤ 8	Absent
5' UTRs	50–1100 bp	40–220 bp	Mostly absent
3' UTRs	10–700 bp	10–30 bp	Mostly absent
<i>Petite</i> -positive or -negative	Positive	Negative	Negative
Nucleoid	Yes	Yes	Yes
Gene expression foci	MIOREX	Unknown	RNA granules
Complex I	No	No	Yes
	Only Ndi1, Nde1 and Nde2	Only Ndi1 and Nde1	
Major CoQ type ¹²⁸	CoQ ₆	CoQ ₁₀	CoQ ₁₀

Note: Other references are mentioned in text; bp, base pairs.

14 active mitochondrial promoters, over 10 times the numbers recorded for the *S. pombe* and human mtDNAs.¹⁸

Considering the aforementioned resemblance to human cells, fission yeast is an attractive model for studies on mitochondrial biogenesis and diseases. Here, we review the progress in establishing such model organism, by (i) summarising our current understanding of mitochondrial gene expression in *S. pombe*, (ii) comparing and contrasting it to the *S. cerevisiae* and human system and (iii) offering critical perspectives on ambiguous areas of knowledge. We also appreciate the contributions of *S. pombe* to, and further accentuate its potential in, fundamental and therapeutic research relating to mitochondria.

2 | MITOCHONDRIAL DNA STRUCTURE AND REPLICATION

2.1 | Despite containing similar gene content to other yeasts, *Schizosaccharomyces pombe* mtDNA is exceptionally compact

The complete sequence of the *S. pombe* genome was published in 2002, encompassing three linear nuclear

chromosomes and one circular mtDNA.¹¹ Annotations of the mtDNA were first made available in 2005, and has since been refined (Figure 1).^{19,20} The mitogenome of fission yeast encodes seven subunits of the OXPHOS complexes (Cytb, Cox1, Cox2, Cox3, Atp6, Atp8 and Atp9), three components of the mitoribosome (Urfa, *rnl* and *rns*), 25 tRNAs and a ribonuclease P (RNase P) RNA (*rnpB*). Similar gene content is observed amongst the five *Schizosaccharomyces* species, with the absence of (i) *rnpB* in *S. japonicus*, and (ii) *trnI(cau)* in *S. cryophilus*, *S. octosporus* and *S. osmophilus*.²¹ However, the mitogenome of fission yeast shows only partial synteny with those of the other *Schizosaccharomyces* species, and is the most compact owing to short intergenic regions. Depending on the strain under study, the *S. pombe* mtDNA could contain up to eight introns, distributed among *cytb*, *cox1* and *cox2* (Table S1).²⁰ For instance, the reference strain (972 h⁻) possesses only *cytb-II*, *cox1-I1b* and *cox1-I2b* (Figure 1),^{19,20} whose successive deletions generated a mitochondrial intron-less strain.²²

The mtDNA gene content of *S. pombe* resembles that of *S. cerevisiae*, though the prior contains one additional tRNA sequence (Figure 1).^{11,16} In contrast, the human mtDNA encodes 13 subunits of the OXPHOS complexes (ND1, ND2, ND3, ND4, ND4L, ND5, ND6, CYTB, COX1, COX2, COX3, ATP6 and ATP8), only the rRNA

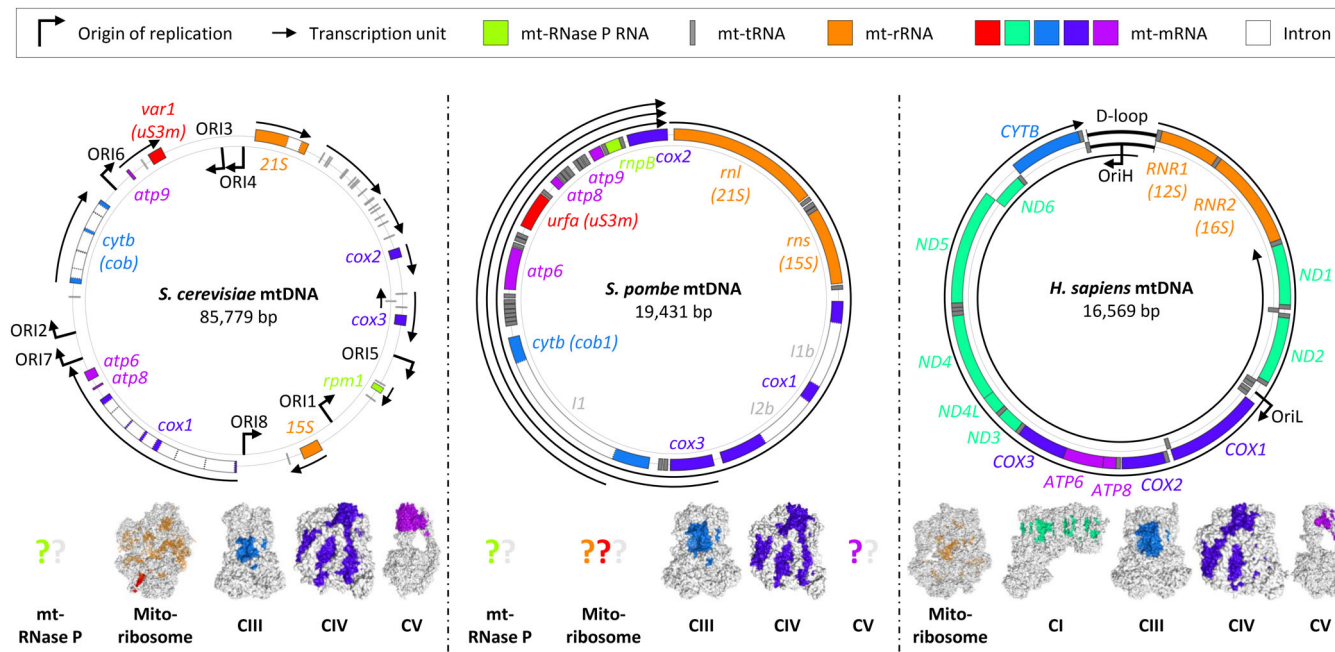


FIGURE 1 Reference mitogenomes of *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* and *Homo sapiens*. Each mitogenome encodes a variable set of mt-tRNAs, and one or several components of the mitochondrial complexes shown beneath. The mitochondrial-encoded RNA and/or proteins are coloured accordingly. Question mark (?), unresolved structure; mt-RNase P, mitochondrial ribonuclease P; CI-V, complexes I-V. *S. cerevisiae* S288C mtDNA (NC_001224),¹⁶ mitoribosome (5MRC),⁷⁶ complex III (1KYO),¹³⁵ complex IV (6YMY)¹³⁶ and complex V (6CP6)¹³⁷; *S. pombe* 972 h⁻ mtDNA (NC_001326),¹⁹ complex III (8Q1B)¹²¹ and complex IV (8C8Q)¹²²; *H. sapiens* mtDNA (NC_012920),¹² mitoribosome (3J9M),⁶⁷ complex I (5XTD),¹³⁸ complex III (5XTE), complex IV (5Z62)¹³⁹ and complex V (8H9T).¹⁴⁰

components of the mitoribosome (*RNR1* and *RNR2*), 22 tRNAs and no RNase P RNA.¹² Distinctively, coding regions are localised to a single mtDNA strand in *S. pombe*, rather than both strands as per *S. cerevisiae* and humans.^{12,16,20} Moreover, although mitochondrial nucleoids have been observed in fission yeast,²³ no apparent mtDNA-packaging protein has been recognised (Table 1).¹ Whilst, *S. cerevisiae* and human nucleoids are respectively maintained by the high mobility group (HMG)-box proteins Abf2 and TFAM, the latter of which also functions in mtDNA transcription.^{24,25}

2.2 | *S. pombe* mtDNA replication abides by the rolling circle model

To initiate rolling circle replication, a single-stranded DNA (ssDNA) invades the circular mitogenome and commences leading-strand synthesis.^{26,27} Simultaneously, the lagging strand is generated as ligated Okazaki fragments. Repeated DNA synthesis produces a linear molecule of several genomic units fused head-to-tail, which may undergo homologous recombination to regenerate the circular genome. The initial ssDNA could result from a previous replication cycle, or from end resection after a double-stranded break. The rolling circle model was supported by the observation that upon pulsed field gel electrophoresis, the *S. pombe* mtDNA appeared primarily as linear molecules of ~5 genome units in vivo.²⁶ Moreover, via two-dimensional agarose gel analysis, the predominant replication intermediates were simple Y-shaped replication forks, contrary to bacterial replication bubbles and human mtDNA displacement loop (D-loop). The *S. cerevisiae* mitogenome is also replicated according to the rolling circle mechanism, while human mtDNA replication emulates the strand displacement model under normal conditions, and the rolling circle mode upon oxidative stress.²⁷ No mitochondrial origins of replication have been published in *S. pombe*, in contrast to the extensively mapped sequences in the other two species (Figure 1).

However, the three organisms consistently recruit the cruciform-endonuclease Cce1, the ssDNA binding protein Rim1/SSBP1 and/or the DNA polymerase gamma (Poly) for mtDNA replication. Firstly, both the fission and budding yeast require Cce1 for the resolution of Holliday junction during homologous recombination.^{28,29} The human homologue of these proteins has not been identified.^{1,30} Secondly, *S. pombe* Rim1 and its homologues (*S. cerevisiae* Rim1 and human SSBP1) have each been demonstrated to interact with ssDNA in vitro and form homotetramers in vivo.^{25,29,31} Thirdly, the incorporation of deoxynucleotide triphosphates (dNTPs) to the

growing mtDNA requires Poly, whose catalytic subunit is named Pog1, Mip1 and POLG in *S. pombe*, *S. cerevisiae* and humans, respectively.^{25,29,32} Surprisingly, the deletion of *pog1* in haploid *S. pombe* was stably obtained by tetrad dissection of a Δ *pog1/pog1* hetero-diploid, that is, *pog1* did not appear essential to viability.³² The haploid strain showed a near abolition of fermentative growth, abnormal cellular and mitochondrial morphology, and minimal mtDNA presence. The authors suggested that the basal mitogenome number could have been carried over from the *pog1*/ Δ *pog1* mother cell. However, upon prolonged incubation, the Δ *pog1* strain continued to proliferate. We proposed that suppressor mutations might have occurred, and produced *petite*-positive revertants. Alternatively, mtDNA replication could be feebly maintained by other DNA polymerases. In *S. cerevisiae*, the DNA polymerase zeta (Pol ζ) contributes to both nuclear and mitochondrial genome maintenance.³³ Each subunit of the Pol ζ heterotrimer (Rev3, Rev7 and Rev1) has an orthologue in *S. pombe*,¹ and such functional duality was also observed for one composition (REV3L) of the human Pol ζ .³³

3 | MAINTENANCE OF MITOCHONDRIAL DNA INTEGRITY

3.1 | Mitogenome integrity is vital to *S. pombe*, a *petite*-negative yeast

Mitochondria are essential for various cellular processes other than respiration. To fulfil such functions, mitochondria must import nuclear-encoded proteins from the cytoplasm, which requires the electrochemical gradient across the inner mitochondrial membrane (IMM).^{17,34} In wild-type *S. pombe* and *S. cerevisiae*, the gradient is accomplished by the OXPHOS complexes III and IV, which pump protons from the mitochondrial matrix to the intermembrane space, and complex V, which utilises the electrochemical gradient to phosphorylate ADP. The yeast mtDNAs encode core components of the former two complexes, alongside three subunits in the transmembrane (F_0) domain of complex V (Figure 1). As a *petite*-negative species (briefly discussed in 'Introduction'), *S. pombe* must maintain the mitogenome integrity to ensure functional OXPHOS complexes, thereby sustaining the IMM potential and supporting life. On the contrary, in *S. cerevisiae* (a *petite*-positive yeast), the electrochemical gradient could also be achieved via the coupling between (i) the antiporter Pet9, which imports ATP into and exports ADP out of the mitochondria, and (ii) the hydrophilic (F_1) domain of complex V, which instead runs in reverse and hydrolyses ATP. As expected,

when both components are functional, mutations in the electron transport chain (ETC) often produce IMM potential comparable to wild-type.³⁵ Upon either Pet9 or F₁ domain malfunction, budding yeast becomes *petite*-negative. For example, the deletion of *YME1* (encoding the catalytic subunit of a mitochondrial protease complex) resulted in a build-up of Inh1 (the F₁ ATPase inhibitory factor), ultimately disrupting the electrochemical gradient in the absence of the mitogenome.³⁶

The orthologues of *S. cerevisiae* Pet9, Yme1 and Inh1 have been identified in *S. pombe*, respectively referred to as Anc1, Yme1 and Inh1.¹ *S. pombe* Anc1 complemented for the loss of Pet9 in *S. cerevisiae*.³⁷ Interestingly, the expression of *S. cerevisiae* *YME1* in *S. pombe* converted the *petite*-negative yeast to *petite*-positive.³⁸ Spontaneous conversions of *S. pombe* to *petite*-positive have also been obtained, termed *ptp1-1* (for *petite*-positive), *ptp2-1*, *rzl4-1* (for *rho zero lethality*), *rzl5-1* and *rzl5-1*.^{13,14} Our unpublished data suggests that some of the *rzl* mutations resided in the F₁ subunits Atp2 and Atp3. The remaining strains contain as yet uncharacterised nuclear mutations. Taken together, the *petite*-negative property of *S. pombe* appears to arise from the F₁ domain, more specifically from its inability to hydrolyse ATP when the mitochondrial-encoded F₀ domain is absent. Consistently, *Kluyveromyces lactis* (similar to fission yeast in terms of mtDNA protein-coding capacity and dependence) could be converted to *petite*-positive by boosting F₁ ATP hydrolysis, induced upon *inh1* deletion, F₁ subunit mutations, or a combination of both.³⁹ Based on these observations, the two-component hypothesis for *petite*-negativity was proposed: *S. pombe* (and *K. lactis*) must possess the mitogenome to build a functional ETC and an F₁-F₀ assembled complex V, either of which is required to maintain the IMM potential and support viability.³⁴

3.2 | Some of the *S. pombe* mtDNA repair factors are shared with the nucleus

Eukaryotic mitogenomes are prone to mutations, deletion and rearrangements. In fission yeast, several mtDNA repair proteins have been recognised either in vitro (Cmb1⁴⁰) or in vivo (Cce1²⁸ and Uve1⁴¹). *S. pombe* Cmb1 is homologous to *S. cerevisiae* Abf2 and human TFAM (previously mentioned in ‘Mitochondrial DNA structure and replication’), and to date, remains as the only HMG-box protein in *S. pombe* mitochondria.¹ Other candidate factors include the mitochondrial isoform of Pfh1 and Exo5. Specifically, the *S. pombe* *pfh1* and *exo5* mRNA each produce a nuclear- and a mitochondrial-targeted protein via alternative translation initiation.^{42,43} The former

isoforms are firmly established to participate in nuclear DNA (nDNA) replication and/or repair. Whilst, the mitochondrial counterparts have been inferred by mutant studies to maintain the mitogenome, though the specific stage(s) of action remain(s) ambiguous. In budding yeast, one of the two homologues of *S. pombe* Pfh1 (termed Pif1) is also present in vivo as a nuclear- and a mitochondrial-localised factor, the latter of which functions in mtDNA replication and repair.²⁹ On the contrary, a single PIF1 in humans contributes to both nDNA and mtDNA replication, while *S. cerevisiae* Exo5 and human EXO5 are solely and respectively involved in mtDNA replication and nDNA repair.^{29,43}

4 | MITOCHONDRIAL TRANSCRIPTION

4.1 | *S. pombe* mtDNA transcription employs universally conserved promoters and proteins

Transcription of the *S. pombe* mitogenome starts at three promoters of increasing initiation efficiency, termed intronic (P_{in}, ATATATGTG), minor (P_{mi}, TTATATGTG) and major (P_{ma}, ATATATGTA) (Figures 1 and 2).^{19,44} These are localised to a single mtDNA strand, and situated upstream of the *rnl* gene, upstream of the *cox3* gene and at the start of the *cytb-11* intron, respectively. The positions of the three promoters were established by in vitro capping of the primary transcripts with Vaccinia virus guanylyl transferase, in the presence of radioactively labelled GTP, and subsequently confirmed via 5' rapid amplification of cDNA ends (5' RACE). Across *S. pombe*, *S. cerevisiae* and humans, the mitochondrial promoter sequences are highly conserved.^{18,19,45} However, their number and strand distribution vary (Figure 1). The human mitogenome contains two promoters, each located to a different strand.⁴⁵ In contrast, 14 mitochondrial promoters have been detected in budding yeast.¹⁸ These show considerably skewed strand dispersal, and three promoters (immediately upstream of ORI2, ORI3 and ORI5) solely synthesise primers for mitogenome replication, that is, they do not generate transcripts.

S. pombe mtDNA transcription requires the RNA polymerase Rpo41, and at least the transcription factor Mtf1.⁴⁴ Similar to the Δ *pog1/pog1* strain (discussed in ‘Mitochondrial DNA structure and replication’), the Δ *rpo41/rpo41* and Δ *mtf1/mtf1* heterozygous diploids could be generated. However, upon tetrad dissection, neither the Δ *rpo41* nor Δ *mtf1* haploid survived after a few division cycles, that is, viability necessitates a functional copy of each gene. During the short proliferation period,

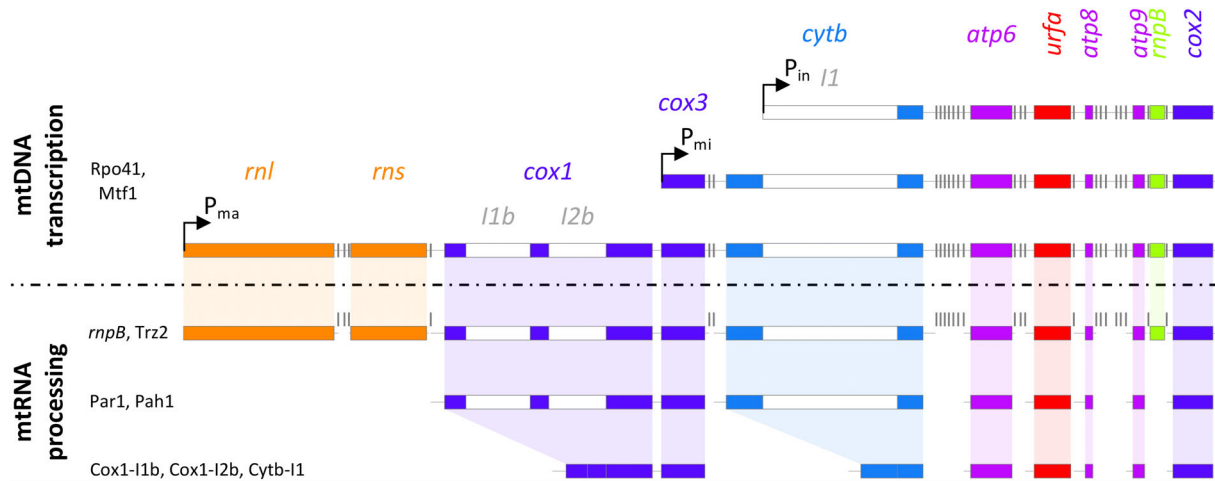


FIGURE 2 *Schizosaccharomyces pombe* mtDNA transcription and mtRNA processing. Transcriptions could be initiated from any of the three promoters, albeit at varying efficiency ($P_{in} < P_{mi} < P_{ma}$). The resulting transcripts are polycistronic, comprising of several mtRNA species (coloured as per Figure 1). Subsequent processing is only shown for the most abundant primary transcript (P_{ma}), and involves the endoribonucleolytic cleavage of mt-tRNAs according to the tRNA punctuation model, the 3' exoribonucleolytic cleavage of mt-mRNAs and the splicing of any introns. For each stage in mtDNA transcription and mtRNA processing, known factors are indicated (more might be uncovered in the future). The mitochondrial 3'-5' exoribonuclease Par1 (Rpm1, SPCC1322.01) should not be confused with the cytoplasmic serine/threonine phosphatase regulatory subunit Par1 (Pbp1, SPCC188.02).

the cells exhibited distorted shape, abnormal septation, minimum IMM potential and dramatically reduced mt-mRNAs. Electrophoretic mobility shift assay (EMSA) confirmed the specific binding of Rpo41 and Mtf1 to P_{in} , P_{mi} and P_{ma} . Promoter binding and transcription in vitro was most efficient when both proteins were present, emphasising the importance of Mtf1 in mitochondrial transcription initiation, and suggesting a direct interaction between *S. pombe* Rpo41 and Mtf1. To date, no experimental results are available on the structures and in vivo functions of these proteins. Concerning *S. cerevisiae* and humans, transcriptions of the mitogenomes respectively recruit Rpo41 and POLRMT (the homologues of *S. pombe* Rpo41), alongside Mtf1 and TFB2M (the homologues of *S. pombe* Mtf1), among others.^{19,45} In each organism, the RNA polymerase has been visualised by cryo-electron microscopy (cryo-EM), and demonstrated to bind the corresponding transcription factor.

Based on AlphaFold2 prediction and cryo-EM structures (when available), the conformation of *S. pombe* Rpo41 and its homologues (*S. cerevisiae* Rpo41 and human POLRMT) are rather conserved.^{19,45,46} Each protein contains an N-terminal extension (NTE) of unknown origin, and a C-terminal catalytic core. A few pentapeptide repeat (PPR) motifs are also present, connecting the two protein domains. The NTE of *S. cerevisiae* Rpo41 has been inferred in mtDNA maintenance, among other functions.^{19,45} Interestingly, a potential interaction between *S. pombe* Rpo41 and the mtDNA repair protein Pfh1 (discussed in 'Maintenance of Mitochondrial DNA

Integrity') has been indicated.⁴⁷ Specifically, upon immunoprecipitation (IP) of Pfh1, Rpo41 was recovered as a co-enrichment by mass spectrometry. However, no confirmatory experiment (e.g. co-IP coupled with Western blot) was conducted, and the binding region on the RNA polymerase (if present) remains to be explored.

5 | MITOCHONDRIAL RNA PROCESSING AND STABILISATION

5.1 | The mt-tRNA punctuation model applies to *S. pombe* and humans

For fission yeast and humans, mtDNA transcription generates two to three large polycistronic transcripts, in which mitochondrial RNase P RNA (*S. pombe* only), mt-rRNAs and mt-mRNAs are virtually always interspersed with mt-tRNAs^{19,44} (Figures 1 and 2.). Consequently, mt-tRNA excision could liberate nearly every constituent from the primary transcripts. This is termed the mt-tRNA punctuation model, and in contrast to the clustered mt-tRNA system observed in budding yeast. Regarding *S. pombe*, the model has been confirmed independently via primer extension and mitochondrial transcriptomics.^{19,48} The former experiment revealed the precise correspondence of each mt-rRNA/mt-mRNA 5' end to the upstream mt-tRNA 3' end. Subsequently, mtRNA sequencing confirmed these observations, and further showed the coincidence between both ends of *rnpB* mRNA and the cleavage sites

of its flanking mt-tRNAs. As exceptions to the mt-tRNA punctuation model, the *cox1-cox3* and *cox2-rnl* intergenic regions are devoid of mt-tRNAs. However, these regions respectively contain P_{mi} and P_{ma} , from which transcriptions compensate for the absence of mt-tRNA processing.

In general, the 5' endoribonucleolytic cleavage of mt-tRNAs is conducted by mitochondrial RNase P, a group of functionally conserved enzyme complexes, albeit compositionally diverse.⁴⁹ Concerning *S. pombe*, homology searches reveal the presence of a mitochondrial-encoded RNase P RNA (*rnpB*).¹⁹ Neither the function of this RNA, nor any protein component of the mitochondrial RNase P has been characterised. The homologues of *rnpB* are present across the *Schizosaccharomyces* taxa, except for *S. japonicus*.²¹ Meanwhile, *S. cerevisiae* mitochondrial RNase P is formed by the mitochondrial-encoded ribozyme *rpm1* (homologous to *S. pombe rnpB*⁵⁰) and the nuclear-encoded PPR protein Rpm2 (no clear *S. pombe* orthologue¹).⁵¹ Whilst, human mitochondrial RNase P does not contain an RNA component, instead comprising three protein subunits: TRMT10C (MRPP1), HSD17B10 (MRPP2) and the catalytic PPR protein PRORP (MRPP3), none of which possesses homologues in the budding or fission yeast.

In contrast to mitochondrial RNase P, members of the mitochondrial long form of ribonuclease Z (RNase Z^L) are both structurally and functionally conserved.⁴⁹ These are referred to as ELAC2 in humans, Trz1 in *S. cerevisiae* and Trz2 in *S. pombe*. While human ELAC2 and *S. cerevisiae* Trz1 carry out 3' processing of both nuclear and mitochondrial tRNAs, *S. pombe* Trz2 strictly functions in the mitochondria.⁵² A mutation in *trz2* resulted in severely impaired 3' mt-tRNA processing, coupled with a build-up of mt-mRNAs.⁴⁸ 5' maturation of mt-tRNAs was also affected, albeit to a lesser extent. This had previously been reported in budding yeast, and might be due to reduced 3' processing of the mt-tRNA directly preceding *RPM1* (or *rnpB* for *S. pombe*). Taken together, these observations have (i) confirmed the function of *S. pombe* Trz2 in 3' mt-tRNA processing, (ii) supported the tRNA punctuation model and (iii) implicated the involvement of *rnpB* in the mitochondrial RNase P machinery.

5.2 | *S. pombe* mt-mRNAs further undergo 3' exonucleolytic cleavage

As mt-tRNAs are cleaved from the primary transcripts, the liberated mt-mRNAs each contain a 3' C-core motif, which appears to be the signal for downstream cleavage by the mitochondrial 3'-5' exoribonuclease Par1 (Rpm1), and the mitochondrial DExH/D box RNA helicase Pah1

(Rpm2).^{19,48,53} Amongst eukaryotes, DExH/D box RNA helicases play crucial roles in both nuclear and mitochondrial RNA metabolism.⁵⁴ Concerning *S. pombe*, the absence of Par1 resulted in the accumulation of mt-mRNAs with unprocessed 3' ends, extending up to the RNase P cleavage site.⁵³ On the contrary, *pah1* deletion brought about random end truncations. Taken together, these results have demonstrated (i) the essential function of Par1 as 3' mt-mRNA exoribonuclease, and (ii) the support role of Pah1 in mt-mRNA end protection, C-core motif recognition and/or the loading of mt-mRNAs onto Par1. Moreover, neither *par1* nor *pah1* overexpression could complement for the absence of Pet127 (an mtRNA catabolic factor conserved in lower eukaryotes), and *vice versa*. Therefore, *S. pombe* Par1 and Pah1 are probably not involved in mtRNA degradation, contrary to their homologues in *S. cerevisiae* (Dss1 and Suv3) and humans (SUPV3L1).⁵⁴ Apart from *S. pombe* Pet127, a possible mtRNA catabolic factor is Pnu1, previously demonstrated to degrade ssDNA and RNA *in vitro*.⁵⁵

5.3 | *S. pombe* mt-mRNA introns are generally spliced by mitochondrial intron-encoded proteins

As mentioned previously, the *S. pombe* *cytb*, *cox1* and *cox2* genes could each contain one or more introns (Table S1).^{20,56} These are classified as either group I or II, based primarily on the predicted secondary structures. The prior group resides exclusively in the *cox1* gene. Except for the recently discovered *cox1-I1b'*, of which no experimental results have been published, mitochondrial group I introns are incapable of autocatalytic splicing *in vitro*. Instead, *cox-I1b* and *cox-I2b* contain reading frames, and translations from the start codon of the upstream exon could yield intron maturases (Figure 2.). The essentiality of these proteins for mitochondrial splicing has been demonstrated by mutant studies. Specifically, defective *cox1* mRNA translation always resulted in the accumulation of un-spliced *cox1* (see 'Mitochondrial specific translation and early complex assembly').⁵⁷⁻⁵⁹ In addition, the intron-encoded proteins participate in homologous (homing) and ectopic insertion (retrotransposition) of the intron sequences, thereby contributing to evolution *via* horizontal intron transfer.^{20,56} For the remaining group I introns, the mechanism of splicing remains ambiguous: the reading frame of *cox1-I1b'* is out-of-frame with the upstream exon, while *cox1-I2a* and *cox1-I3* appear to be non-coding.

On the contrary to those from group I, mitochondrial introns of group II are distributed among *cytb*, *cox1* and *cox2*. However, these also appear to encode proteins with

maturase, endonuclease and reverse transcriptase activities, respectively responsible for intron splicing, homing and retrotransposition. Autocatalytic splicing of *cytb-I1* has been refuted in vitro, while that of other group II introns remains to be explored.²² Recently, *via* mass spectrometry, a polypeptide uniquely corresponding to *cytb-I1* has been unambiguously detected, and shown to enrich among mitoribosomal proteins and mitochondrial translational activators (Supplementary Data in⁵⁷ and unpublished). On the one hand, such observation confirmed the in vivo translation of the *cytb-I1* reading frame. Furthermore, because *cytb-I1* mRNA locates at the beginning of the P_{in} primary transcript (Figure 2.), its translation might employ an internal initiation site, rather than an upstream start codon as per *cox-I1b* and *cox-I2b*. On the other hand, a potential interaction between *Cytb-I1* and the mitochondrial translation machinery suggests a functional coupling between mt-mRNA splicing and translation.

5.4 | *S. pombe* mtRNA modification is poorly understood

After Trz2-mediated endoribonucleolytic cleavage, to produce mitochondrial aminoacyl-tRNAs, the free 3' ends of mt-tRNAs are joined to a CC series by Cca1, appended with an A by Cca2 and eventually attached to a specific amino acid *via* the appropriate aminoacyl-tRNA ligase.⁶⁰ Based on sequence homology, 38 aminoacyl-tRNA ligases have been recognised in fission yeast, among which 17 are mitochondrial-specific, and five probably localised to both the mitochondria and cytoplasm (Table S2).¹ On the one hand, similar to humans and unique among yeasts, *S. pombe* contains two valyl-tRNA ligases, each functioning in a specific cellular compartment.⁶¹ On the other hand, fission and budding yeast might resemble one another regarding mitochondrial glutamyl-tRNA synthesis. Neither species contains a specific mitochondrial glutamyl-tRNA ligase.^{1,62} Instead, *S. cerevisiae* mitochondria first generate glutamyl-tRNA using Mse1, then trans-amidate the molecule to glutamyl-tRNA *via* the Her2/Pet112/Gtf1 complex.⁶² The homologues of these factors in *S. pombe*, respectively termed Mse1 and Gta1/2/3, are functionally uncharacterised.¹

Sequence homology has also revealed the presence of several mt-tRNA/mt-rRNA methyltransferases and pseudo-uridine synthases, potentially involved in specific mtRNA base modifications.¹ These processes have yet to be investigated in *S. pombe*, albeit well established in other eukaryotes. Mature mt-mRNAs in fission yeast are subjected to neither 5' 7-methylguanylate capping nor 3' polyadenylation, consistent with those of budding

yeast.^{19,48} On the contrary, 3' poly(A) tails have been reported in human mature mt-mRNAs.

5.5 | Several *S. pombe* penta-tricopeptide repeat proteins are involved in mtRNA-specific stabilisation

PPR proteins constitute a family of RNA-binding factors ubiquitous amongst eukaryotes.^{63,64} Regarding fission yeast, 12 members have been identified by domain prediction algorithms.^{57,58,65} Each member contains between two (for Rpo41) and at least 16 PPR motifs (Ppr5). Increasing the number of PPR motifs enables the recognition of longer RNA sequences, ultimately improving the binding specificity. So far, *S. pombe* PPR proteins reside exclusively in the mitochondria, where they contribute to transcription (Rpo41; previously discussed in 'Mitochondrial transcription'), mt-rRNA and mt-mRNA stabilisation (Ppr3, Ppr1, Ppr7 and Ppr6; addressed here) and translation (see 'Mitochondrial general translation factors' for Ppr10, Ppr2 and Ppr5; see 'Mitochondrial specific translation and early complex assembly' for Ppr4, Mtf2 and Cbp8). One member (Ppr8) remains functionally ambiguous.⁵⁸

S. pombe Ppr3 has been demonstrated to preserve 15S rRNA, consistent with its homologue in *S. cerevisiae* (Ccm1 or Dmr1).⁶³ The absence of *ppr3* in *S. pombe* mitochondria was associated with severe respiratory deficiency, a strong decrease in full-length (coupled with an accumulation of degraded) 15S rRNA and negligible mitochondrial translation.⁵⁸ There was also visible reduction in the remaining mtRNAs, especially *cytb* mRNA. These results suggested that (i) Ppr3 plays an important role in mt-SSU biogenesis and (ii) the protein might possess more than one mtRNA targets. Similarly, *S. cerevisiae* Ccm1 participates in both 15S rRNA stabilisation, and *cytb-b14* and *cox1-a14* intron splicing.⁶⁶ On the contrary, the closest human homologue of *S. pombe* Ppr3 is PTCD3 (mS39), a component of the mt-SSU.^{58,67}

Several of the other PPR members have been demonstrated to stabilise mt-mRNAs. These are Ppr1 (maintaining *cox2* and *cox3* mRNAs), Ppr7 and Ppr6 (stabilising *atp6* and *atp9* mRNAs, respectively). The absence of *ppr1* resulted in severe respiratory growth defect, major reductions in *cox2* and *cox3* mRNA and no detectable translation of these species.⁵⁸ Whilst, *S. pombe* deleted for either *ppr7* or *ppr6* showed sensitivity to antimycin A and minimal respiratory growth. Because antimycin A inhibits complex III, the former phenotype indicated a complex V defect, as per the two-component hypothesis (previously discussed in 'Maintenance of mitochondrial DNA integrity').³⁴ Furthermore, *atp6* mRNA was barely detectable

in the *Δppr7* strain, and *atp9* mRNA could not be visualised by Northern blot upon *Δppr6*. In both mutants, the remaining mt-mRNAs were generally decreased (including *urfa*), and the synthesis of every mitochondrial-encoded protein was downgraded. These might be secondary defects, resulting from complex V dysfunction. To date, the homologues of *S. pombe* Ppr1 and Ppr7 in *S. cerevisiae* have not been identified. Meanwhile, the homologue of *S. pombe* Ppr6 in *S. cerevisiae* (*Aep2*) also functions in *atp9* mRNA stabilisation.⁶³

6 | THE MITORIBOSOME

Given the endosymbiotic origin of membrane-bound organelles, it comes as no surprise that the mitochondrial translation machinery generally resembles the prokaryotic (rather than the eukaryotic cytoplasmic) system. For example, various antibiotics could inhibit both the organellar and the bacterial ribosomes, while showing no effects against the eukaryotic cytosolic counterpart.⁶⁸ The majority of general translational factors are also conserved between mitochondria and prokaryotes (see ‘Mitochondrial general translation factors’).

However, through evolution, the mitochondrial translation machinery has accumulated unique features. Foremost, the mitoribosome possesses a higher protein:rRNA mass ratio (Table 2). Secondly, because the bacterial Shine–Dalgarno sequence is absent in mt-mRNAs, mitochondria must adopt other strategies to initiate translation (e.g. translational activators, see ‘Mitochondrial specific translation and early complex assembly’). Thirdly, certain mitochondrial codons deviate from the universal genetic code (Table 3). Surprisingly, such difference is minimal in *S. pombe*: the TGA codon encodes for tryptophan in the yeast

mitochondria, instead of the universal ‘stop’. Consequently, upon a relocation of the mitochondrial *urfa* gene to the nucleus, the TGA codon must be replaced by TGG to produce a functional protein.⁶⁹ However, TGA only occurs in the *urfa* and intronic transcripts, that is, rarely compared to the other tryptophan codon (TGG).⁷⁰ The sole mitochondrial *trnW(cca)* has been proposed to recognise both TGA and TGG, albeit at varying efficiency.

6.1 | The majority of universal mitoribosomal proteins bear sequence homologues in *S. pombe*

No structural information is currently available for the *S. pombe* mitoribosome. However, in collaboration with PomBase, we have managed to assemble a comprehensive list of putative proteins, based on (i) sequence homology with the human and/or *S. cerevisiae* mitoribosomal constituents, and (ii) in vivo interactions with the *S. pombe* translational activator Mrh5 (see ‘Mitochondrial specific translation and early complex assembly’) (Table S3).^{1,57} No homologue has been identified for two *S. cerevisiae* and 19 human mitoribosomal proteins. Structural and functional studies might retrieve more divergent orthologues, alongside any *Schizosaccharomyces*-specific components. So far, only four proteins of the *S. pombe* mitoribosomal small subunit (mt-SSU), and one of the mitoribosomal large subunit (mt-LSU) have been experimentally explored. Respectively, these are termed Urfa (uS3m), Bot1 (mS45), Mrp51 and Mug178 (bS1m isoforms) and Aco2-Mrp149 (Aco2-bL21m) tandem fusion protein.

Urfa is the only mitoribosomal protein encoded by the mtDNA (Figure 1). The function of Urfa in mitochondrial

TABLE 2 Overall components of the bacterial and mitochondrial ribosomes.

	<i>E. coli</i>	<i>S. pombe</i>	<i>H. sapiens</i>	<i>S. cerevisiae</i>
SSU				
Total number of proteins	21	≥33	30	34
Of which mitochondrial-specific	-	12	14	14
Small rRNA (nt)	1542	1421	954	1648
LSU				
Proteins	33	≥46	52	46
Of which mitochondrial-specific	-	15	23	16
Large rRNA (nt)	3024	2827	1559	3294
Other RNA	-	?	tRNA ^{Val/Phe}	-

Note: Cryo-EM structures have been published for the cytosolic ribosome of *E. coli*,¹²⁹ and the mitoribosomes of *H. sapiens*^{67,130} and *S. cerevisiae*.⁷⁶ In contrast, the *S. pombe* mitoribosomal composition was solely inferred by sequence homology (see Table S3).

Codon	Universal	Mitochondrial		
		<i>S. pombe</i>	<i>H. sapiens</i>	<i>S. cerevisiae</i>
AUA	Isoleucine	Isoleucine ^a	Methionine	Methionine
CTN	Leucine	Leucine	Leucine	Threonine
TGA	Stop	Tryptophan	Tryptophan	Tryptophan
UAA/UAG	Stop	Stop ^b	Stop	Stop
AGA/AGG	Arginine	Arginine	Stop	Arginine

Note: Bold, codons specific to mitochondria.

^a*S. pombe* mitochondria contain *trnI(cau)* and *trnI(gau)*, the prior of which decodes the AUA codon as isoleucine.²¹ A mitochondrial *trnI(cau)* is absent in *S. cryophilus*, *S. octosporus*, *S. osmophilus*,²⁰ *S. cerevisiae*¹⁶ and *H. sapiens*.¹²

^bIn *S. pombe* mitochondria, UAA is the primary stop codon, while UAG only appears in some intronic reading frames.²⁰

TABLE 3 Comparison between the universal and mitochondrial genetic codes.

translation remains ambiguous, though its contribution to mitogenome maintenance has been inferred.^{69,71,72} A single *Urfa* residue substitution enhanced the occurrence of respiratory-deficient progeny, which contained mtDNA point mutations and/or deletions (the ‘mutator’ phenotype). Whilst, a non-sense *urfa* mutation produced offspring with varying degrees of respiratory deficiency, ranging from normal to no respiratory growth.^{69,71} Such segregation was recorded across generations (i.e. stably inherited), and could be cured by expressing from a nuclear locus the wild-type, mitochondrial-targeted *Urfa*. Similarly, the *S. cerevisiae* homologue *Var1* (uS3m) is encoded by the mitogenome, and constitutes the mt-SSU (Figure 1). Interestingly, the relocation of *var1* to the nucleus promoted mtDNA instability under stress, probably as a strategy to enhance cellular survival.⁷³ The protein was further shown to co-sediment with the mitochondrial nucleoids, suggesting a physical interaction.

Among the nuclear-encoded constituents of the *S. pombe* mitoribosome, Bot1 was the first ever identified. The protein co-sedimented with the mt-SSU, and contains clear sequence homology to *S. cerevisiae* Mrps35 (mS45).⁷⁴ The abolition of *bot1* could be obtained by tetrad dissection of a $\Delta bot1/bot1$ hetero-diploid, and resulted in bottle-shaped haploids (hence the name Bot1), which briefly proliferated before reaching lethality. Similarly, basal *bot1* expression (from a thiamine repressive promoter) was associated with altered cellular morphology, abnormal actin distribution, mitochondrial fragmentation and severely reduced mitochondrial translation. On the contrary, *S. pombe* overproducing Bot1 showed increased cell size, and enhanced oxygen consumption under fermentable condition, though similar mitochondrial translation to the wild-type strain. Morphology alterations commonly occurs upon respiratory defects,^{44,75} and did not suggest an extra-mitoribosomal function of Bot1.

6.2 | Structural heterogeneity enables *S. pombe* mitoribosome to regulate translation

The search for the homologues of *S. cerevisiae* Mrp51 (bS1m) and human MRPS28 (bS1m) yielded two nuclear-encoded proteins in fission yeast, termed Mrp51 and Mug178.¹ These co-sedimented and co-immunoprecipitated with the mt-SSU protein Bot1, albeit never co-immunoprecipitated with one another.⁵⁷ In wild-type *S. pombe*, Mrp51 was highly expressed, especially under fermentable condition. In vivo absence of this protein caused lethality. On the contrary, Mug178 showed relatively low and stable expression across the growth conditions. Upon *mug178* deletion, cells were viable, albeit deficient in respiratory growth, Cytb accumulation, *cox3* translation and complex IV activity. Taken together, these results demonstrated the presence of two mitoribosomal populations: one contained the bS1m isoform Mrp51, and fulfilled a wider role in mitochondrial translation; while the other instead contained Mug178, and rather specialised in *cytb* and *cox3* mRNA translation. Together with transcript-specific translational activators, the two mitoribosomal populations coordinate the stoichiometric synthesis of OXPHOS complex subunits (see ‘Mitochondrial specific translation and early complex assembly’). Interestingly, *S. cerevisiae* Mrp51 and human MRPS28 both constitute the mt-mRNA exit channel, which in budding yeast, appears as a recruitment platform for translational activators.^{67,76} The prior protein was further shown to suppress mutations in the 5’ UTR of *cox2* and *cox3* mRNA.⁷⁷

6.3 | Several tandem fusion proteins from *S. pombe* appear crucial to the mitoribosome

S. pombe mitochondria contain an abnormally high number of nuclear-encoded tandem fusion proteins. Moreover,

TABLE 4 *Schizosaccharomyces pombe* mitochondrial tandem fusion proteins.

PomBase ID	N-terminal domain	N-terminal function	C-terminal domain	C-terminal function	Internal cleavage
SPBP4H10.15 (1-918)	Aco2 (67-721)	Lys biosynthesis ^{Sc} Citric acid cycle ^{Hp}	Mrpl49 (bL21m) (812-914)	mt-LSU subunit	- ⁷⁸
SPAC1420.04c (1-753)	Rsm22 (140-463)	mt-SSU assembly ^{Sc, Hp}	Cox11 (588-739)	Complex IV assembly ^{Sc, Hp}	+ ⁸⁰
SPAC19B12.13 (1-753)	Rsm22 (140-463)	mt-SSU assembly ^{Sc, Hp}	Cox11 (588-739)	Complex IV assembly ^{Sc, Hp}	?
SPAC1782.04 ^a (1-175)	Mrx6 (40-94)	mtDNA maintenance ^{Sc}	Cox24 (mS38) (144-175)	mt-SSU subunit <i>cox1</i> splicing ^{Sc}	?
SPAC31A2.06 (1-542)	Rsf ^b (93-216)	Mitoribosome silencing ^{Sc}	Atp25? (?-?)	<i>atp9</i> mRNA stabilisation ^{Sc}	+ ^{Sc}
SPAC22E12.10c (1-616)	Cox15 (100-457)	Heam <i>a</i> biosynthesis ^{Sc, Hp}	Yah1 (539-590)	Ferredoxin ^{Sc}	+ ¹³¹
SPAC15E1.04 (1-625)	Hal3 (32-213)	Coenzyme A biosynthesis	Cdc21 (331-625)	dTMP biosynthesis	- ¹³²
SPCC1223.08c (1-461)	Fsh3 (1-213)	Serine hydrolase	Dfr1 (234-409)	Folate metabolism	?
SPAC4G9.09c (1-885)	Kinase (40-94)	Arginine biosynthesis	Reductase (144-175)	Arginine biosynthesis	+ ^{Sc}
SPAC22A12.08 (1-595)	Hydrolase (42-346)	?	Crld1 (399-507)	Cardiolipin synthase	+ ¹³³

Note: Numbers in brackets indicate start and end residue. Proteins related to the mitoribosome are shaded. ^{Sc} and ^{Hp}, respectively inferred from *S. cerevisiae* and *H. sapiens* homologue.¹

^aSPAC1782.04 is exceptionally compact compared to the other *S. pombe* tandem fusion proteins, and to its predicted *S. cerevisiae* homologues: Mrx6 (524 residues in length) and Qri5 (Cox24 or mS38, 111 residues in length).³⁰

^bThe ribosome silencing factor (Rsf) domain is homologous to human mitochondrial assembly of ribosomal large subunit protein 1 (MALSU1).¹ The tandem fusion protein is also present in *S. cerevisiae*, probably as a strategy to prevent the Rsf domain from interfering with the cytosolic translation machinery.¹³⁴

among the 10 proteins recognised so far, half are structurally and/or functionally connected to the mitoribosome (Table 4).¹ For instance, Aco2-Mrpl49 (SPBP4H10.15) contains an N-terminal aconitase, and a C-terminus homologous to *S. cerevisiae* Mrpl49 (bL21m) and human MRPL21 (bL21m). The *spb4h10.15* transcript is subjected to alternative selection of poly(A) site.⁷⁸ Upon addition of a poly(A) tail ~400 nucleotides (nt) upstream of the 3' end, a stop codon is introduced, producing only the Aco2 domain upon translation. On the contrary, when a poly(A) tail is positioned after the 3' end, the mRNA is translated into the pre-Aco2-Mrpl49 polypeptide. No internal cleavage of the tandem fusion protein was observed in the mitochondria. As *S. pombe* mitochondria also contain another aconitase (Aco1),¹ Aco2 is essential to viability solely because of its association with the Mrpl49 domain.⁷⁸ Consistently, Mrpl49 alone could sustain mitochondrial translation, albeit at reduced efficiency compared to Aco2-Mrpl49. Thus, tandem fusion might serve to optimise the stability and/or import of the mt-LSU constituent. Interestingly, an interactomics study on *S. cerevisiae* mitochondria

has revealed a potential interaction between Aco2 and the mt-LSU.⁷⁹

Meanwhile, the *S. pombe* mitochondrial Rsm22-Cox11 is present in vivo as two isoforms (SPAC1420.04c and SPAC19B12.13). The prior has been demonstrated to undergo cleavage after mitochondrial transport, releasing the two domains.⁸⁰ Thus, the tandem fusion might serve to coordinate the expression and/or import of Rsm22 and Cox11, rather than to persistently tether the two proteins. No study has been conducted on the second isoform. The homologues of *S. pombe* Rsm22 in *S. cerevisiae* (Rsm22) and humans (METTL17) each contains an iron-sulphur cluster, and is essential for mt-SSU assembly.⁸¹ Whilst, *S. cerevisiae* Cox11 co-fractionated with the mitoribosome, and functions in the (apparently co-translational) shuttle of copper to Cox1.⁸² Human COX11 similarly delivers copper to COX1, and further participates in the concerted biosynthesis and/or incorporation of complex IV metal redox centres.⁸³ Taken together, mitochondrial tandem fusion proteins could be a mechanism to couple the various stages of mitochondrial gene expression. In humans and

budding yeast, mitochondrial gene expression is spatiotemporally orchestrated within specialised compartments, respectively referred to as RNA granules and mitochondrial organization of gene expression (MIOREX) (Table 1).^{84,85} These are dynamic, visible by microscopy and composed of numerous factors involved in mitochondrial transcription, RNA processing and translation. Perhaps, equivalent foci are also present in *S. pombe*.

7 | MITOCHONDRIAL GENERAL TRANSLATION FACTORS

7.1 | *S. pombe* Mti2 and Mti3 play partially overlapping roles in translation initiation

In prokaryotes, translation initiation requires the methionyl-tRNA formyltransferase FMT, and the initiation factors 1–3 (IF1–3).⁸⁶ While FMT formylates methionyl-tRNA, IF1 blocks the product from entering the ribosomal A site. IF2 then positions formylmethionyl-tRNA in the ribosomal P site, and IF3 prevents the premature association of the ribosomal subunits. Except for IF1, the homologues of these proteins have each been recognised in *S. pombe*, *S. cerevisiae* and humans.¹ However, human mitochondrial IF2 (MTIF2) contains a 37-residue insertion, capable of occupying the ribosomal A site and further stabilising mt-mRNAs.⁸⁷ An insert sequence is also present in *S. cerevisiae*⁸⁸ and *S. pombe* mitochondrial IF2 (Mti2) (unpublished), albeit shorter and functionally uncharacterised.

Regarding fission yeast mitochondria, neither FMT (Fmt1), IF2 (Mti2) nor IF3 (Mti3) is required for viability.⁸⁹ Normal proliferation was observed in the absence of either Fmt1 or Mti3. In contrast, upon *mti2* deletion, both fermentative and respiratory growth were negatively affected, the mtDNA level progressively decreased and mitochondrial translation was barely detectable.⁸⁹ These phenotypes were exacerbated upon an additional *mti3* deletion (Figure 3). Furthermore, both Mti2 and Mti3 co-sedimented slightly with the assembled mitoribosome, and primarily with the mt-SSU. Mti2 remained enriched alongside the mt-SSU in the absence of Mti3. Whilst, *mti2* deletion abolished any potential interaction between Mti3 and the mitoribosome. Taken together, these results suggested that (i) *S. pombe* mitochondrial translation could be initiated by both formylated and unformylated methionyl-tRNA, (ii) Mti2 and Mti3 might share certain functions and (iii) Mti2 plays a much more prominent role, and might be required for Mti3-mitoribosomal association. The absence of *S. pombe* Mti2 could be

complemented by overexpressing its *S. cerevisiae* homologue (Ifm1), indicating a functional conservation.

7.2 | *S. pombe* translation elongation represents an intermediary between *S. cerevisiae* and humans

In bacterial cytoplasm and human mitochondria, translation elongation involves the delivery of an aminoacyl-tRNA to the ribosomal A site, and the translocation of mRNA and aminoacyl-tRNAs within the ribosome.^{86,90} Respectively, these are mediated by elongation factor Tu (EF-Tu, TUFM) and G (EF-G, GFM1), both of which belong to the GTPase protein family. However, only the prior requires a GTP/GDP exchange enzyme, termed elongation factor Ts (EF-Ts, TSFM). In addition, another GTPase protein (LepA, GUF1) is crucial for the fidelity (accuracy) of translation elongation.⁹¹ Each factor contains a structural homologue in *S. pombe* mitochondria (Figure 3).¹ So far, only Tuf1 (mitochondrial EF-Tu) and Tsf1 (mitochondrial EF-Ts) have been functionally characterised.⁹² The deletion of *tuf1* could be conducted solely on the *petite*-positive background *ptp1-1* (previously mentioned in ‘Maintenance of mitochondrial DNA integrity’), that is, a functional copy of the gene is absolutely required for viability. The $\Delta tuf1$ *ptp1-1* strain exhibited drastic mtDNA depletion, though most clones stably maintained 1–2% of residual mtDNA, perhaps as storage copies. In contrast, *S. pombe* could survive in the absence of Tsf1, albeit severely deficient in respiratory growth and mitochondrial translation. The basal translational activity was sufficient to maintain a wild-type mtDNA level.

Interestingly, while both mitochondrial EF-Tu and EF-Ts are present in human and *S. pombe* mitochondria, only the former was found in *S. cerevisiae* (Tuf1). In other words, *S. cerevisiae* Tuf1 functions independently of a GTP/GDP exchange factor. The overexpression of *S. cerevisiae* Tuf1 complemented for *S. pombe* *tuf1* deletion (Figure 3). Furthermore, *S. pombe* Tsf1 could be rendered redundant by either overproducing Tuf1, or introducing mutations around its GTP-binding site. These results suggested a strong, albeit incomplete dependency of *S. pombe* Tuf1 on its GTP/GDP exchange factor. On the other hand, human TSFM could functionally replace its homologue in fission yeast. In contrast, human TUFM complemented neither *S. pombe* nor *S. cerevisiae* $\Delta tuf1$ mutants, even when its own GTP/GDP exchange factor was co-expressed. Thus, while human mitochondrial EF-Ts could activate GTP/GDP exchange on *S. pombe* mitochondrial EF-Tu, human mitochondrial EF-Tu appears incapable of delivering aminoacyl-tRNAs to the *S. pombe* mitoribosome.

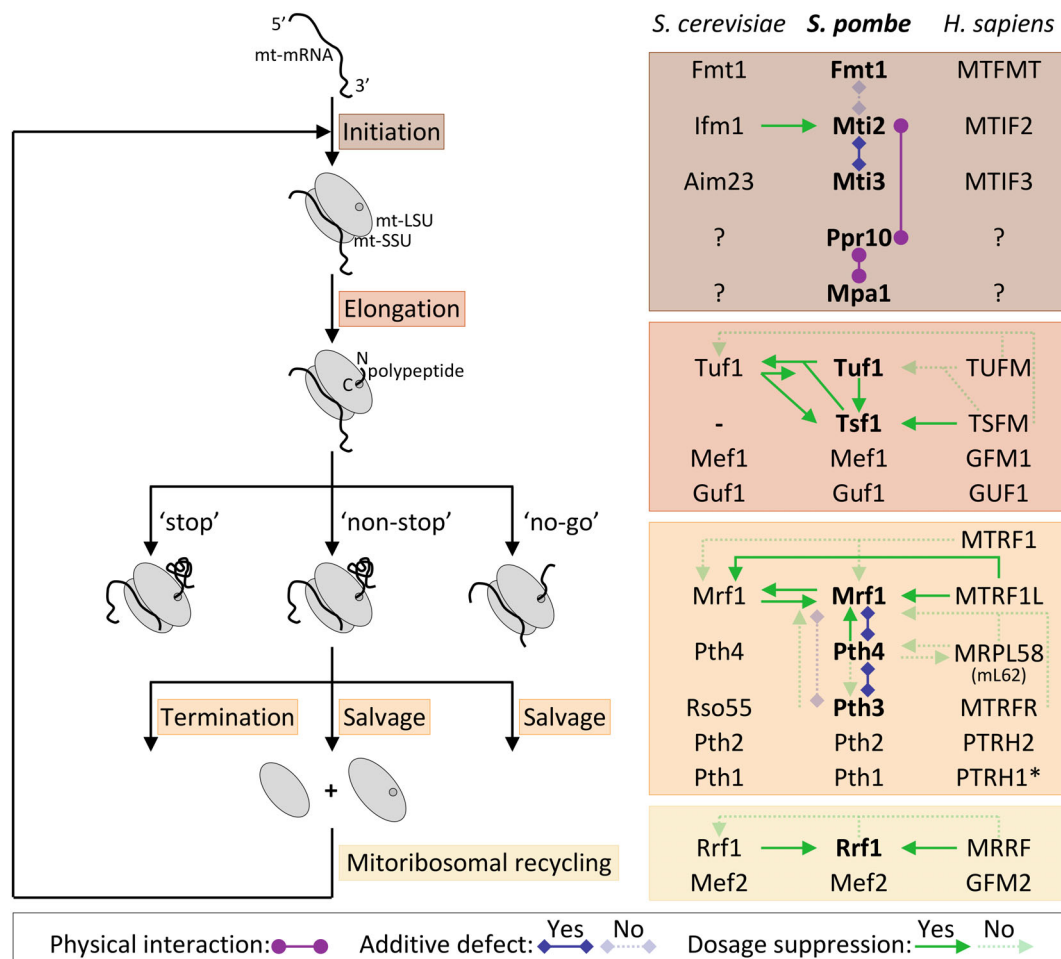


FIGURE 3 General translation factors in *Schizosaccharomyces pombe* mitochondria. The majority of these factors contain structural homologues in *Saccharomyces cerevisiae* and/or humans (names as per UniProt). Notably, *S. pombe* and *S. cerevisiae* Mrf1 are homologous to two mitoribosomal release factors in human mitochondria (MTRF1 and MTRF1L). Whilst, the human homologue of yeast Pth1 rather acts on the cytosolic ribosome (asterisk, *).¹⁴¹ Based on physical and genetic interaction studies, numerous *S. pombe* factors (bold) were functionally confirmed and assigned to a specific translational stage. So far, human diseases have been associated with MTFMT, TUFM, TSFM, GFM1, GUF1, GFM2, PTRH2 and MTRFR. The names of some human factors might vary between UniProt and publications: MTRF1L (mtRF1a), MTRFR (C12orf65) and MRPL58 (ICT1). Homologues of *S. pombe* Ppr10, Mpa1 and Tsf1 have either not been identified (question mark,?) or proven absent (hyphen, -).

7.3 | An interplay of factors acts in *S. pombe* translation termination, salvage and mitoribosome recycling

During translation termination, bacteria recruit the class I release factors 1 (RF1) and 2 (RF2) for stop codon recognition and peptidyl-tRNA hydrolysis.⁸⁶ Both proteins carry a conserved glycine-glycine-glutamine (GGQ) motif, responsible for cleaving the ester bond between the nascent polypeptide and the tRNA in-ribosome. Subsequently, RF1 and RF2 are discharged from the ribosome, either *via* the class II release factor 3 (RF3, a GTPase) or spontaneously (for RF2). In some cases, the translation machinery is stalled on the mRNA, and must be salvaged by peptidyl-tRNA hydrolase (PTH), among other factors.⁹³ Contrary to

RF1 and RF2, PTH does not possess the GGQ motif, hence could only hydrolyse peptidyl-tRNA already released from the ribosome. Regarding *S. pombe* mitochondria, a class I release factor (Mrf1) and several peptidyl-tRNA hydrolases (Pth1-4) have been recovered *in silico*.¹ Pth1 and Pth2 are respectively homologous to the bacterial and archaeal PTH, and neither contain a GGQ motif⁹⁴ nor appear essential for viability.² No further experimental data is available. Interestingly, the *S. cerevisiae* homologue of *S. pombe* Pth2, apart from its peptidyl-hydrolase activity, further participates in the removal of non-imported proteins from the translocase of the outer mitochondrial membrane (TOM).⁹⁵

On the contrary, *S. pombe* Mrf1, Pth3 and Pth4 each contains a GGQ motif. The independent abolition of

these proteins respectively produced cells with severe, slight and no respiratory deficiency.^{94,96} Increasing the *pth4* copy number showed no effect on the Δ *pth3* strain, though considerably improved the respiratory growth and mitochondrial translation of Δ *mrfl* (Figure 3).⁹⁴ Whilst, overproducing Pth3 could not complement for *mrfl* deletion. To further investigate the genetic interactions, double mutants were constructed. Additive defect was only observed for Δ *pth4* Δ *pth3* and Δ *pth4* Δ *mrfl*. Taken together, these results indicated a partial overlap in function between *S. pombe* Pth4 and Mrfl. Recently, *S. cerevisiae* Rso55 (homologous to *S. pombe* Pth3) and Pth4 have been demonstrated to hydrolyse peptidyl-tRNA in the mitoribosome, preferentially and respectively salvaging the 'no-go' (e.g. under antibiotics inhibition) and 'non-stop' mitoribosome (e.g. loaded with an mt-mRNA without a stop codon).⁹⁷ Regarding human cells, two mitochondrial release factors have been identified, termed MTRF1L (MTRF1a) and MTRF1.⁹⁶ While the prior significantly complemented for *mrfl* deletion in the fission and budding yeast, the latter could achieve neither, and was subsequently shown to instead decode AGG and AGA as 'stop' codons.⁹⁸ Other genetic interactions have also been explored, revealing an intricate, partially conserved network of factors involved in the final stages of mitochondrial translation (Figure 3).

After dissociation from the peptidyl-tRNA, the bacterial LSU and SSU are recovered by the ribosome recycling factor RRF, alongside EF-G and IF3 (discussed above).⁸⁶ *S. pombe* mitochondria contain a mitoribosome recycling factor (Rrf1)⁹⁹ and a GTPase protein (Mef2, paralogous to the mitochondrial EF-G Mef1),¹ none of which is essential for viability.² Upon *S. pombe* *rrf1* deletion, impaired proliferation on respiratory medium was observed, and could be reverted by overproducing the human homologue MRRF (Figure 3).⁹⁹ On the contrary, the overexpression of neither fission yeast *rrf1* nor human MRRF complemented for the absence of budding yeast *rrf1*. Thus, *S. pombe* Rrf1 is more functionally related to human MRRF than the *S. cerevisiae* homologue.

7.4 | Three PPR proteins are important for *S. pombe* mitochondrial translation

Apart from their involvements in mtDNA transcription and mtRNA stabilisation (previously and respectively discussed in 'Mitochondrial transcription' and 'Mitochondrial RNA processing and stabilisation'), *S. pombe* PPR proteins also act as general translation factors. On the one hand, Ppr10 participates in mitochondrial translation initiation. The deletion of *ppr10* abolished growth on both respiratory medium, and fermentable medium

supplemented with antimycin A.⁶⁵ Mitochondrial translation showed severe reduction, and mitochondrial-encoded OXPHOS subunits were barely detectable. Moreover, mature *cytb* and *cox1* mRNA were less abundant, a result of deprived intron-encoded maturases.¹⁰⁰ Tandem affinity purification (TAP) of Ppr10 recovered every mt-mRNAs, several mt-LSU and mt-SSU proteins and a novel nuclear-encoded, mitochondrial-localised protein Mpa1.^{65,101} Consistently, both Ppr10 and Mpa1 co-sedimented with the assembled mitoribosome. Deletion of the two central PPR motifs in Ppr10 compromised its interaction with Mpa1. In addition, Ppr10 co-immunoprecipitated with the mitochondrial translation initiation factor Mti2 (discussed above). Upon either *ppr10* or *mpa1* deletion, Mti2 (and Mti3) no longer co-sedimented with the mt-SSU.¹⁰¹ Taken together, these data suggested that Ppr10 and Mpa1 cooperatively enhance mitochondrial translation initiation, by physically connecting Mti2 and Mti3 to the mt-SSU.

On the other hand, the contributions of *S. pombe* Ppr2 and Ppr5 to mitochondrial translation remain enigmatic. Upon *ppr2* deletion, cells barely showed any respiratory growth or mitochondrial translation.⁵⁸ However, the associated Northern blot and prolonged ³⁵S labelling profile were similar to wild-type. Thus, the absence of Ppr2 might have lowered the efficiency, without affecting the fidelity, of the mitochondrial translation machinery. *S. pombe* Ppr2 could be the structural homologue of human MRPS27 (mS27, an mt-SSU protein) or PTC1 (an *RNR2* stabilisation factor).^{102,103} Based on the low expression of Ppr2 in vivo,¹ we would favour the second hypothesis. On the contrary, the absence of *ppr5* did not produce any visible defects on cellular proliferation and mtRNA accumulation.⁵⁸ Surprisingly, mitochondrial translation was enhanced for Δ *ppr5*, and suppressed in *ppr5* overexpression. Therefore, the corresponding protein plays a general, inhibitory role on mitochondrial translation via unestablished mechanisms.

8 | MITOCHONDRIAL SPECIFIC TRANSLATION AND EARLY COMPLEX ASSEMBLY

8.1 | *S. pombe* translational activators cooperate with the mitoribosome to balance Cytb and Cox1 production

During bacterial translation initiation, the 16S rRNA binds to the Shine–Dalgarno sequence, located within the mRNA 5' UTR.⁸⁶ To date, no Shine–Dalgarno sequence has been detected in the mt-mRNAs of *S. pombe*, *S. cerevisiae* and humans. Concerning budding yeast,

mitochondrial translation initiation instead employs nuclear-encoded, transcript-specific translation activators.^{104,105} These proteins facilitate interactions between the mt-mRNA 5' UTRs and the mitoribosome, and in some cases, further participate in translational autoregulation. Similar factors have also been uncovered in fission yeast, through the search for either (i) *S. pombe* PPR proteins (Ppr4), (ii) the homologue (Cbp7) of an *S. cerevisiae* mitochondrial translational activator (Cbs2) or (iii) the physical and genetic interactors (Cbp8, Mtf2, Mrh5 and Sls1) of the *S. pombe* mitochondrial translation machinery.

The *cox1* translational activators encompass Ppr4, Mtf2, Mrh5 and Sls1. While Ppr4 and Mtf2 both belong to the PPR protein family, Mrh5 is a DExH/D box RNA helicase. No obvious domain has been found for Sls1. The consequences of *ppr4*, *mtf2*, *mrh5* or *sls1* deletion were investigated on two genetic backgrounds: intronless and intron-containing mtDNA.^{57–59,106} Across the strains under study, severe respiratory growth defect was observed, coupled with abolished *cox1* mRNA translation, un- or barely detectable Cox1 and Cox2, and a secondary defect on Cytb accumulation. While the intronless strains might also exhibit a slight reduction in mature *cox1* mRNA, the intron-containing mutants consistently accumulated un-spliced *cox1* species. Furthermore, *via* co-IP and mass spectrometry, the four proteins were shown to bind each other and the mt-SSU (at least through Mrh5) without interacting with Cox1.⁵⁷ In another study, Sls1 co-precipitated with the *cox1* mRNA.¹⁰⁶ Thus, *S. pombe* Ppr4, Mtf2, Mrh5 and Sls1 form a stable complex of translational activators, essential for the synthesis of both Cox1 and its associated intron-encoded maturases^{57,106} (Figure 4).

Meanwhile, Cbp7 and Cbp8 play crucial roles in the normal accumulation and translation of *S. pombe cytb* mRNA. Respectively, the proteins are orthologous to the *S. cerevisiae* translational activators Cbs2 and Cbp1 (a PPR protein).^{57,104,105} The absence of either Cbp7 or Cbp8 resulted in severe respiratory deficiency, reduced *cytb* mRNA level, barely detectable Cytb amount, decreased complex III activity and minimal presence of supercomplex III/IV.⁵⁷ Unexpectedly, in both cases, defect on Cytb synthesis was negligible, and the protein appeared resistant to degradation. Perhaps, mitochondrial translation without *cbp7* or *cbp8* produced misfolded and/or aggregated protein, capable of escaping (i) detection from both in vivo mitochondrial proteases and in vitro Cytb antibody, and (ii) supercomplex integration. In addition, Cbp7 and Cbp8 were shown to form a stable complex *via* co-IP, coupled with either mass spectrometry or Western blot. The prior factor also roughly co-sedimented with the mt-SSU protein Bot1. However, upon the IP of Cbp7, neither mitoribosomal proteins nor

Cytb were significantly co-enriched. Thus, Cbp7 and Cbp8 appear less probable as complex III assembly factors, and more credible as cooperative *cytb* translational activators, interacting transiently or indirectly with the mitoribosome (Figure 4).

Together with the Mug178/Mrp51 mitoribosomal isoforms (previously discussed in 'The mitoribosome'), *cytb* and *cox1* translational activators regulate the stoichiometric production of OXPHOS complex subunits (Figure 4).⁵⁷ This was deduced from two sets of observations. Firstly, upon *mug178* deletion, additional absence of either *cytb* translational activator (Cbp7 or Cbp8) exacerbated the respiratory growth defect, and rendered *cytb* mRNA translation undetectable. In contrast, overexpression of *mug178* (but not *mrp51*) improved the respiratory growth and Cytb accumulation of $\Delta cbp7$ strain. Thus, Mug178 and the Cbp7/8 complex collaborate in *cytb* mRNA translation. Secondly, the respiratory growth deficiency associated with *mug178*, *cbp7* or *cbp8* deletion was suppressed by the same chromosomal mutations, occurred in either Mrp51 or one of the *cox1* translational activators (Ppr4, Mtf2 or Mrh5). The general rescue mechanism was enhanced Cytb synthesis, coupled with lowered productions of one or several complex IV subunits.

8.2 | *S. pombe* transcript-specific translation might not adhere to the autoregulation model

In *S. cerevisiae* mitochondria, Cbp3 and Cbp6 function as both *cytb* translational activators and early complex III assembly factors, and generate a negative feedback loop on *cytb* mRNA translation.^{104,105} Whilst, the *S. pombe* (and human) homologues of these proteins solely contribute to the correct assembly of complex III^{107,108} (Figure 3). Upon the absence of either Cbp3 or Cbp6, *S. pombe* respiratory growth was abolished, though *cytb* mRNA remained at normal cellular levels. The $\Delta cbp6$ strain was further analysed, revealing no deficiency in *cytb* translation, albeit undetectable Cytb subunit and its associating complex III. Thus, Cbp6 (and perhaps also Cbp3) is crucial for Cytb stabilisation, hence complex III assembly.

Similarly, while *S. cerevisiae* Mss51 mediates the autoregulation of *cox1* mRNA translation,^{104,105} the *S. pombe* homologue functions solely in early complex IV assembly¹⁰⁷ (Figure 4). The deletion of *S. pombe mss51* resulted in severe respiratory growth defect, coupled with enhanced Cox1 and Cox2 degradation, as well as dramatically reduced complex IV presence and activity. The accumulation and translation of *cox1* and *cox2* mRNAs showed no or very little

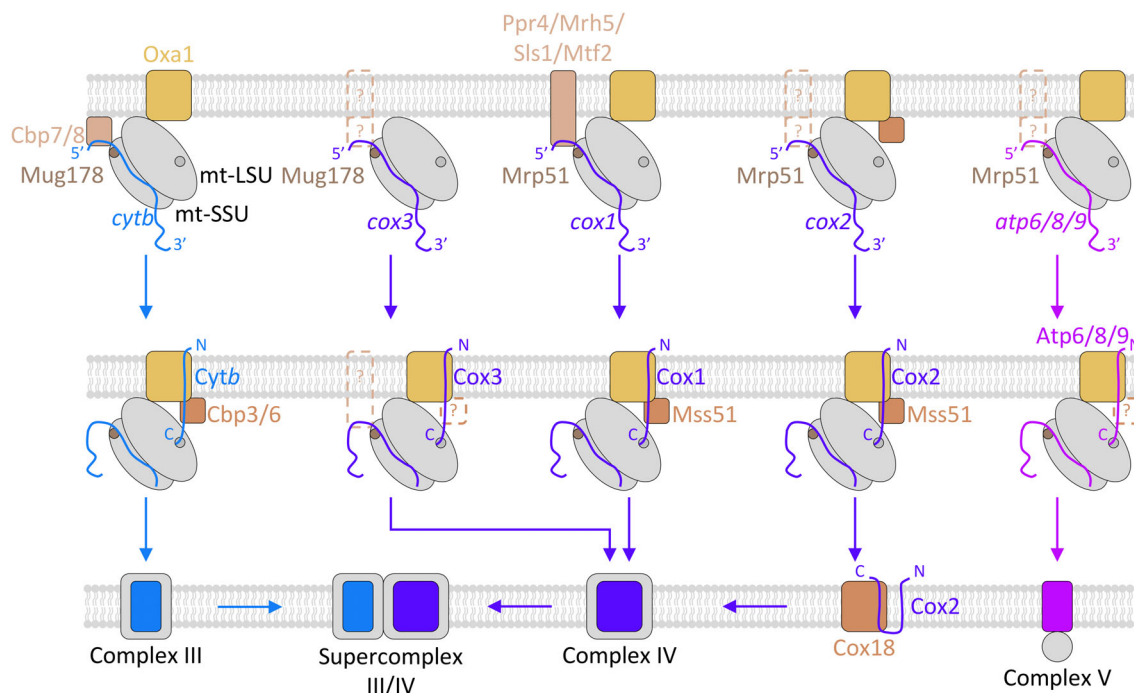


FIGURE 4 *Schizosaccharomyces pombe* mitochondrial transcript-specific translation and early OXPHOS complex assembly. *S. pombe* mitochondria contain two mitoribosomal populations. These are distinct by virtue of the bS1m isoform (Mug178 or Mrp51), and cooperate with translational activators (Cbp7/8 and Ppr4/Mrh5/Sls1/Mtf2) to initiate the translation of specific mt-mRNAs. During and after mitochondrial translation, the nascent polypeptides are subjected to stabilisation and IMM insertion by general (Oxa1) and protein-specific factors (Cbp3/6, Mss51 and Cox18). Eventually, mitochondrial- and nuclear-encoded subunits are assembled into OXPHOS complexes and supercomplexes. Unknown translational activators and complex-specific assembly factors are shown as question marks (?). Some representations have been inferred from *Saccharomyces cerevisiae* and/or humans, and remain to be confirmed in *S. pombe*: the mitoribosome is tethered to the inner mitochondrial membrane (IMM) via the mt-LSU and Oxa1; translational activators and early complex assembly factors are either peripheral or integral IMM proteins; and Cox18 specifically inserts the C-terminus of Cox2 to the IMM.

deficiency. Thus, *S. pombe* Mss51 functions primarily in the stabilisation and assembly of complex IV subunits. The homologue of *S. pombe* Mss51 in humans (MSS51 or ZMYND17) is instead a mitochondrial metabolic regulator.¹⁰⁹

8.3 | Members of the YidC/ALB3/OXA1/COX18 family play vital roles in early OXPHOS complex assembly

Another assembly factor of *S. pombe* complex IV is Cox18.¹¹⁰ The absence of this protein abolished growth on respiratory medium, and destabilised Cox2, despite showing a similar mitochondrial translation profile to the wild-type. Cox18 belongs to the YidC/ALB3/OXA1/COX18 membrane insertase family, whose members are evolutionarily conserved from prokaryotes to eukaryotes.¹¹¹ *S. cerevisiae* Cox18 and human COX18 also participate in complex IV assembly, more specifically in the translocation of the Cox2/COX2 C-terminus in the intermembrane space. Overexpression of the corresponding genes partially complemented for *S. pombe* *cox18* deletion.¹¹⁰

The other YidC/ALB3/OXA1/COX18 mitochondrial member is OXA1. In *S. cerevisiae* and humans, Oxa1/OXA1L is responsible for the IMM translocation of Cox2/COX2 N- and C-terminus, and of other mitochondrial-encoded and some nuclear-encoded proteins.¹¹¹ To perform these functions, the protein interacts with the mitoribosome, and several OXPHOS complex assembly factors (e.g. *S. cerevisiae* Cbp3/6 and Mss51), among other proteins. *S. pombe* Oxa1 is present in vivo as two isoforms, both of which could partially complement for a disruption in the sole *S. cerevisiae* OXA1.¹¹² The isoforms are encoded on different chromosomes, and as diverged from one another as they are from the *S. cerevisiae* and human homologues. Upon a disruption in the *S. pombe* *oxa1* on chromosome 1 (SPAC9G1.04, isoform I), normal growth was observed on fermentable and respiratory medium, coupled with slight reductions in complex III and IV activities. On the contrary, disruption of the chromosome II *oxa1* (SPBP4H10.03, isoform II) resulted in severe respiratory deficiencies: neither growth on respiratory medium nor resistance to antimycin A, absence of complex III and IV cytochrome spectral absorption and

substantial reductions in complex III and IV activities. The overexpression of isoform I could partially rescue the growth phenotypes, and the double isoform disruptions were lethal, regardless of the presence or absence of the *ptp1-1 petite*-positive mutation. Moreover, overexpression of neither *oxa1* isoforms could complement for *cox18* deletion, consistent with observations on the *S. cerevisiae* homologues.¹¹¹ Therefore, despite close structural homology, the YidC/ALB3/OXA1/COX18 mitochondrial members within each species exhibit certain functional divergence. Isoform I of *S. pombe* Oxa1 could be a backup for isoform II. Alternatively, the proteins might associate with different mitoribosomal populations (previously discussed in ‘The mitoribosome’). The presence of two *oxa1* genes is not a hallmark of *petite*-negative yeasts, because *K. lactis* contains a single *oxa1* coding sequence.

9 | CONTRIBUTIONS OF *S. POMBE* TO MITOCHONDRIAL RESEARCH

9.1 | *S. pombe* is a tool for mitochondrial biomedical research

S. pombe has been successfully employed in biomedical research, whose applications are relevant to mitochondrial disorders. Firstly, studies conducted on fission yeast contributed to the recognition of (i) a novel cancer susceptibility gene (*pif1*),¹¹³ (ii) the toxicity off-target (POLG) of sunitinib (an anticancer molecule)¹¹⁴ and (iii) the action mechanism of osthole (another chemotherapeutic).¹¹⁵ Secondly, using the *S. pombe* single-gene deletion library, multiple proteins have been found to promote resistance against doxorubicin, a chemotherapeutic agent.¹¹⁶ Among the significant factors were Ppr1, Cbp6 and Cox6, whose roles are *cox2* and *cox3* mRNA stabilisation, complex III assembly and complex IV subunit, respectively (previously discussed in ‘Mitochondrial RNA processing and stabilisation’ and ‘Mitochondrial specific translation and early complex assembly’). The study also revealed an extensive network of molecules, residing in numerous cellular compartments, and acting in conjunction to ensure cellular survival in the presence of doxorubicin. Thirdly, *S. pombe* has also assisted disease modelling and drug repurposing. For example, human OPA1 functions in mitochondrial fusion, and is structurally and functionally homologous to *S. pombe* Msp1.¹⁴ Mutations in the prior could cause dominant optic atrophy, a neurodegenerative disorder with no effective treatment. Upon screening of a drug library on an *S. pombe msp1* mutant, hexestrol and clomifene were shown to suppress the associated phenotypes (respiratory growth deficiency, mitochondrial fragmentation and/or mtDNA depletion).¹¹⁷ Because hexestrol

and clomifene have already been used on humans, the drugs could be repurposed for OPA1-, and perhaps other mitochondrial fusion-related disorders.

9.2 | *S. pombe* is a highly pertinent model and tool to study mitochondrial translation

Fission yeast has established itself as a valuable model for fundamental research, especially regarding mitochondrial translation. Compared to *S. cerevisiae*, *S. pombe* better resembles human cells in terms of general translation factors. For example, mitochondrial EF-Ts are present in fission yeast (Tsf1) and humans (TSFM), though absent in budding yeast (previously discussed in ‘Mitochondrial general translation factors’) (Figure 3).⁹² Concerning mitoribosomal recycling factors, human MRRF could complement for *S. pombe rrf1* deletion, albeit had no effect on *S. cerevisiae ΔRRF1*.⁹⁹ Whilst, the human release factor MTRF1L, when expressed with its own mitochondrial targeting sequence, better accumulated in fission yeast (rather than budding yeast) mitochondria.⁹⁶ Upon the absence of either human *MTRF1L* or *S. pombe mrf1*, cells exhibited respiratory defects less severe than *S. cerevisiae ΔMRF1*. This might be attributed to the lengths of mt-mRNA 3' UTRs, which range from hundreds of base pairs in budding yeast, to a couple of dozens in fission yeast and mostly absent in humans (Table 1). Consistently, the double deletion of *S. pombe pah1* and *mrf1* was lethal,⁹⁴ the former of which functions in the 3' exoribonucleolytic cleavage of mt-mRNAs (previously discussed in ‘Mitochondrial RNA processing and stabilisation’). In other words, by increasing the length of mt-mRNA 3' UTRs, fission yeast becomes more dependent on Mrf1.

Although *S. pombe* is *petite*-negative, the majority of respiratory mutants are viable. Moreover, strong mitochondrial defects often select for mitogenome conservation in this species, rather than mtDNA instability as per *S. cerevisiae*. For example, fission yeast devoid of *mti2*, *tsf1* or *mrf1* stably maintained a pool of residual mtDNA, despite minimal mitochondrial translation (previously discussed in ‘Mitochondrial general translation factors’).^{89,92,96} In some cases, respiratory mutations are lethal. These could be confirmed by tetrad dissection of a hetero-diploid containing a wild-type allele (e.g. *Δbot1/bot1*),⁷⁴ previously discussed in ‘The mitoribosome’, and/or investigated in a *petite*-positive background (e.g. *tuf1 ptp1-1*)⁹². In the latter example, a strong mtDNA depletion homogeneously occurred across the population (previously discussed in ‘Mitochondrial general translation factors’). However, the residual mitogenome was

wild-type, and brought about cellular proliferation once mitochondrial translation was restored. By retaining some wild-type mtDNA, *S. pombe* respiratory mutants enable genetic interaction studies (reversion, complementation, suppression, etc.), which are vital for functional characterisation.

Research on *S. pombe* mitochondrial translation encounters three major drawbacks. Foremost, ^{35}S labelling of newly synthesised proteins is rather inefficient in fission yeast, rendering short pulse difficult to implement. In our experience, this could be improved by replacing cycloheximide with anisomycin for cytosolic translation inhibition.⁵⁷ Secondly, because *S. pombe* mitochondrial transformation has yet to be achieved, mitochondrial translation defects could not be studied using reporter genes. An example is *Arg8^m*, which has been extensively applied to *S. cerevisiae*.¹¹⁸ Once mitochondrial transformation is established for fission yeast, the expression of heterologous genes would greatly benefit from the near universal genetic code in *S. pombe* mitochondria. Thirdly, similar to *S. cerevisiae*, *S. pombe* contains no complex I, instead possessing nuclear-encoded, single-polypeptide NADH dehydrogenases (Table 1).^{1,30} Consequently, any study concerning human complex I must employ an alternative model (e.g. *Yarrowia lipolytica*, *Neurospora crassa* or *Podospora anserina*).

9.3 | *S. pombe* is a tool to investigate novel concepts in mitoribosomal biology

As previously discussed (in ‘The mitoribosome’), *S. pombe* mitochondria contain at least two populations of mitoribosomes, which carry either the Mrp51 or Mug178 isoform, and preferentially translate *cytb* and *cox3* mRNA, or the remaining mt-mRNAs, respectively.⁵⁷ Previously, only cytoplasmic ribosomes were shown to induce transcript-specific translation *via* compositional paralogues.¹¹⁹ For instance, to synthesise nuclear-encoded, mitochondrial-localised proteins, *S. cerevisiae* cytoplasmic ribosomes must contain the **b** isoforms of uL1, uL2 and eS26. Considering the nomenclature of known cytosolic ribosomal paralogues, and the functions of *S. pombe* Mrp51 and Mug178, we propose a second name for these isoforms: bS1ma and bS1mb, respectively.

Mitoribosomes had been demonstrated to regulate translation, though not *via* structural heterogeneity. Specifically, in *S. cerevisiae*, the absence of the mt-SSU protein Cox24 (mS38) modified the decoding centre of the mitoribosome, and was associated with primary defects in *cox1-3* mRNA translation initiation.¹²⁰ Such phenotypes occurred regardless of modifications in translational activators, and overexpression of *cox24* showed

wild-type (rather than enhanced) Cox1-3 synthesis. Moreover, the position of Cox24 within the mitoribosome would have prevented any interaction of the mitoribosomal protein with translational activators, and only one population of mitoribosome has been detected in *S. cerevisiae* mitochondria.⁷⁶ Therefore, the preference towards *cox1-3* mRNA translation is an intrinsic property of *S. cerevisiae* mitoribosome, mediated by Cox24, and dependent on neither translational activators nor structural heterogeneity. It would be particularly interesting to investigate the role of the Mrx6-Cox24 (Mrx6-mS38) tandem fusion protein (Table 4) in the two *S. pombe* mitoribosomal populations.

10 | CONCLUSION AND PERSPECTIVES

Fission yeast is an invaluable model for studies on mitochondrial biogenesis and diseases, due to the availability of experimental and database support, and its inherent resemblance to human cells. Notably, the two organisms share a similar structure of, and dependency on, the mitogenomes. Expression of these genomes generally employs conserved mechanisms (e.g. mt-tRNA punctuation) and protein machinery (e.g. general translation factors). However, compared to the amount of knowledge from *S. cerevisiae* and humans, our understanding of *S. pombe* mitochondrial gene expression is still at an early stage. The participating factors remain obscure for numerous processes, and only two multimeric protein complexes have had their structures resolved: OXPHOS complex III (published as part of supercomplex III/IV)¹²¹ and complex IV (retrieved as either a supercomplex III/IV constituent¹²¹ or a stand-alone complex¹²²).

Further application of fission yeast to mitochondrial research necessitates structural information on the mitoribosome. To date, the recognition of *S. pombe* mitoribosomal constituents have solely or predominantly relied on sequence homology. Structural resolution of the mitoribosome would (i) contribute to the functional confirmation of these proteins, (ii) enable the identification of *Schizosaccharomyces*-specific components, (iii) further elucidate the biological significance of the bS1m and Oxa1 isoforms and (iv) provide information on the physical partners of and conformational changes within the mitoribosome. Previously, cryo-EM analyses have uncovered several novel ligands (e.g. iron-sulphur clusters) in the human mitoribosome,¹²³ and a protein recruitment platform on the *S. cerevisiae* counterpart.⁷⁶ The former discovery emphasises the importance of functional coupling in mitochondria, more specifically between mitochondrial gene expression and iron metabolism. Interestingly, the

absence of several *S. pombe* mitochondrial PPR proteins has been shown to perturb iron homeostasis.^{124,125}

Research efforts should also be dedicated to other elements in *S. pombe* mitochondrial biogenesis. Firstly, studies on the leucine-tyrosine-arginine motif (LYRM) should recover novel complex assembly factors, and/or improve our understanding of the aforementioned functional coupling. In humans, mitochondrial LYRM proteins are critical to the assembly and function of multimeric protein complexes, especially those containing iron-sulphur clusters.¹²⁶ Several members have been recognised in budding and fission yeast, none of which are functionally characterised.^{1,30} Secondly, while replication origins, nucleoids and gene expression foci have been well established in *S. cerevisiae* and human mitochondria, they remain understudied or undocumented in fission yeast. Thirdly, various *S. pombe* mitochondrial complexes are structurally and/or functionally ambiguous, including the mitochondrial RNase P, the mitochondrial contact site and cristae organising system (MICOS) and the endoplasmic reticulum-mitochondria encounter structure (ERMES). In addition, *S. pombe* mitophagy is only starting to be investigated, using for instance, the general translation factor Tuf1.¹²⁷ In conclusion, considering its versatility, originality and biological relevance, fission yeast is a powerful model for biomedical and fundamental studies related to mitochondria. We are convinced that through time, as our understanding of *S. pombe* mitochondrial biogenesis improves, the model system would carry on contributing to these areas of research, and gain even more traction in the scientific community.

ORCID

Nhu Dinh  <https://orcid.org/0009-0000-1804-2151>

Nathalie Bonnefoy  <https://orcid.org/0000-0002-5068-8849>

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SUPPORTING INFORMATION

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