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Review

RNA maturation in mitochondria of *S. cerevisiae* and *S. pombe*

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Abstract

Although the gene content is rather conserved, the genomes in mitochondria of yeasts vary dramatically in size [Clark-Walker, G.D., Evans, R.J., Hoeben, P., McArthur, C.R., 1985. Basis of diversity in yeast mitochondrial DNAs. In: Quagliariello, E.C., Palmieri, F., Saccone, C., Kroon, A.M. (Eds.). Achievements and Perspectives of Mitochondrial Research 2. Science Publishers, Amsterdam, pp. 71-78] and in the number of transcription units. Since the fidelity and processivity of the mitochondrial single-subunit phage-like RNA polymerase present in yeast mitochondria are certainly limited, one might speculate that the density of transcription initiation sites on the mitochondrial genomes is one of the factors influencing the genome size. In an effort to find common features among the apparent idiosyncrasies of *Saccharomyces cerevisiae* (with its extremely large mtDNA) and *Schizosaccharomyces pombe* (with its extremely small mitochondrial genome), the aim of this review is to compare recent data about transcription and generation of 5′ and 3′ ends of mature RNA transcripts in *S. cerevisiae* and in *S. pombe*. Both organisms are two attractive model systems enabling investigation of various aspects of mitochondrial genetics.

Keywords: Mitochondrial transcriptome; Promoters; Upstream RNA processing signals; Downstream RNA processing signals; tRNA punctuation model

1. Transcription and RNA processing in *S. cerevisiae* mitochondria

In-vitro-capping of 5′ termini of primary transcripts has been exploited to map the transcriptional initiation sites on the large mitochondrial genome in the petite-positive budding yeast *Saccharomyces cerevisiae* (Christianson et al., 1982; Christianson and Rabinowitz, 1983; Edwards et al., 1983). At least 19 highly-conserved sequence motifs of nine nucleotides exist that serve as initiation sites of transcription (Foury et al., 1998). Transcription of protein and rRNA coding genes is usually initiated from the terminal A residue of the motif ATATAAGTA, whereas TTATAAGTA is characteristic of tRNA gene transcription (Christianson and Rabinowitz, 1983). The function of these mitochondrial nonanucleotide promoters is to recruit the core RNA polymerase (Rpo41p) plus the supplementary specificity factor Mtf1p for the initiation of RNA synthesis (Jang and Jaehning, 1991; Karlok et al., 2002). This mode of transcription initiation seems to be conserved in mitochondria of yeasts since identical or very similar nonanucleotide promoter sequences have been identified in *K. lactis* (Ragnini and Frontali, 1994), *Torulopsis glabrata* (Clark-Walker et al., 1985; Koszul et al., 2003), *Williopsis* (*Hansenula*) *mrakii* (Drissi et al., 1993, 1994) and *Yarrowia lipolytica* (Kerscher et al., 2001). Following the widely-held consensus that all mitochondria in extant eucaryotes can be traced back to a single endosymbiotic event, one would expect to see particular regulatory elements in the promoter regions that promote modulation of transcription rates also on yeast mitochondrial genomes. Instead, the context of flanking sequences seems to be the only mechanism that affects the relative strength of the budding yeast promoters (Biswas and Getz, 1986).

Abbreviations: mtDNA, mitochondrial DNA; *S. pombe*, *Schizosaccharomyces pombe*; *cox1,2,3*, subunits 1–3 of the mitochondrial cytochrome oxidase complex; *atp6,8,9*, subunits 6, 8, 9 of the mitochondrial ATPase complex; *rnl*, ribosomal RNA of large subunit; LSU RNA, ribosomal RNA of small subunit (*rns*); mtP-RNA, mitochondrial RNAse P RNA (*rnpB*); P_{ma}, P_{mi}, major and minor mitochondrial promoter in fission yeast; 5′ UTL, 5′ untranslated leader sequence.

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1.1. Processing of mitochondrial precursor RNAs

The maturation of the precursor RNAs is much more elaborate in *S. cerevisiae* mitochondria than in animal mitochondria. Most primary transcripts from *S. cerevisiae* mitochondrial promoters are polycistronic and are typically composed of two or more coding sequences including various combinations of rRNAs, tRNAs and mRNAs (for review see Tzagoloff and Myers, 1986). These polycistronic primary transcripts require subsequent complex processing to generate the mature RNA molecules. The tRNAs included in precursor RNAs can be located either 5′ or 3′ of mRNAs and are the most important mitochondrial RNA processing signals. The tRNAs are recognized and cleaved by tRNA processing enzymes, like the RNase P and presumably an RNase Z homolog, which thus liberate the flanking RNA molecule(s) from the polycistronic precursor RNAs. This mechanism of RNA processing is known as the 'RNA punctuation mode' and is similarly used in a wide range of fungal mitochondria including *Neurospora crassa* (Burger et al., 1985), *Aspergillus nidulans* (Dyson et al., 1989), as well as in green (Wolff and Kück, 1996) and red algae (Richard et al., 1998) and (most developed) in animal mitochondria (Ojala et al., 1980).

The 3′ termini of mitochondrial mRNAs in budding yeast are specified by the dodecamer motif 5′-AAUAA(U/C)AUUCUU-3′ in the 3′ UTR region (Hofmann et al., 1993; Osinga et al., 1984). This motif serves as a target for a 3′ processing process promoted by a degradosome complex (mtEXO). The complex itself is composed of the DExA motif containing RNA helicase Suv3p and the 3′–5′ exoribonuclease Dss1p (Dziembowski et al., 1998). The role of a 55-kDa RNA binding protein that specifically binds to the dodecamer sequence (DBP) is yet speculative (Li and Zassenhaus, 1999). Other RNase activities such as the oligoribonuclease Ynt20p (Hanekamp and Thorsness, 1999) or the non-specific ribonuclease Nuc1p (Vincent et al., 1988) might be involved in the general RNA metabolism within mitochondria but its specific functions remain to be clarified. In the absence of polyadenylation, recognizable regulatory sequence elements or other mechanisms for modulation of mitochondrial transcription, it would appear that RNA maturation (e.g., endonucleolytic cleavage, trimming of 5′ and 3′ ends, splicing, etc.) and degradation should have a central role in the control of mitochondrial gene expression.

2. The transcription system in fission yeast mitochondria

Unlike the petite-positive yeast *S. cerevisiae*, the wild type of the petite-negative fission yeast *Schizosaccharomyces pombe* (*S. pombe*) is strictly required to maintain mitochondrial functions and mitochondrial DNA integrity (Haffter and Fox, 1992; Massardo et al., 1994). The mitochondrial contribution to mitochondrial functions in *S. pombe* is expressed by a very small genome encoding eight proteins (for reviews see Paquin et al., 1997; Schäfer, 2003), a set of RNAs involved in translation (*rnl*, *rns*, a complete set of 25 tRNAs) and the mtP-RNA (*rnpB;* Seif et al., 2003). All the genes are tightly packed and located on one DNA strand.

2.1. RNA processing in fission yeast resembles RNA maturation in animal mitochondria

While the mitochondrial RNAs in yeasts are usually transcribed from numerous promoters and maturated in complex processing pathways, studies mainly from HeLa cells have led to a simple model of how the mature rRNAs, tRNAs, and mRNAs are generated in vertebrate mitochondria (reviewed in Tracy and Stern, 1995; Tzagoloff and Myers, 1986). Here, the tRNAs demarcate the starts and ends of the mature RNAs in the transcript precursors that originate from a few promoters. However, the detailed analysis of the mitochondrial transcriptome in *S. pombe* recovered that the transcription system in fission yeast mitochondria combines typical fungus-like mechanisms with features that are more similar to that in animal mitochondria (Schäfer et al., 2005). As described below, the machinery in *S. pombe* is reduced to a small subset of mechanisms involved in RNA maturation. The advantage of this simple but efficient organisation is that it requires of only a limited number of proteins.

2.2. Yeast-like nonanucleotide motifs serve as transcription sites and form the 5′ ends of mature rnl and cox3 RNAs

A search for promoters on the mtDNA of *S. pombe* recovered four sequence motifs that are almost identical to the nonanucleotide promoter motifs found on mtDNA of *S. cerevisiae* and other distantly related yeast species. Two of the four predicted motifs exhibit significant transcriptional activity in *S. pombe* in in-vitro capping experiments (Schäfer et al., 2005). Transcription on both promoters gives rise to two long primary transcripts. Due to an apparent lack of transcription termination signals, the two promoters (P_{ma} and P_{mi}) are likely to initiate synthesis of RNAs with a length of 19.4 and 10.5 kb as predicted from the mtDNA map.

Several RNA processing mechanisms can be expected to excise tRNAs, rRNAs and mRNAs from these large precursor RNAs in *S. pombe*. While tRNA genes are located in the intergenic region between most genes (Fig. 1), ***cox1*** and ***cox3*** as well as ***cox2*** and ***rnl*** are not separated by a tRNA gene. As reported in Schäfer et al. (2005), the first A at the 5′ termini of ***rnl*** and ***cox3*** RNAs coincides with the 3′ end of the promoter motifs P_{ma} or P_{mi} (Fig. 3). This clearly indicates a dual role of the transcription initiation sites in fission yeast, because these sites are not only active promoters required for RNA synthesis but also involved in fixing 5′ ends of mature ***rnl*** and ***cox3*** RNA; further 5′