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Review

Yeast and human mitochondrial helicases☆

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ABSTRACT

Mitochondria are semiautonomous organelles which contain their own genome. Both maintenance and expression of mitochondrial DNA require activity of RNA and DNA helicases. In *Saccharomyces cerevisiae* the nuclear genome encodes four DExH/D superfamily members (*MSS116*, *SUV3*, *MRH4*, *IRC3*) that act as helicases and/or RNA chaperones. Their activity is necessary for mitochondrial RNA splicing, degradation, translation and genome maintenance. In humans the ortholog of *SUV3* (*hSUV3*, *SUPV3L1*) so far is the best described mitochondrial RNA helicase. The enzyme, together with the matrix-localized pool of PNPase (*PNPT1*), forms an RNA-degrading complex called the mitochondrial degradosome, which localizes to distinct structures (D-foci). Global regulation of mitochondrially encoded genes can be achieved by changing mitochondrial DNA copy number. This way the proteins involved in its replication, like the Twinkle helicase (*c10orf2*), can indirectly regulate gene expression. Here, we describe yeast and human mitochondrial helicases that are directly involved in mitochondrial RNA metabolism, and present other helicases that participate in mitochondrial DNA replication and maintenance. This article is part of a Special Issue entitled: The Biology of RNA helicases — Modulation for life.

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1. RNA helicases in yeast mitochondria

Saccharomyces cerevisiae to this day remains the most extensively studied model organism for research on the mitochondrial genome maintenance and expression (see [1] for a recent review). In contrast to human mitochondrial genome, the *S. cerevisiae* mitochondrial DNA (mtDNA) is transcribed from several (up to 13) promoters, the transcripts are not polyadenylated and contain long UTR sequences. In addition, three mitochondrial genes contain introns: 21SrRNA, COX1 and COB, and their number is strain-dependent [1]. In budding yeast the nuclear genome contains four genes encoding mitochondrially-targeted proteins belonging to the large DExH/D superfamily of NTP-dependent RNA remodelling enzymes acting as helicases and/or RNA chaperones and RNP remodelling factors: *MSS116* (*YDR194C*), *SUV3* (*YPL029W*), *MRH4* (*YGL064C*) and *IRC3* (*YDR332W*). They all contain the general SF2 NTP-dependent helicase core composed of two RecA-like domains [2–4], and are further subdivided based on the sequence of one of the conserved motifs–motif II (Walker motif B) into the DEAD, DEAH and DExH/D subfamilies [5,6]. Mss116p and Mrh4p contain the canonical DEAD tetrapeptide; Irc3p belongs to the DEAH family; whereas the sequence of Suv3p contains an atypical DEIQ variant of the DExH/D motif. All four proteins were shown to be involved in the functioning of the mitochondrial genetic system (Fig. 1). For Mss116p and Suv3p there is a substantial body of evidence demonstrating their roles in the mitochondrial RNA (mtRNA) metabolism both *in vivo* and *in vitro*. The data on Mrh4p and Irc3p are still far from complete, nevertheless they also point to an important function in the organellar genome maintenance and/or expression.

1.1. Mss116p — a model DEAD-box helicase involved in splicing

The *MSS116* gene was identified in a genetic screen for nuclear-encoded factors involved in splicing of the intron-containing transcripts of mitochondrial genes [7], and shown to encode a protein belonging to the DEAD family of RNA helicases. Whereas some group I and II introns can self-splice *in vitro* under generally non-physiological conditions [8], *in vivo* their self-splicing depends on accessory protein factors. Among these factors are intron-encoded maturases, and nuclear-encoded proteins, either acting on specific introns or generally affecting all intron-containing transcripts [1]. Mss116p clearly belongs to the latter group, as it was shown to be required for efficient splicing of all the mitochondrial group I and II introns, including those that do not require the assistance of an intron-encoded maturase [9]. Involvement of Mss116p in splicing is,

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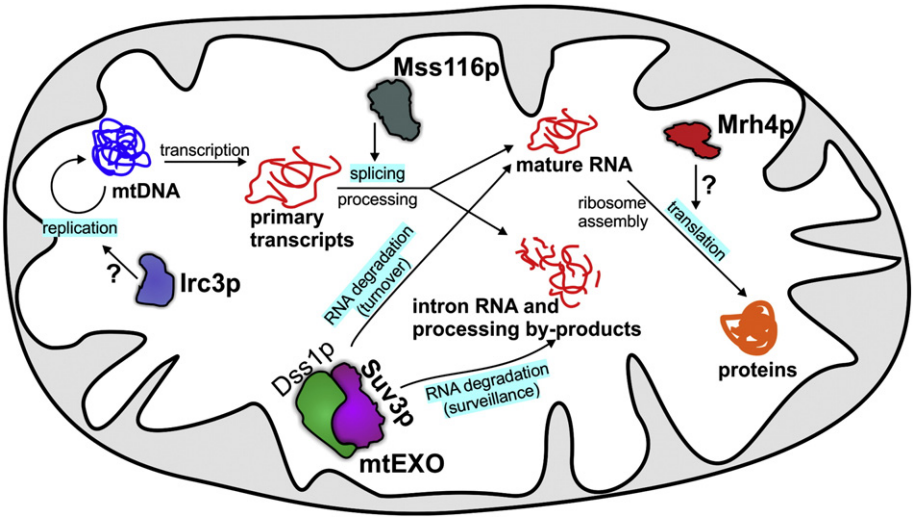

Fig. 1. Functions of RNA helicases in the mitochondrial gene expression in *S. cerevisiae*. Processes wherein an involvement of an RNA helicase is suggested are highlighted in blue. Names of proteins or complexes with a demonstrated or predicted RNA helicase activity are in bold. Complexes that do not contain an RNA helicase protein were omitted for clarity. Question marks indicate that the function is putative and based on limited experimental evidence.

at least in part, independent of the mitochondrial translation, as splicing of the bi1 (first intron of *COB*), ai5γ (seventh intron of *COX1*) and ω (the only intron of the *LSU-rRNA*) introns does not require a maturase. Splicing of these introns is, however, clearly affected by the lack of Mss116p, even in ρ^- petites, where mitochondrial translation does not occur at all [9]. Nevertheless, Mss116p seems to be also involved in translation independent of its function in splicing, as its deletion in strains devoid of all the introns in mtDNA still results in a respiratory-deficient phenotype, depending on the genetic background either at all growth conditions [7], or in a cold-sensitive manner at 18–24 °C [9], with the synthesis of Cox1p and Cox3p being most visibly affected.

Recent studies provided significant insights into the mechanistic and structural aspect of Mss116p activity, making it an important model in research on the DEAD-box protein function [10,11]. Mss116p *in vitro* is an RNA-dependent ATPase and can unwind short RNA/RNA duplexes without requiring either 5′ or 3′ ssRNA overhangs [12,13]. In the splicing of a group I intron — ai5β, Mss116p acts in an ATP-dependent manner by promoting a change in the structure of a catalytically active intermediate formed in the first step by the intron RNA with a specific splicing factor Mrs1p, thus facilitating the second step — exon ligation [14]. Similarly, in maturase-dependent splicing of the ai2 group II intron, Mss116p acts after the maturase protein binds the intron RNA and assists in forming the catalytically active RNP ribozyme [9].

Much attention has been devoted to the study of Mss116p involvement in the splicing of short group II introns that do not encode maturases, exemplified by the ai5γ intron. Mss116p alone is sufficient to promote self-splicing of the ai5γ intron *in vitro* in conditions of salt and Mg^{2+} concentrations approaching physiological [12,13]. The folding of ai5γ ribozymes under such conditions is slow and hindered by a significant energy barrier [15] and Mss116p is believed to assist in the folding of the ribozyme into the active structure. Studies *in vivo* demonstrated that the absence of Mss116p activity results in the loss of ai5γ tertiary structure in *mss116Δ* strains, with only the secondary structure of the intron remaining [16].

Different mechanisms were proposed to explain the role of Mss116p in ai5γ intron folding. One explanation is built around the RNA duplex unwinding activity of Mss116p and suggests that the main function of the protein is in resolving undesirable secondary structure elements ("kinetic traps") that hinder proper folding into the native state [11,17–21]. The actual mechanism would involve local structure destabilization by strand separation without directional movement of the helicase [21]. In this model the Mss116 protein can disrupt short duplexes by using a single cycle of ATP-dependent structure rearrangement while remaining tethered to the target RNA or RNP complex [17,18,21].

The alternative explanation postulates that Mss116p assists in the folding of the compact catalytic core structure of the intron by binding to the RNA and stabilization of the weak on-pathway folding intermediates [13,22–26]. Such mechanism is supported by studies involving single-molecule analysis of intron folding, showing that Mss116p promotes the formation of an early compact intermediate of the folding pathway in an ATP-independent manner [24], followed by an ATP-dependent step that consists of recycling the RNA-bound protein [23,27] or resolution of one or more small misfolded structures that arise after the formation of the compact intermediate.

The exact mechanistic roles of Mss116p in intron splicing are thus quite complex, and both RNA duplex unwinding and folding intermediate stabilization are likely to play a role in its biological activity. RNA helix unwinding is probably important in folding of the long precursors that contain both exonic and intronic sequences, whereas single stranded structure stabilization is critical for folding of the compact ribozyme core [10,18,25,26].

Studying of the mechanistic aspects of Mss116p function has been greatly facilitated by the progress made in understanding the underlying protein structure. Analysis of a high resolution crystal structure of Mss116p in complex with a RNA oligonucleotide and ATP analogues [28] indicated that the helicase core cooperates with the C-terminal domain typical for a subclass of DEAD-box proteins like Mss116p and *Neurospora crassa* CYT-19 [29] in performing the protein function. The conserved helicase core induces a bend in the bound RNA substrate in a manner typical for DEAD-box helicases, while the C-terminal extension induces a second bend, thus resulting in the formation of two wedges that crimp the bound RNA [28]. The C-terminal extension thus has two functions — stabilizing the helicase core and contributing to RNA binding [28,29].

The DEAD-box RNA helicases do not exhibit significant sequence specificity, and are partially interchangeable, sometimes in spite of non-overlapping function *in vivo*. The CYT-19 mitochondrial DEAD-box helicase of *N. crassa* stimulates splicing of the yeast ai5γ intron *in vitro* and is also capable of complementing the phenotype in *mss116Δ* strains *in vivo* [9,12,13,18]. Remarkably, the cytoplasmic DEAD-box helicase Ded1p involved in translation initiation is also capable of promoting *in*

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