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Review

Mechanism of transcription initiation by the yeast mitochondrial RNA polymerase ☆

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ABSTRACT

Mitochondria are the major supplier of cellular energy in the form of ATP. Defects in normal ATP production due to dysfunctions in mitochondrial gene expression are responsible for many mitochondrial and aging related disorders. Mitochondria carry their own DNA genome which is transcribed by relatively simple transcriptional machinery consisting of the mitochondrial RNAP (mtRNAP) and one or more transcription factors. The mtRNAPs are remarkably similar in sequence and structure to single-subunit bacteriophage T7 RNAP but they require accessory transcription factors for promoter-specific initiation. Comparison of the mechanisms of T7 RNAP and mtRNAP provides a framework to better understand how mtRNAP and the transcription factors work together to facilitate promoter selection, DNA melting, initiating nucleotide binding, and promoter clearance. This review focuses primarily on the mechanistic characterization of transcription initiation by the yeast *Saccharomyces cerevisiae* mtRNAP (Rpo41) and its transcription factor (Mtf1) drawing insights from the homologous T7 and the human mitochondrial transcription systems. We discuss regulatory mechanisms of mitochondrial transcription and the idea that the mtRNAP acts as the *in vivo* ATP "sensor" to regulate gene expression. This article is part of a Special Issue entitled: Mitochondrial Gene Expression.

1. Introduction

The primary function of the mitochondria in the eukaryotic cells is to synthesize ATP. Hydrolysis of ATP releases energy that can be enzymatically coupled to drive biochemical reactions of the metabolic pathways. Mitochondria are indispensable organelles of eukaryotic cells because they also play important roles in processes such as cell growth, differentiation, apoptosis, signaling, and innate immunity [1–5]. There is mounting evidence that dysfunctions in mitochondria are responsible for many neurological and muscular degenerative diseases as well as pathologies associated with human aging [6,7]. Many of these mitochondrial (mt) related diseases are associated with defects in normal ATP production caused either by mutations in mt DNA itself or mutations in nuclear-encoded proteins that maintain the integrity of mt DNA [8–11]. These inherited diseases are thought to be as frequent as 1 in 4000 individuals, but they are difficult to diagnose due to wide range of symptoms and lack of reliable screening methods [12].

The mt genome is a circular double-stranded DNA that is widely believed to have originated from an ancient invasion by an α-proteobacterium-like ancestor into an archaea type host [13–15]. The mt DNA genome sizes vary greatly among various organisms, ranging from one of the smallest 16.5 kb intron-less human mt genome to the much larger 75 kb yeast *Saccharomyces cerevisiae* mt (Ymt) genome. Despite different genome sizes, the primary function of mt DNA is to code for proteins that synthesize ATP via the oxidative phosphorylation pathway and for components of mitochondrial ribosome. Mitochondria are not self-sufficient however and they depend on the nuclear DNA. A large number of proteins for ATP synthesis and all of the proteins required for mt DNA maintenance, including components of mt DNA replication, transcription, and repair are encoded by the nuclear DNA, and the proteins are synthesized in the cytoplasm and imported into the mitochondria.

The transcriptional machinery of mitochondria is distinct from the nuclear. The mt transcriptional machinery is much simpler and consists of two or three components: the mt RNA polymerase (mtRNAP) and one or two accessory transcription factors. The mtRNAP shows sequence and structural similarity to the bacteriophage T7/T3 class of single-subunit RNAPs [16,17]. However, unlike phage RNAPs that catalyze all the steps of transcription without requiring any accessory factors [18], the mtRNAP depends on accessory transcription factors for initiation [19–21]. Since multi-subunit RNAPs, which transcribe bacterial and nuclear genomes, also rely on accessory transcription factors [22–25], the mtRNAPs provide a mechanistic link between single and multi-subunit RNAPs. This leads to interesting questions on the evolution of mt transcription.

Much of our understanding of the mechanism and regulation of mt DNA transcription, including that of human mt DNA, is derived

Abbreviations: mtRNAP, mitochondrial RNAP; Ymt, yeast mitochondrial; Mtf1, mitochondrial transcription factor 1; nt, nucleotides; aa, amino acids; SAM, S-adenosyl-L-methionine; K_d, equilibrium dissociation constant; FRET, fluorescence resonance energy transfer; 2-AP, 2-aminopurine; K_m, Michaelis–Menten constant

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from studies of Ymt transcription. Biochemical studies of Ymt transcription are facilitated by the simpler two-component organization of Ymt transcription machinery and the genetic studies by the ease of creating and screening mutants in yeast. The two components of the Ymt transcription machinery, Rpo41 (the catalytic mtRNAP component) and Mtf1 (transcription factor), are homologous to human POLRMT and TFB2M, respectively [26–29], and sufficient for catalyzing transcription *in vitro* [30]. However, human mt transcription requires TFAM, a homolog of Ymt Abf2 protein [31], which both activates and regulates transcription by POLRMT-TFB2M complex [32].

The focus in this review is on recent structural and biophysical studies of mt transcription and comparisons to the mechanisms of T7 RNAP. Experimental evidence presented here primarily addresses the Ymt transcription machinery with parallels drawn from the players of the homologous human mt transcription. Mitochondrial DNA transcription has been a topic of many excellent and comprehensive reviews [19,33–35]. Comparison of mtRNAP and T7 RNAP provides a better understanding of the roles of mtRNAP and transcription accessory factors. We discuss similarities and differences between the RNAPs of the two in promoter DNA recognition, discrimination against non-promoter DNA sequences, promoter melting, nucleotide binding, and promoter clearance. Mitochondrial transcription is known to play a key role in regulating mt gene expression, especially in response to changing ATP levels. We discuss a model that postulates that the Ymt transcriptional machinery serves as the *in vivo* ATP "sensor" to regulate gene expression in mitochondria.

2. Mitochondrial DNA transcriptional machinery

2.1. The mitochondrial RNA polymerase

The mtRNAP protein can be divided into several domains including the C-terminal domain, N-terminal domain, and N-terminal extension based on amino acid sequence similarity to the single-subunit T7 RNAP. A homology model of Ymt Rpo41 was created from sequence and structural similarity to T7 RNAP-promoter complex (Fig. 1A, B) [36,37]. This remarkable similarity between Rpo41 and T7 RNAP was confirmed by the recently determined crystal structure of human POLRMT (218–1230 region, Fig. 1C) [38]. The C-terminal domain of Rpo41 (~800 aa from a total of 1351 aa) shows 49–77% sequence identity in 8 regions to the C-terminal domain of T7 RNAP (~740 aa from a total of 883 aa), and this type of similarity is generally observed for other mtRNAPs [16,37,39]. The N-terminal domain (~200 aa) has low sequence similarity, but the crystal structure of POLRMT shows that parts of it are structurally similar to the N-terminal domain of T7 RNAP. The N-terminal extension (~250 aa) is the most variable region in mtRNAPs and it is completely missing in T7 RNAP.

2.1.1. The C-terminal domain of mtRNAP

The C-terminal domain of mtRNAPs (~800 aa) contains the elements to bind the template DNA, NTP, and Mg^{2+} and contains the catalytic site for the nucleotidyl transfer reaction to make RNA. The human POLRMT structure shows that this domain resembles a right hand with palm, thumb, and fingers subdomains (Fig. 1A–C), which are conserved elements in Pol-A family polymerases. A unique feature of T7 RNAP in this family of polymerases is the presence of specific insertions in the fingers subdomain that aid in sequence specific recognition of the promoter sequence. T7 RNAP contains a prominent insertion in the fingers subdomain called the promoter specificity loop, which forms an antiparallel β-ribbon that inserts into the major groove of the promoter DNA, where it makes base-specific interactions with −7 and −8 positions [40,41]. Sequence and structure alignments have identified promoter specificity loops in Rpo41 and POLRMT, and biochemical studies with Rpo41 have confirmed its role in promoter recognition. Mutational and chemical-nuclease studies indicate that the promoter specificity loop in Rpo41 (1127–1149, Fig. 1D) binds the Ymt promoter between −1 and −8 [36,37]. Residues in the promoter specificity loop, namely Glu-1224, Lys-1127, Gln-1135, Gln-1129 and Thr-1136, are crucial for promoter-specific transcription, and the latter two amino acids interact with the −7 position of the Ymt promoter [37,42]. Many of the amino acids in the proposed promoter specificity loop of POLRMT (1086–1105, Fig. 1D) are missing in the crystal structure [38]. It may indicate that the promoter specificity loop assumes a defined structure only in the presence of the promoter DNA.

The fingers subdomain of T7 RNAP contains a second insertion called the fingers flap. The fingers flap domain has been proposed to interact with the non-template strand in the initiation and elongation complex [43,44]. Although, its function is not completely defined, the fingers flap has been proposed to aid promoter melting during initiation and to control processivity during elongation [45,46]. Sequence alignment shows that the fingers flap region is missing in Rpo41 [36]. Based on the finding that Mtf1 interacts with the non-template strand, an interesting possibility was suggested that Mtf1 adopts the role of fingers flap region in promoter melting (Section 4).

2.1.2. The N-terminal domain of mtRNAP

The N-terminal domain of mtRNAP (~200 aa) shows moderate sequence similarity to the N-terminal domain of T7 RNAP. The N-terminal domain of T7 RNAP contains several elements that bind to the promoter in the initiation complex. Two specific elements, the AT-rich recognition loop and the intercalating hairpin, interact with the T7 promoter in the initiation complex [44,47]. The AT-rich recognition loop binds into the minor groove of the upstream promoter region between −13 and −17 causing a slight bend in the DNA. The tip of the intercalating hairpin acts as a wedge and positions at the single-stranded/double-stranded junction between −4 and −5 to stabilize the melted promoter [40,48]. Homology modeling and crystal structure have identified such elements in Rpo41 and human POLRMT [36–38]. The intercalating hairpin (Rpo41:617–630; POLRMT:610–620) appears to be important in promoter melting because deletion of five residues at the tip of the intercalating hairpin in POLRMT inactivates transcription from duplex but not pre-melted promoter [38]. The function of the AT-rich recognition loop (Rpo41: 481–497; POLRMT: 450–470, Fig. 1D) is less clear as single mutations in this region do not affect transcription by POLRMT [38].

2.1.3. The N-terminal extension of mtRNAP

The N-terminal extension (~300 aa) of mtRNAP is the most variable region among various species and it is not present in T7 RNAP. It contains the signal peptide for targeting into mitochondria, which is cleaved off after import by mitochondrial processing peptidases. Deletion of 1–100 or −185 aa in the N-terminal extension of Rpo41 has little effect on Ymt transcription, *in vitro* or *in vivo* [39,49]. Remarkably, deletion of 1–270 aa in Rpo41 increases the efficiency of *in vitro* transcription by reducing abortive synthesis without affecting synthesis of the full-length transcript [39]. Hence, it was proposed that the N-terminal extension of Rpo41 is autoinhibitory and its deletion relieves this effect. A larger deletion of 1–380 aa greatly decreases transcription from duplex promoter, but not from pre-melted promoter [39].

Based on these studies, it was proposed that the 270–380 region is important for promoter melting. Furthermore, protein–protein cross-linking studies showed the Rpo41 deletion mutants bind Mtf1 more weakly, and hence it was proposed that the N-terminal extension may be involved in interactions with Mtf1 [39]. However, direct interactions of the isolated N-terminal extension regions with Mtf1 could not be demonstrated [50], which could be because the isolated extension regions do not fold properly on their own. The crystal structure