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TEFM Enhances Transcription Elongation by Modifying mtRNAP Pausing Dynamics

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ABSTRACT Regulation of transcription elongation is one of the key mechanisms employed to control gene expression. The single-subunit mitochondrial RNA polymerase (mtRNAP) transcribes mitochondrial genes, such as those related to ATP synthesis. We investigated how mitochondrial transcription elongation factor (TEFM) enhances mtRNAP transcription elongation using a single-molecule optical-tweezers transcription assay, which follows transcription dynamics in real time and allows the separation of pause-free elongation from transcriptional pauses. We found that TEFM enhances the stall force of mtRNAP. Although TEFM does not change the pause-free elongation rate, it enhances mtRNAP transcription elongation by reducing the frequency of long-lived pauses and shortening their durations. Furthermore, we demonstrate how mtRNAP passes through the conserved sequence block II, which is the key sequence for the switch between DNA replication and transcription in mitochondria. Our findings elucidate how both TEFM and mitochondrial genomic DNA sequences directly control the transcription elongation dynamics of mtRNAP.

INTRODUCTION

Mitochondrial genomic DNA (mtDNA) is a circular 16.5-kb DNA and encodes 13 genes related to ATP synthesis, 22 transfer RNAs, and 2 ribosomal RNAs. Mitochondrial RNA polymerase (mtRNAP) is a single-subunit enzyme evolutionarily related to T3 and T7 bacteriophage RNA polymerases, which differ from the 12-subunit nuclear RNA polymerase II (Pol II) (1).

The mtRNAP transcription initiation process requires mitochondrial transcription factor A (TFAM) and mitochondrial transcription factor B2 (TFBIIM) to bind to mitochondrial promoters. TFAM, which works as a transcription initiation factor as well as an mtDNA packaging protein, binds upstream of the promoters and induces a DNA U-turn (2–4). mtRNAP is then recruited to the promoter initiation site, followed by TFBIIM, to form the initiation complex (5). Yeast TFBIIM ortholog mitochondrial transcription factor Mtf1 plays a similar role in yeast (6,7). These features are different from the Pol II transcription initiation process, which is not coupled to DNA replication and requires many transcription initiation factors, such as TFIIA, TFIIB, and TFIID. In addition, mtRNAP transcription initiation is also different from that of T7 RNA polymerase, which does not require any transcription initiation factors (8,9). With regard to the transcription elongation process, mtRNAP and nuclear Pol II seem to share more similarities. Compared to the T7 RNA polymerase, mtRNAP transcribes much slower (129 ± 8 nt/s for T7 RNA polymerase vs. 25 ± 2 nt/s for yeast mtRNAP (RPO41)) in both bulk biochemical and single-molecule optical-tweezers transcription elongation assays (10,11). mtRNAP transcription elongation speed as well as pause frequency and durations are similar to those of Pol II (11,12).

Mitochondrial transcription elongation factor (TEFM) is a transcription elongation factor only present in vertebrate and was first identified as a nuclear transcription factor Spt6-like protein (13). Both TEFM and Spt6 contain two tandemly repeated helix-hairpin-helix motifs and one RNase H-fold, but their RNase H-fold type and the helix-hairpin-helix positions are different (13). TEFM enhances the processivity of transcription elongation by binding to mtRNAP, the DNA, and the nascent RNA and stabilizing the mtRNAP transcription elongation complex (13–16). TEFM knockdown in human bone osteosarcoma cells

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largely decreased promoter-distal mitochondrial transcripts and reduced respiratory chain component abundance (13).

In addition to a general transcription enhancing effect, TEFM is required for mtRNAP to pass through the conserved sequence block II (CSBII), which is the mtRNAP transcription termination sequence. Within CSBII, a DNA-RNA G-quadruplex structure is formed to promote termination (14,16). It has been suggested that transcription termination at CSBII may stimulate DNA replication initiation by producing a replication primer (16,17). Therefore, TEFM may be important for regulating the switch between transcription and DNA replication of mitochondrial DNA. A recent structural study shows that TEFM binds mtRNAP near the RNA exit channel, and its binding has been proposed to prevent the formation of the DNA-RNA G-quadruplex (15).

Here, we studied the effects of TEFM on transcription elongation using a single-molecule optical-tweezers assay. We found that TEFM does not change pause-free velocity and short-lived pauses but enhances transcription elongation by reducing long-lived pause frequencies and long-lived pause durations. Furthermore, our data suggest that TEFM increases the probability of mtRNAP's passage through CSBII by preventing the formation of the RNA-DNA hybrid G-quadruplex. These findings elucidate the mechanism of how TEFM regulates transcription elongation on mitochondrial DNA at the single-molecule level.

MATERIALS AND METHODS

Protein expression and purification

Human mtRNAP was cloned in a modified pET28 vector, His- and Avi-tagged at its N-terminus, and expressed with a BirA expression plasmid (Avidity) in *Escherichia coli* BL21(DE3) cells. Expression was induced at 16°C for 20 h in the presence of 50 μM biotin and 0.2 mM isopropyl β-D-1-thiogalactopyranoside. Harvested cells were resuspended in lysis buffer (50 mM NaH_2PO_4 (pH 8.0), 300 mM NaCl, 10 mM imidazole, 5 mM 2-mercaptoethanol, and 1 mM phenylmethane sulfonyl fluoride) followed by sonication. The samples were loaded onto SoftLink Soft Release Avidin Resin (Promega, Madison, WI), Ni^{2+} nitrilotriaetic acid agarose beads (Qiagen, Hilden, Germany), and then onto a Hitrap Heparin column (General Electric Healthcare, Chicago, IL). Recombinant TEFM was expressed in *E. coli* BL21(DE3) codonplus cells and purified using established methods (14).

In vitro biochemical transcription elongation assay

To biochemically examine the elongation activity of mtRNAP in the absence or presence of TEFM, we made a transcription elongation complex by hybridizing a 3 pmol of [^{32}P] -γATP labeled synthetic 9-nt (nucleotide) RNA (5′-GACGCCCGA-3′) to 1.5 pmol of the template DNA (5′-P-CTC TTCCCGGGTTCGCCTTGTCTCGGGCGTCGGCTGTAAGTATCCTAT ACCGTCGGCAT-3′), subsequently adding 1.5 pmol of mtRNAP, and annealing 7.5 pmol of the other DNA strand (5′-CCGACGGTATAGGA TACTTACAGCCGACGCCCGAGACAAGGCGAACCCGGGAAGAGG TT -3′) following the method of Zamft et al. (11) and Kireeva et al. (18). Synthesized mtDNA containing the CSBII sequence (245 bp, with *Dra*III site between +161 and +386 in the mitochondrial genome, BGI) (Table S1) was used as the downstream template. The template was ligated with the mtRNAP elongation complex in 1% polyethylene glycol (PEG-8000), 20 mM Tris-HCl (pH 7.9), 40 mM KCl, 10 mM $MgCl_2$, 10 μM $ZnCl_2$, and 2 mM 2-mercaptoethanol at 25°C. The mtRNAP elongation complexes (50 nM) were generated by incubating 50 μM each of rCTP, rGTP, and rATP at 30°C for 5 min. Then, the elongation complexes were incubated with 200 nM TEFM for 10 min and chased with 1 mM of ribonucleoside triphosphates at 30°C for 15 min. To stop the reaction, we added an equal volume of urea-containing loading dye (8 M urea, 89 mM Tris-HCl (pH 8.0), 89 mM boric acid, 50 mM EDTA, and 0.25% bromophenol blue) to the samples and heated it at 65°C for 20 min. We then ran the reaction samples in a denaturing urea acrylamide gel. Gels were scanned and quantified by a Typhoon phosphorimager (GE Healthcare, Chicago, IL).

Single-molecule transcription elongation assay

Single-molecule transcription elongation assays using the optical tweezers were performed in passive force mode as previously reported (11,19). Briefly, the mtRNAP elongation complex (1.5 nM) was conjugated to a bead coated with avidin (Spherotech, Lake Forest, IL). The mtRNAP elongation complex was then attached to a digoxigenin-labeled λ-DNA template, which was later conjugated to a second bead coated with antidigoxigenin antibody (Spherotech). We then flowed a 1-mM NTP mixture into the sample chamber. For experiments in the presence of TEFM, 200 nM TEFM was added to the NTP solution. The opposing force experiments were performed using a 4974-bp λ-DNA template (the sequence information can be found in Table S1). Only mtRNAP molecules that transcribed up to a force higher than 4 pN were considered active. Assisting force experiments were performed using the synthesized mtDNA containing the CSBII sequence (Table S1) and started at around 9 pN.

Pause analysis

Raw position data (1 kHz) were averaged by decimation to 20 Hz and then smoothed using a first-order Savitzky-Golay filter with a time constant of 1 s. The dwell times were measured from the filtered data by recording the time it took for the polymerase to advance 3 bp. A pause threshold was selected as a multiple of the mean dwell time. For most traces, the pause threshold was ~1 s per 3 bp. Regions of traces with dwell times longer than this pause threshold were considered pauses. Only pauses with durations between 1 and 60 s were considered for further analysis.

RESULTS

TEFM significantly enhances the stall force of mtRNAP from 6.7 pN to 10.3 pN

Similar to the nuclear transcription elongation process, mitochondrial transcription is controlled by transcription elongation factors. To examine the effects of TEFM on mtRNAP elongation, we performed in vitro single-molecule transcription elongation assays using optical tweezers (Fig. 1 *A*). We first used an opposing-load geometry, in which the force on the enzyme increases as transcription progresses (Figs. 1, *A* and *B* and S1). This geometry hinders mtRNAP transcription elongation and thus facilitates the measurement of the enhancing effects of TEFM. Specifically, as the force load increases, the probability of mtRNAP pause entry increases and, eventually, transcription stalls. In general, the maximal force against which a motor can transcribe is referred to as its stall force. The stall

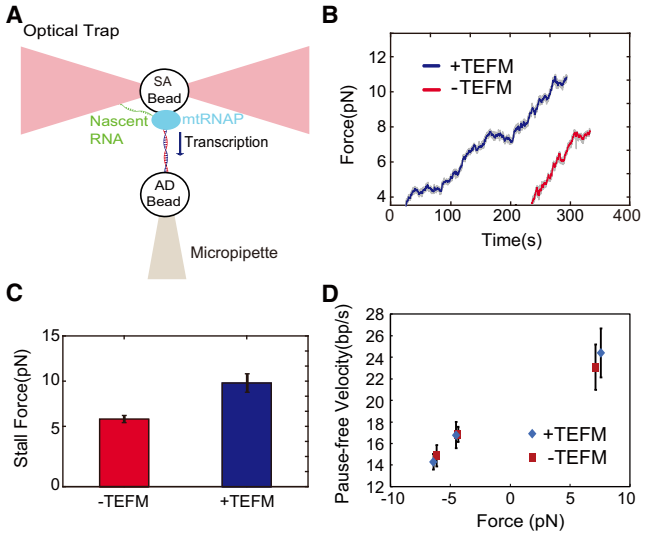

FIGURE 1 TEFM increases the stall force but does not change the pause-free velocity of mtRNAP. (*A*) Geometry of the single-molecule opposing-force transcription elongation assay. The elongation complex is bound to a streptavidin-coated bead (SA), whereas the handle DNA is bound to an anti-digoxigenin-coated bead (AD). During transcription elongation, mtRNAP translocates along the DNA, increasing the opposing force acting on the enzyme. The SA bead movement is detected by the optical trap. (*B*) Representative mtRNAP traces in the absence (*red*) and presence (*blue*) of TEFM. The gray lines correspond to 20-Hz decimated data, whereas the red and blue lines correspond to data filtered (Savitzky-Golay) to 1 Hz. (*C*) The mean stall force in the absence (*red*) and presence (*blue*) of TEFM. (*D*) The pause-free velocities of the mtRNAPs are plotted against the applied force (error bars indicate means ± standard error). Positive and negative force values indicate assisting and opposing forces, respectively. $n = 18$ traces for each experimental condition in (*C*) and (*D*).

forces of RNA polymerase are quite different among species. *E. coli* RNA polymerase transcribes up to 20 pN, whereas yeast Pol II transcribes up to only 8 pN (12,20). The mean stall force of human mtRNAP was found to be 6.7 ± 0.3 pN, which is very similar to that of yeast mtRNAP (11), and it increased to 10.3 ± 1.1 pN in the presence of TEFM (Fig. 1 *C*).

Only long-lived mtRNAP transcriptional pauses are shortened and reduced in frequency by TEFM

The overall elongation speed of mtRNAP in the presence of TEFM (3.81 ± 0.37 nt/s) is faster than that in absence of TEFM (2.35 ± 0.24 nt/s). These results indicate that TEFM enhances overall mtRNAP elongation consistent with previous studies (13). However, transcription elongation consists of pause-free translocation interrupted by pauses, which can be distinguished by the optical-tweezers transcription assay. Therefore, we sought to determine which of these variables are modulated by TEFM. We found that pause-free transcription elongation velocities for mtRNAP in the absence and presence of TEFM are the same within experimental error at around 14–15 nt/s in the 5.5- to 8-pN opposing force range and 16–17 nt/s in the 3- to 5.5-pN opposing force range (Fig. 1 *D*). Furthermore, pause-free elongation speed increases to 23–24 nt/s in the 5- to 9-pN assisting force range, but, again, no significant difference in the pause-free velocity was observed in the presence of TEFM under this condition (Fig. 1 *D*).

The observation that TEFM increases the overall elongation speed without affecting the pause-free velocity indicates that TEFM may influence the pausing dynamics of mtRNAP. The cumulative pause duration distributions (1–60 s) in the absence and presence of TEFM were first compared. This evaluation revealed that TEFM may shorten long-lived pauses (pause durations longer than 4 s) without affecting short-lived pauses (Fig. S2 *A*). To test this hypothesis, we compared long-lived and short-lived pauses (pause durations shorter than 4 s). Similar to other RNA polymerases, we observed that pause densities for mtRNAP were force sensitive (Fig. 2, *A* and *B*; Table S2). Interestingly, only long-lived pause densities were reduced, and their mean duration was shortened in the presence of TEFM at all force ranges (Fig. 2, *B* and *D*; Table S2). We observed no significant effects of TEFM on short-lived pauses (Fig. 2, *A* and *C*; Table S2). These trends were also observed in the cumulative pause-duration distribution plots (Fig. S2, *B* and *C*). These data suggest that TEFM mainly affects force-sensitive long pauses (e.g., long pauses that involve resolving an RNA secondary structure).

Furthermore, we estimated the density of pauses shorter than 1 s (only pauses longer than 1 s can be experimentally detected) using a double exponential fit to the pause duration distribution (Fig. S3, *A–D*; Table S2) (21). Interestingly, we found that the density of such pauses increased in the

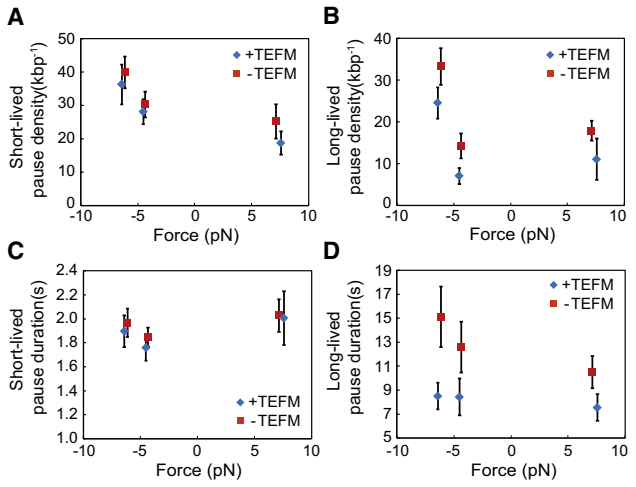

FIGURE 2 TEFM reduces only long-lived pause frequencies and durations. Mean (*A*) short- (≤4 s) and (*B*) long- (>4 s) lived pause densities of mtRNAP in the absence and presence of TEFM as a function of force. Error bars represent means ± standard error. Mean (*C*) short- (≤4 s) and (*D*) long- (>4 s) lived pause durations of mtRNAP in the absence and presence of as a function of force. Table S2 shows the number of pauses (*N*) used for each condition.

presence of TEFM (Fig. S3, *E* and *F*). This result suggests that TEFM might shift long pauses to sub-1-s pauses.

TEFM prevents the formation of the RNA-DNA hybrid G-quadruplex within the mitochondrial CSBII transcription termination sequence

CSBII is a very important transcription regulation sequence present in human mitochondrial DNA. CSBII contains a G-rich motif sequence that plays a crucial role during transcription termination. When mtRNAP transcribes through CSBII, repeated guanines within CSBII and the nascent RNA transcript form a G-quadruplex with the upstream DNA sequence (17). The G-quadruplex formation at CSBII ultimately triggers transcription termination. In addition to the DNA-RNA G-quadruplex formation, there is also a pretermination site at CSBII, UUAUU. Both DNA-RNA G-quadruplex and the pretermination site play an important role in transcription termination (22). However, in the presence of TEFM, mtRNAP can proceed with transcription (14,16). Given the importance of this transcription regulation sequence, we sought to investigate the impact of TEFM on transcription elongation on CSBII.

Consistent with previous observations, we observed that mtRNAP transcription was repressed at CSBII and that TEFM enhanced transcriptional passage through CSBII (Fig. S4) (14). Transcription dynamics at CSBII were monitored using the optical-tweezers assay (Fig. 3 *A*). We observed that 76% of mtRNAP molecules stopped at the CSBII sequence in the absence of TEFM (Fig. 3 *B*). When mtRNAP entered CSBII, a shortening of the DNA tether length was observed (Fig. 3 *B*, *lower panel*). The average shortening length was 15.5 ± 0.7 bp (n = 17; Fig. S5), which is consistent with the predicted size of the DNA-RNA G-quadruplex structure (17). After the G-quadruplex was formed, the majority of mtRNAP molecules did not resume elongation (n = 13), and only a few passed through CSBII (n = 4). In such rare recovery cases, the tether length change rate immediately after recovery was 50.0 ± 2.5 bp/s, which is significantly higher than the pause-free velocity of the motor. This observation also points to the formation (tether shortening) followed by disruption (tether extension) of the G-quadruplex rather than to a large backtrack followed by recovery. In the presence of TEFM, the passage through CSBII significantly increased as previously reported (Fig. 3 *B*) (14,16). Interestingly, we did not observe any large tether-shortening events at the CSBII region in the presence of TEFM (Fig. 3 *B*, events shown with *). This result suggests that TEFM plays a role in preventing the formation of the G-quadruplex and supports the prediction based on the crystal structure of the mtRNAP elongation complex with TEFM (15). Further analysis showed that TEFM reduced the dwell times at all regions, especially at the CSBII, from 1.54 ± 0.26 s/nt in absence of TEFM to 0.69 ± 0.22 s/nt in the presence of

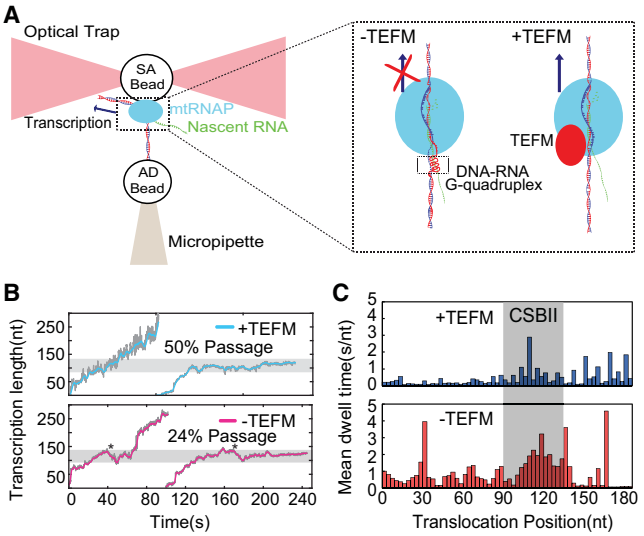

FIGURE 3 TEFM prevents formation of the G-quadruplex and reduces dwell times at the CSBII sequence. (*A*) Geometry of the single-molecule transcription elongation assay on the CSBII sequence. During transcription elongation, mtRNAP translocates along the DNA, decreasing the assisting force acting on the enzyme. The inset illustrates the mtRNAP elongation complex within the CSBII region in the absence (*left*) and presence (*right*) of TEFM. (*B*) Example traces of mtRNAP transcription elongation on the CSBII sequence. The gray region corresponds to CSBII. The * indicates the appearance of tether-shortening events likely associated with G-quadruplex formation. The gray lines correspond to 20-Hz decimated data, whereas the red and blue lines correspond to data filtered (Savitzky-Golay) to 1 Hz. (*C*) Dwell times as a function of position in the absence and presence of TEFM. The gray region corresponds to the CSBII sequence. n = 17 traces in the absence of TEFM, and n = 12 traces in the presence of TEFM for (*B*) and (*C*). AD, antidigoxygenin-coated bead; SA, streptavidin-coated bead.

TEFM (Fig. 3 *C*; Table S3). Interestingly, TEFM reduced pause durations and densities within CSBII to levels similar to those outside CSBII (Fig. 4, *A* and *B*; Table S4).

DISCUSSION

One notable aspect of the dynamics of RNA polymerases is their propensity to pause during elongation. Pausing has

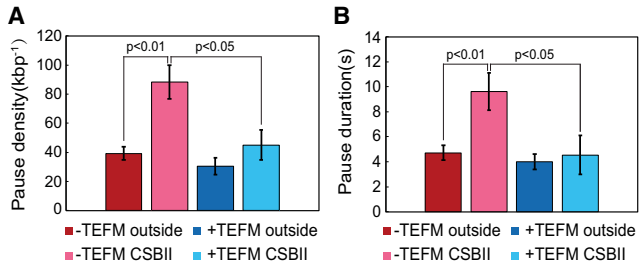

FIGURE 4 TEFM abolishes increased pause durations and densities within CSBII. (*A*) Mean pause densities within and outside the CSBII region. n = 17 in the absence of TEFM, and n = 12 in the presence of TEFM. (*B*) Mean pause durations within and outside the CSBII region The p-value corresponds to the Student's t-test. Table S4 shows the number of pauses used for each condition.

been reported in most RNA polymerases and represents a crucial mechanism of transcriptional regulation (23). For instance, transcription elongation factors TFIIS and TFIIF enhance Pol II transcription elongation by modulating pausing without affecting its pause-free velocity (24,25). In a similar fashion, the results presented in this study demonstrate that TEFM modulates the pausing dynamics of mtRNAP by decreasing the duration and frequency of long-lived pauses. Furthermore, we found that TEFM prevents the formation of the DNA-RNA G-quadruplex at the CSBII sequence, which is an important element for the switch between DNA replication and transcription in mitochondria.

The Pol II transcription process can be divided into five main steps: 1) translocation, 2) NTP binding, 3) NTP sequestration/conformation change, 4) bond formation, and 5) pyrophosphate release, with step 4 being the rate-limiting step (26). From a recent crystal structural study, the post-translocated state of the mtRNAP elongation complex is stabilized by TEFM relative to the pretranslocated state, a result that indicates that TEFM might favor forward translocation (15). However, the catalytic step has been shown to be rate limiting in Pol II (26). Therefore, the overall pause-free velocity of mtRNAP may remain unchanged with TEFM addition for this reason. Additional investigations are needed to determine whether TEFM affects some of the on-pathway transcription steps in addition to the effect on pausing dynamics described here.

TEFM stabilizes the mtRNAP transcription elongation complex by interacting with nascent RNA and DNA both upstream and downstream of the elongation complex (15,16). Our results show that TEFM attenuates long-lived pauses without affecting short-lived pauses. These data, in combination with the structural study of TEFM, suggest that TEFM may reduce nascent RNA (sequence)-dependent pauses. We also observed that short-lived pauses are force insensitive. This result suggests that mtRNAP may have backtrack-independent pauses. For instance, *E. coli* RNAP has been shown to experience sequence-independent pauses not associated with backtracking (elemental pauses), characterized by a half-translocated state of the enzyme (27–32). Further studies are needed to confirm whether several types of pauses for mtRNAP exist and to determine how the DNA sequence influences mtRNAP transcription elongation, pausing, and the effect of TEFM. Taken together, these further studies would help elucidate how mitochondrial gene expression is controlled.

Furthermore, we found that TEFM enhanced mtRNAP passage through CSBII, likely preventing the formation of the DNA-RNA G-quadruplex within this sequence. Mitochondrial DNA contains ~200 putative DNA-DNA G-quadruplex sequences (33,34) whose presence has been recently proven using a fluorescent anticancer reagent (35). DNA-DNA G-quadruplexes constitute very strong transcriptional barriers in front of or behind the mtRNAP elongation complex (36). Mitochondrial DNA helicase Twinkle has been reported to unwind G-quadruplex structures, allowing transcription through G-rich sequences (34). In addition to the DNA-RNA G-quadruplex formation, there is also a pretermination site at CSBII, UUAUU. Both play an important role in termination (22). Furthermore, other factors affect transcription elongation within CSBII. TFAM, a transcription initiation factor that binds to mtDNA, acts as a transcriptional repressor and preferentially binds to the G-quadruplex structure and stimulates the G-quadruplex barrier (37). Further studies are necessary to elucidate how TEFM contributes to mtRNAP transcription elongation through the TFAM-protected G-quadruplex structure and whether its binding plays a role in the dynamic switching between mitochondrial DNA replication and transcription.

SUPPORTING MATERIAL

Five figures and four tables are available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(18)31224-4.

AUTHOR CONTRIBUTIONS

H.Y., C.X., H.J., and T.I. designed the research. H.Y., C.X., and H.J. performed the experiments. G.X. and S.D. maintained the instrument. H.Y., C.X., G.X., Y.C., and T.I. analyzed the data. T.I. wrote the manuscript with contributions from all coauthors.

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REFERENCES

1. Masters, B. S., L. L. Stohl, and D. A. Clayton. 1987. Yeast mitochondrial RNA polymerase is homologous to those encoded by bacteriophages T3 and T7. *Cell.* 51:89–99.
2. Ngo, H. B., G. A. Lovely, …, D. C. Chan. 2014. Distinct structural features of TFAM drive mitochondrial DNA packaging versus transcriptional activation. *Nat. Commun.* 5:3077.
3. Ngo, H. B., J. T. Kaiser, and D. C. Chan. 2011. The mitochondrial transcription and packaging factor Tfam imposes a U-turn on mitochondrial DNA. *Nat. Struct. Mol. Biol.* 18:1290–1296.
4. Malarkey, C. S., M. Bestwick, …, M. E. Churchill. 2012. Transcriptional activation by mitochondrial transcription factor A involves preferential distortion of promoter DNA. *Nucleic Acids Res.* 40:614–624.
5. Morozov, Y. I., A. V. Parshin, …, D. Temiakov. 2015. A model for transcription initiation in human mitochondria. *Nucleic Acids Res.* 43:3726–3735.
6. Falkenberg, M., N. G. Larsson, and C. M. Gustafsson. 2007. DNA replication and transcription in mammalian mitochondria. *Annu. Rev. Biochem.* 76:679–699.

7. Deshpande, A. P., and S. S. Patel. 2012. Mechanism of transcription initiation by the yeast mitochondrial RNA polymerase. *Biochim. Biophys. Acta.* 1819:930–938.
8. Luse, D. S. 2014. The RNA polymerase II preinitiation complex. Through what pathway is the complex assembled? *Transcription.* 5:e27050.
9. Sousa, R., and S. Mukherjee. 2003. T7 RNA polymerase. *Prog. Nucleic Acid Res. Mol. Biol.* 73:1–41.
10. Thomen, P., P. J. Lopez, and F. Heslot. 2005. Unravelling the mechanism of RNA-polymerase forward motion by using mechanical force. *Phys. Rev. Lett.* 94:128102.
11. Zamft, B., L. Bintu, …, C. Bustamante. 2012. Nascent RNA structure modulates the transcriptional dynamics of RNA polymerases. *Proc. Natl. Acad. Sci. USA.* 109:8948–8953.
12. Galburt, E. A., S. W. Grill, …, C. Bustamante. 2007. Backtracking determines the force sensitivity of RNAP II in a factor-dependent manner. *Nature.* 446:820–823.
13. Minczuk, M., J. He, …, I. J. Holt. 2011. TEFM (c17orf42) is necessary for transcription of human mtDNA. *Nucleic Acids Res.* 39:4284–4299.
14. Posse, V., S. Shahzad, …, C. M. Gustafsson. 2015. TEFM is a potent stimulator of mitochondrial transcription elongation in vitro. *Nucleic Acids Res.* 43:2615–2624.
15. Hillen, H. S., A. V. Parshin, …, D. Temiakov. 2017. Mechanism of transcription anti-termination in human mitochondria. *Cell.* 171:1082–1093.e13.
16. Agaronyan, K., Y. I. Morozov, …, D. Temiakov. 2015. Mitochondrial biology. Replication-transcription switch in human mitochondria. *Science.* 347:548–551.
17. Zheng, K. W., R. Y. Wu, …, Z. Tan. 2014. A competitive formation of DNA:RNA hybrid G-quadruplex is responsible to the mitochondrial transcription termination at the DNA replication priming site. *Nucleic Acids Res.* 42:10832–10844.
18. Kireeva, M. L., L. Lubkowska, …, M. Kashlev. 2003. Assays and affinity purification of biotinylated and nonbiotinylated forms of double-tagged core RNA polymerase II from Saccharomyces cerevisiae. *Methods Enzymol.* 370:138–155.
19. Smith, S. B., Y. Cui, and C. Bustamante. 2003. Optical-trap force transducer that operates by direct measurement of light momentum. *Methods Enzymol.* 361:134–162.
20. Forde, N. R., D. Izhaky, …, C. Bustamante. 2002. Using mechanical force to probe the mechanism of pausing and arrest during continuous elongation by Escherichia coli RNA polymerase. *Proc. Natl. Acad. Sci. USA.* 99:11682–11687.
21. Zhou, J., K. S. Ha, …, S. M. Block. 2011. Applied force provides insight into transcriptional pausing and its modulation by transcription factor NusA. *Mol. Cell.* 44:635–646.
22. Wanrooij, P. H., J. P. Uhler, …, C. M. Gustafsson. 2010. G-quadruplex structures in RNA stimulate mitochondrial transcription termination and primer formation. *Proc. Natl. Acad. Sci. USA.* 107:16072–16077.
23. Kwak, H., and J. T. Lis. 2013. Control of transcriptional elongation. *Annu. Rev. Genet.* 47:483–508.
24. Ishibashi, T., M. Dangkulwanich, …, C. Bustamante. 2014. Transcription factors IIS and IIF enhance transcription efficiency by differentially modifying RNA polymerase pausing dynamics. *Proc. Natl. Acad. Sci. USA.* 111:3419–3424.
25. Schweikhard, V., C. Meng, …, S. M. Block. 2014. Transcription factors TFIIF and TFIIS promote transcript elongation by RNA polymerase II by synergistic and independent mechanisms. *Proc. Natl. Acad. Sci. USA.* 111:6642–6647.
26. Dangkulwanich, M., T. Ishibashi, …, C. J. Bustamante. 2013. Complete dissection of transcription elongation reveals slow translocation of RNA polymerase II in a linear ratchet mechanism. *eLife.* 2:e00971.
27. Herbert, K. M., A. La Porta, …, S. M. Block. 2006. Sequence-resolved detection of pausing by single RNA polymerase molecules. *Cell.* 125:1083–1094.
28. Sydow, J. F., and P. Cramer. 2009. RNA polymerase fidelity and transcriptional proofreading. *Curr. Opin. Struct. Biol.* 19:732–739.
29. Toulokhonov, I., J. Zhang, …, R. Landick. 2007. A central role of the RNA polymerase trigger loop in active-site rearrangement during transcriptional pausing. *Mol. Cell.* 27:406–419.
30. Artsimovitch, I., and R. Landick. 2000. Pausing by bacterial RNA polymerase is mediated by mechanistically distinct classes of signals. *Proc. Natl. Acad. Sci. USA.* 97:7090–7095.
31. Neuman, K. C., E. A. Abbondanzieri, …, S. M. Block. 2003. Ubiquitous transcriptional pausing is independent of RNA polymerase backtracking. *Cell.* 115:437–447.
32. Kang, J. Y., T. V. Mishanina, …, R. Landick. 2018. RNA polymerase accommodates a pause RNA hairpin by global conformational rearrangements that prolong pausing. *Mol. Cell.* 69:802–815.e1.
33. Dong, D. W., F. Pereira, …, B. A. Kaufman. 2014. Association of G-quadruplex forming sequences with human mtDNA deletion breakpoints. *BMC Genomics.* 15:677.
34. Bharti, S. K., J. A. Sommers, …, R. M. Brosh, Jr. 2014. DNA sequences proximal to human mitochondrial DNA deletion breakpoints prevalent in human disease form G-quadruplexes, a class of DNA structures inefficiently unwound by the mitochondrial replicative Twinkle helicase. *J. Biol. Chem.* 289:29975–29993.
35. Huang, W. C., T. Y. Tseng, …, T. C. Chang. 2015. Direct evidence of mitochondrial G-quadruplex DNA by using fluorescent anti-cancer agents. *Nucleic Acids Res.* 43:10102–10113.
36. Agarwal, T., S. Roy, …, S. Maiti. 2014. In the sense of transcription regulation by G-quadruplexes: asymmetric effects in sense and antisense strands. *Biochemistry.* 53:3711–3718.
37. Lyonnais, S., A. Tarrés-Solé, …, M. Solà. 2017. The human mitochondrial transcription factor A is a versatile G-quadruplex binding protein. *Sci. Rep.* 7:43992.