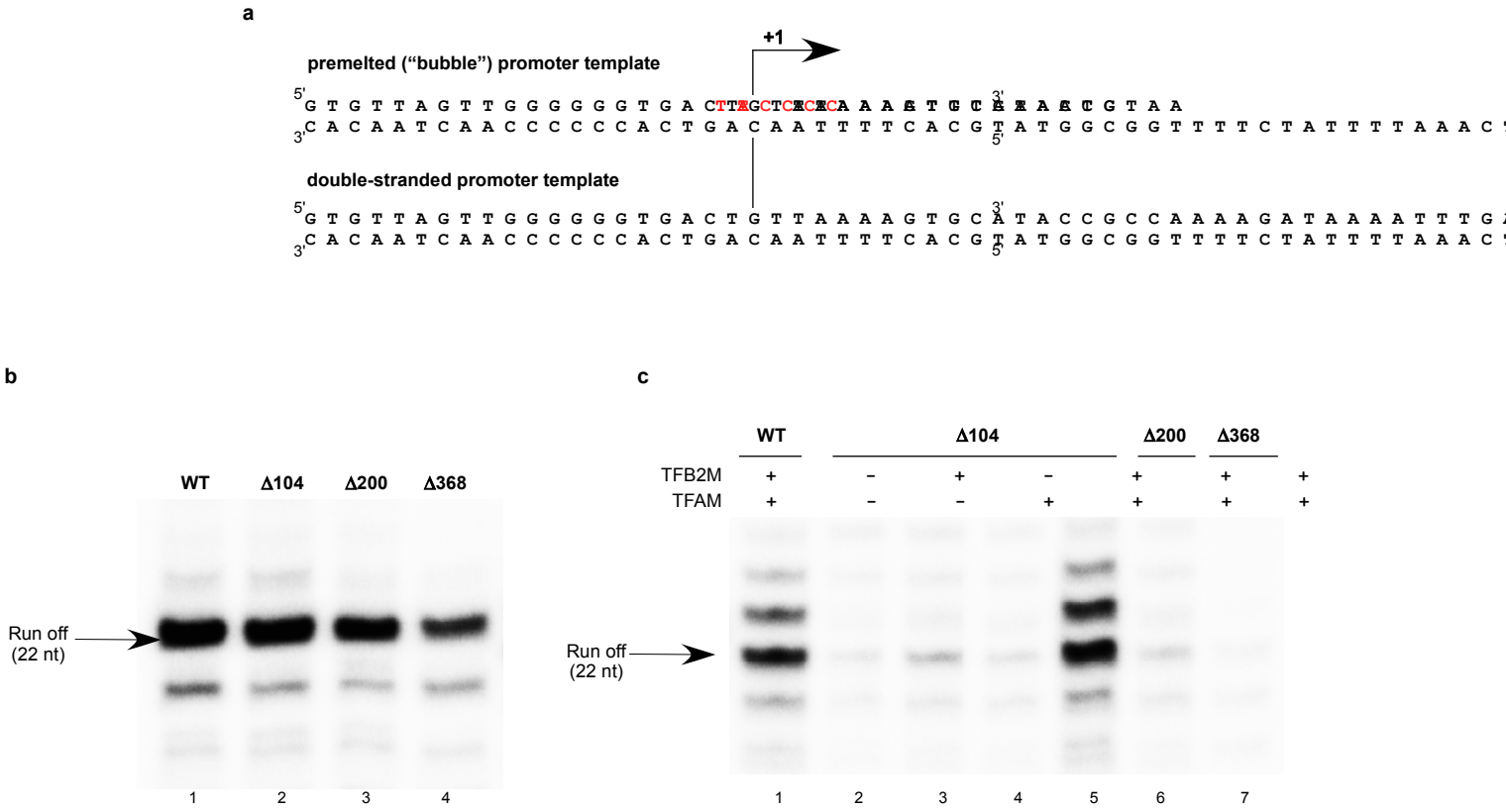
doi:10.1038/nature10435



Supplementary Figure 1. Activity of the N-terminal deletion mutants of human mtRNAP.

a. Templates used in the transcription assays. The light strand promoter (LSP) templates were prepared using synthetic oligonucleotides as described[1]. The pre-melted ("bubble") template contains a 7 bps mismatched region in the non-template strand (highlighted in red).

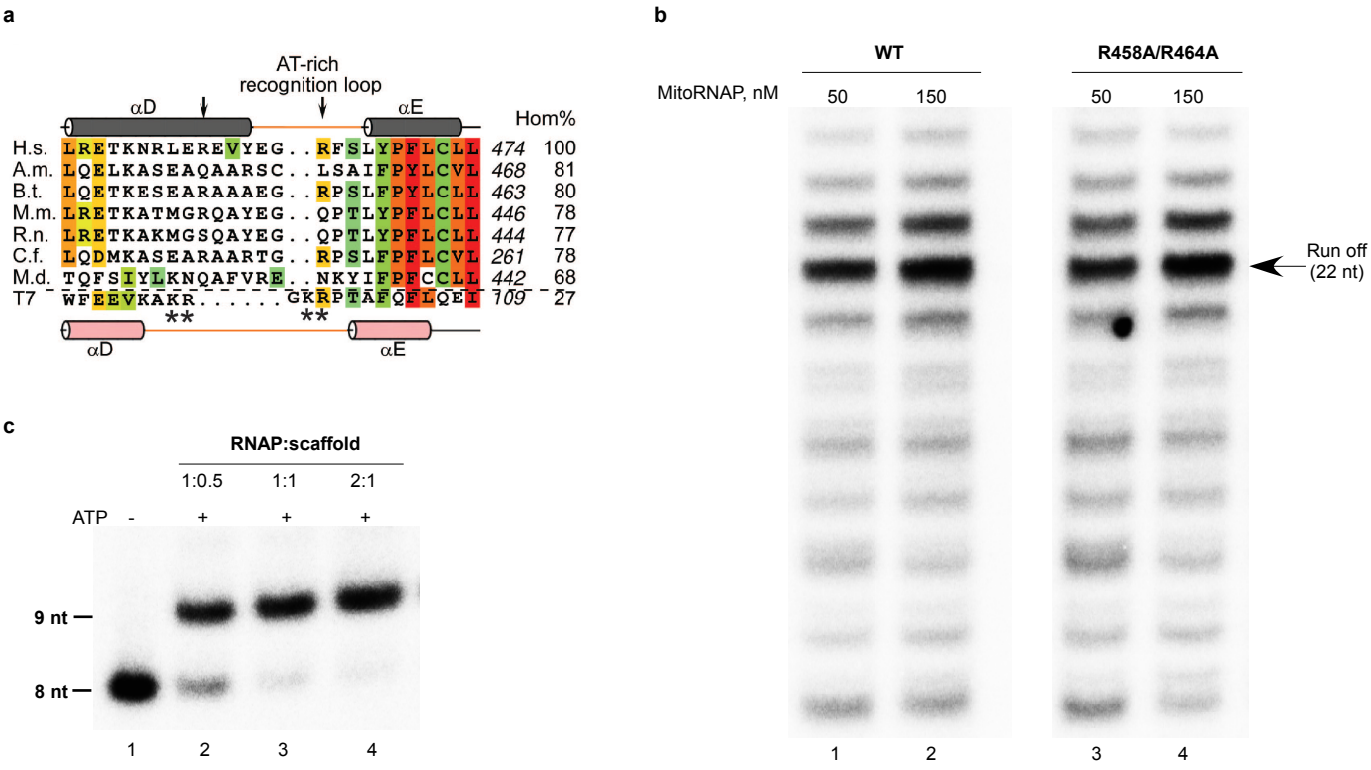
b. Activity of deletion mutants on a pre-melted "bubble" promoter template. Transcription reactions were carried out as described[1] using wild-type (WT), Δ104 (residues 105-1230), Δ200 (201-1230) and Δ368 (369-1230) mtRNAPs for 30 min at 35°C in the absence of transcription factors TFAM and TFB2M.

c. Activity of deletion mutants on a double-stranded promoter template. Transcription reactions were carried out with WT (lane 1) and mutant mtRNAPs (lane 2-7) in the presence or absence of TFAM and TFB2M as previously described[1].

Table 1 Data collection and refinement statistics (**Molecular replacement**)

	Human mitoRNAP
Data collection	
Space group	I 4_1
Cell dimensions	
a, *b*, *c* (Å)	211.3,211.3,60.5
α, β, γ (°)	90, 90, 90
Resolution (Å)	58.1 (2.5) *
R_{merge}	4.7 (63.1)
$I/\sigma I$	15.9 (1.9)
Completeness (%)	99.8 (99.4)
Redundancy	3.8 (3.8)
Refinement	
Resolution (Å)	58.10-2.50
No. reflections	46505
R_{work}/ R_{free}	18.55/ 23.1
No. atoms	
Protein	7588
Ligand/ion	167
Water	145
B-factors	
Protein	89.41
Ligand/ion	101
Water	64.96
R.m.s deviations	
Bond lengths (Å)	0.009
Bond angles (°)	1.12

Number of crystals for each structure should be noted in footnote.
*Highest resolution shell is shown in parenthesis.

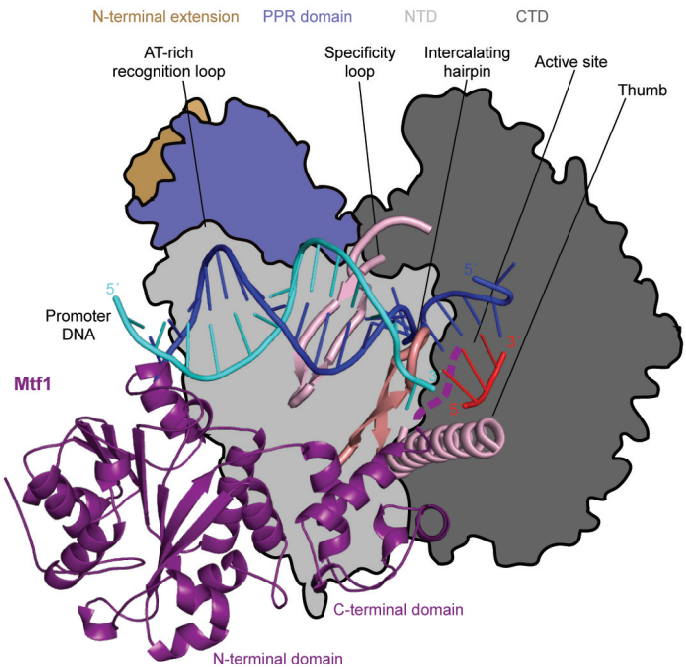


Supplemental Figure 2. Substitution of residues R458 and R464 in the AT-rich recognition loop of human mtRNAP does not affect transcription activity.

a. Sequence alignment of AT-rich recognition loop and adjacent helices in mammalian mtRNAPs and in T7 RNAP. Abbreviations: H.s., Homo sapiens; A.m., Ailuropoda melanoleuca; B.t., Bos taurus; M.m., Mus musculus; R.n., Rattus norvegicus; C.f., Canis lupus familiaris; M.d., Monodelphis domestica. The colour scheme is as in Fig. 2. Percent homology is indicated on the right. The positions of non-conserved and partly conserved arginines in human mtRNAP AT-rich recognition loop are indicated by arrows. Asterisks indicate residues in T7 RNAP involved in promoter interactions.

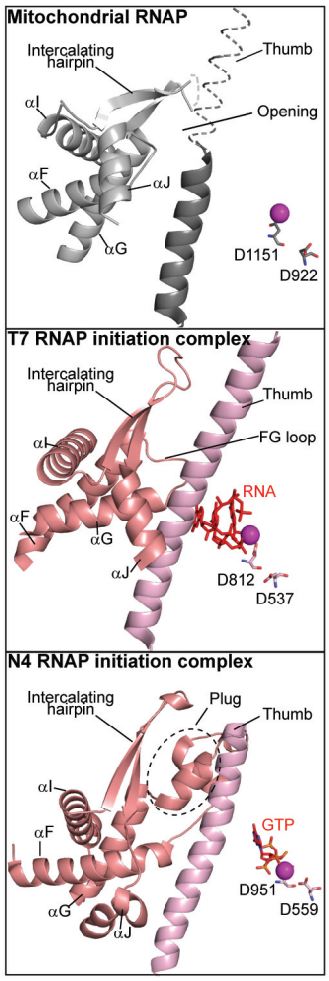
b. R458A/R464A mutant mitoRNAP is fully catalytically active. Elongation complex formed using the mutant mtRNAP and R8/TS1/NT1 scaffold containing P^{32}-labeled RNA (0.25 μM) was extended in the presence of 10 μM ATP for 2 min at 35°C as previously described[2].

c. Activity of the WT and R458A/R464A mitoRNAP on LSP promoter template. Transcription assays were performed as described in Supplemental Figure 1 in the presence of TFAM (50 nM) and TFB2M (150 nM) for 30 min at 35°C.



Supplemental Figure 3. Model of mitochondrial transcription IC.

Since the human TFB2M structure is unknown, we combined the structure of its yeast homolog Mtf1[3], with the human mtRNAP that is expected to closely resemble its yeast counterpart. Mtf1 was modelled onto mtRNAP without generating steric clashes, taking into account that the Mtf1 N-terminal region contains RNAP-binding determinants[4,5], that the Mtf1 C-terminal domain reaches the active site[6], that the 16 most C-terminal residues of Mtf1 contact promoter DNA[3,6], that Mtf1 binding involves the specificity loop and intercalating hairpin[7], and that Mf1 interacts mostly with the promoter at positions -7 to +3[6]. We further assumed that binding of Mtf1 involves the NTD of mtRNAP that likely undergoes refolding upon the transition from IC to EC as in T7 RNAP, thereby displacing Mtf1 upon RNA synthesis[8]. Positioning of Mtf1 enables the C-terminal tail of Mtf1 to use the passage between the thumb and the intercalating hairpin to reach the active site. The Mtf1 yeast homolog structure (PDB 1I4W) is shown as a violet ribbon. MtRNAP is shown as an outline. The promoter DNA was modelled as described in Fig. 4. The intercalating hairpin and specificity loop (light pink ribbon) are shown to occupy positions as in T7 RNAP IC. The model remains tentative since mtRNAP and T7 RNAP structures are in different conformational and functional states and mtRNAP may undergo conformational changes upon binding to nucleic acids.



Supplemental Figure 4. Opening for TFB2M insertion region passage to the mtRNAP active site.

Regions in mitochondrial, T7 and N4 RNAPs involving the thumb, intercalating hairpin and adjacent helices are presented in the same orientation of the corresponding CTDs. Note that only in mtRNAP there is an opening for the Mtf1/TFB2M insertion region to reach the active site[1,6].

Supplementary References

1 Sologub, M., Litonin, D., Anikin, M., Mustaev, A. & Temiakov, D. TFB2 is a transient component of the catalytic site of the human mitochondrial RNA polymerase. Cell 139, 934-944 (2009).

2 Temiakov, D., Anikin, M. & McAllister, W. T. Characterization of T7 RNA polymerase transcription complexes assembled on nucleic acid scaffolds. Journal of Biological Chemistry 277, 47035-47043 (2002).

3 Schubot, F. D. et al. Crystal structure of the transcription factor sc-mtTFB offers insights into mitochondrial transcription. Protein Sci 10, 1980-1988 (2001).

4 Shadel, G. S. & Clayton, D. A. A Saccharomyces cerevisiae mitochondrial transcription factor, sc-mtTFB, shares features with sigma factors but is functionally distinct. Mol Cell Biol 15, 2101-2108 (1995).

5 Cliften, P. F., Park, J. Y., Davis, B. P., Jang, S. H. & Jaehning, J. A. Identification of three regions essential for interaction between a sigma-like factor and core RNA polymerase. Genes Dev 11, 2897-2909 (1997).

6 Savkina, M., Temiakov, D., McAllister, W. T. & Anikin, M. Multiple functions of yeast mitochondrial transcription factor Mtf1p during initiation. J Biol Chem 285, 3957-3964 (2010).

7 Cliften, P. F., Jang, S. H. & Jaehning, J. A. Identifying a core RNA polymerase surface critical for interactions with a sigma-like specificity factor. Mol Cell Biol 20, 7013-7023 (2000).

8 Mangus, D. A., Jang, S. H. & Jaehning, J. A. Release of the yeast mitochondrial RNA polymerase specificity factor from transcription complexes. J Biol Chem 269, 26568-26574 (1994).