Mutations in the Yeast Mitochondrial RNA Polymerase Specificity Factor, Mtf1, Verify an Essential Role in Promoter Utilization*

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The yeast mitochondrial RNA polymerase (RNAP) is a two-subunit enzyme composed of a catalytic core (Rpo41) and a specificity factor (Mtf1) encoded by nuclear genes. Neither subunit on its own interacts with promoter DNA, but the combined holo-RNAP recognizes and selectively initiates from promoters related to the consensus sequence ATATAAGTA. To pursue the question of why Rpo41, which resembles the single polypeptide RNAPs from bacteriophage T7 and T3, requires a separate specificity factor, we analyzed a collection of Mtf1 point mutations that confer an in vivo petite phenotype. These mutant proteins are able to interact with Rpo41 and are capable of nearly wild type levels of initiation on a consensus promoter-containing template (14 S rRNA). However, the petite phenotype of two mutants can be explained by the fact that they exhibit dramatic transcriptional defects on non-consensus promoters. Y54F is incapable of transcribing the weak tRNACys promoter, and C192F cannot transcribe either promoters. Y54F is incapable of transcribing the weak bind to the simple nonanucleotide promoter (consensus ATATAAGTA) and initiate transcription (6). Mtf1 remains associated with Rpo41 during initiation and in the early stages of elongation; this association changes dramatically after a short RNA chain is synthesized and Rpo41 enters into the elongation mode. At this point Mtf1 is readily released from the elongating RNAP (6). In the absence of Mtf1, Rpo41 can initiate transcription non-selectively from a synthetic DNA polymer template poly[d(A-T)]. However, for promoter recognition and selective initiation, Mtf1 is absolutely required (5, 7, 8). Neither the core RNAP nor the specificity factor alone interacts significantly with promoter containing DNA (6, 9).

Rpo41 is a 150-kDa protein with striking amino acid sequence similarity to the T7 and T3 bacteriophage single polypeptide RNAPs (10). Several genes encoding T7-like Rpo41 homologues have been identified in other eukaryotes (reviewed in Ref. 2), and although there are only a few cases where the gene product has been demonstrated to have RNAP activity (11), or to be important for in vivo mitochondrial function (12, 13), there is growing evidence that all higher eukaryotes have an Rpo41-like core mitochondrial RNAP. It is intriguing that a recombinant form of the human Rpo41 homologue is transcriptionally active in vitro on synthetic polymeric templates (11) but inactive on a human mitochondrial promoter and is presumed to be missing an Mtf1-like specificity factor (14).

We are left with an important unanswered question: why do the single polypeptide mitochondrial RNAPs differ from the bacteriophage RNAPs in requiring a dissociable specificity factor? Early mitochondria used a bacterial-like multisubunit RNAP and, presumably, a sigma-like specificity factor for transcription of promoters that still resembled those from bacteria (15). In this regard, it is interesting that Mtf1 has some amino acid sequence similarity to the family of bacterial sigma factors (5), and the AT-rich yeast mitochondrial promoter is recognized as a −10 promoter element by the bacterial RNAP (7). Some of the amino acids shared by sigma factor and Mtf1 include residues important for interactions with their respective core RNAPs (16). Like sigma factors, Mtf1 is only required for initiation (6), and its association with the core RNAP suppresses nonspecific interactions with DNA (7, 9).

The limited amino acid sequence similarity with sigma factors may not reflect a similar three-dimensional structure. The recent report of an Mtf1 crystal structure has revealed clear similarity to the family of RNA and DNA methyltransferases (17). Based on this similarity, Schubot et al. (17) speculate that Mtf1 may not be a promoter specificity factor but may instead bind to Rpo41, converting the enzyme to a promoter recognizing form, or that Mtf1 may act to bind the nascent RNA chain. In this work we have analyzed a collection of point mutants in Mtf1 that are defective for in vivo function but that retain the ability to form a stable complex with Rpo41 and to suppress its
nonspecific interactions with DNA. Among these mutants we have identified two, Y54F and C192F, that are still capable of directing transcription from a consensus promoter but that have lost the ability to transcribe non-consensus variants. These results strongly support the theory that Mtf1 itself, like sigma factor, plays a central role in mitochondrial promoter recognition and the initiation of transcription.

EXPERIMENTAL PROCEDURES

Purification of Recombinant Mtf1—Plasmid DNAs containing GST-Mtf1 fusion constructs (16) were transformed into Escherichia coli BL21(DE3) codon plus RIL cells (Stratagene). Cells were grown in a buffered YT medium containing 1.4% yeast extract, 0.8% Tryptone, 75 mM KH2PO4, 8.8 mM KH2PO4, and 1% glucose. One-liter cultures inoculated to an A600 of 0.1 were incubated in a 20 °C shaking water bath for 6 h and induced for 11 h with 0.1 mM isopropyl-1-thio-β-D-galactopyranoside. Cultures were harvested by centrifugation; cell pellets were washed with 100 mM Tris, pH 7.9, resuspended in 150 ml of T50 (100 mM Tris, pH 7.9, 2 mM EDTA, 5% glycerol, 10 mM MgCl2, 0.1 mM DTT, 50 mM KCl), and stored at −80 °C. To purify GST-Mtf1, the cells were thawed and the buffer was adjusted to 2 mM DTT, 1 mg/ml lysozyme, and protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 0.35 μg/ml bestatin, 0.4 μg/ml pepstatin, 0.5 μg/ml leupeptin, and 20 μg/ml aprotinin) and incubated on ice for 1 h. This mixture was centrifuged at 8,000 × g for 1 h then added to 2 ml of glutathione-agarose (Pierce) equilibrated with T50 plus 2 mM DTT and protease inhibitors. The supernatant was then loaded into a 10-ml disposable column. The column was washed with 100 ml of T50 plus 2 mM DTT and protease inhibitors and then eluted with 50 ml of T50 plus 2 mM DTT, protease inhibitors, and 10 mM reduced glutathione. Peak protein containing fractions were pooled and dialyzed against 150 mM Tris, pH 7.9, 100 mM KCl, 1% Nonidet P-40, and protease inhibitors (see previous section) by dialysis against M(0) buffer (20 mM Tris, pH 7.9, 5% glycerol, 1 mM EDTA, 1 mM K2HPO4, 8.8 mM KH2PO4, and 1% glucose). One-liter cultures of strains containing promoters were grown to mid-log phase in a 1-L fermenter at 30 °C then transferred to a 80 °C shaking water bath for 5 min and stopped by adding 25 μl of formamide dye mix containing 50 mM EDTA, and the products were resolved on 7.5% polyacrylamide gels (InVitrogen).

Purification of Recombinant Rpo41—A 4078-bp Rpo41 fragment generated from yeast strain pH22 (16) was cloned into the baculovirus transfer vector pACHLTA (BD PharMingen). The primers used for this PCR reaction were: StuI, 5′-GAAGGCGCTATGCGACCCGGCGTAT-AAAAAT; and SacI, 5′-GCTCTGAGACGGCTTCCAGAGAATAATTATGAGGAC. Transfection of Sf9 insect cells and growth of HIS-Rpo41-producing clones was carried out by the University of Colorado Health Sciences Tissue Culture Core Facility. A cell pellet from a 500-ml culture of productively infected Sf9 cells was processed to obtain the HIS-Rpo41 protein. Cells were lysed in 50 mM Tris, pH 7.9, 100 mM KCl, 1% Nonidet P-40, and protease inhibitors (see previous section) by vortexing and then sonicated (Fisher 500 Sonic Dismembrator, large tip) with four 1-min pulses. Nonidet P-40 was added to 0.5%, and the extract was centrifuged at 35,000 × g for 45 min at 4 °C. The supernatant was filtered through a 0.22-μm PES filter and then incubated with 5 ml of glutathione-agarose (Pierce) equilibrated with T(50) plus 2 mM DTT and protease inhibitors. This mixture was stirred in the cold for 1.5 h and then loaded into a 10-ml disposable column. The column was washed with 100 ml of T(50) plus 2 mM DTT and protease inhibitors and then eluted with 50 ml of T(50) plus 2 mM DTT, protease inhibitors, and 10 mM reduced glutathione. Peak protein containing fractions were pooled and dialyzed against three 1-liter changes of T(50) containing 10% glycerol, 0.1 mM DTT, and protease inhibitors. Aliquots were frozen in liquid nitrogen and stored at −80 °C. The purity of the samples was assessed by electrophoresis on 4–12% Bis-Tris NuPAGE acrylamide gels (Invitrogen).

Specific primers were used to create DNA fragments, which were then cloned using a TA cloning kit with vector pCR 2.1 (Invitrogen). Primer sequences were: 14 S rRNA #365, 5′-GGACTATTATTACCTTTTT, #366, 5′-CCATAAAATTACAGCCAG; COX2 #85, 5′-AAAAGGTGGGGTTTGGTGA; #86, 5′-TTGTGATACCATCATTAGT; tRNA35′-CTAATTTTATATATTGTC; and #82, 5′-CTCTTAAATGATAGTTGCAATC. Ligated products were transformed into E. coli DH5α cells. Constructs were confirmed by DNA sequencing by the UCHSC Cancer Center Core Facility. One-liter cultures of strains containing promoters were grown to mid-log phase in a 1-L fermenter for plasmid DNA using a Qiagen Giga kit. Strain identifications are: 14 S rRNA-pJJ1305, COX2-pJJ1109, and tRNA35′-pJJ1110. Plasmid DNA was linearized with BanHI and then purified using Qiagen Q-500 tips.

In Vitro Selective Transcription Reactions—The 20-μl reactions contained 50 mM Tris, pH 7.9, 20 mM MgCl2, 1 mM DTT, 500 μM each of ATP, CTP, and GTP, 100 μM UTP, 10 μM [α-32P]UTP (1000 cpm/μM UTP), 20 μg/ml linearized template, 0.16–0.8 pmol of GST-Mtf1, and 0.41 pmol of HIS-Rpo41. Enzyme samples were diluted with T(50) containing 50 μg/ml bovine serum albumin and 1 mM DTT immediately prior to use. Reactions were incubated at 30 °C for 10 min and stopped by adding 25 μl of formamide dye mix containing 50 mM EDTA, and the products were resolved on 7% urea, 8% polyacrylamide gels. For the minus CTP reactions to compare activity on linear and supercoiled templates, conditions were as described above except for the absence of CTP and an increase in the specific activity of [α-32P]UTP to 2000 cpm/μM. Products were resolved on 7% urea, 11% polyacrylamide gels. For dinucleotide reactions, the appropriate (corresponding to +1 and +2 bases of the transcript) or inappropriate dinucleotides (Sigma Chemical Co.) were present at 50 μM. Autoradiographs were quantitated with Molecular Dynamics ImageQuant software, version 1.2.

RESULTS

In Vivo Transcription Analysis of Petite Mtf1 Point Mutations—We have previously described a collection of point mutations in the yeast MTF1 gene produced by PCR mutagenesis and identified as defective in mitochondrial function (petite phenotype) by a plasmid shuffle protocol (16). The two-hybrid technique was used to discriminate among these mutations those that had lost the ability to interact with Rpo41 (16). The functional defects of the remaining six mutations were not defined, but, based on both two-hybrid interactions and in vitro protein/protein interaction assays (summarized in Table I), all of these mutant strains efficiently interact with Rpo41 as well as do wild type Mtf1. Also included in Table I is information on the L53H mutant, a non-interacting, temperature-sensitive (ts) petite mutation of the amino acid that is immediately adjacent to the interacting, but petite T54F mutation; this non-interacting mutation serves as a control in several of the experiments below.

To elucidate the defects in the interacting mutant proteins summarized in Table I, we first used promoter-selective in vitro transcription reactions. These run-off transcription reactions that contain a yeast mitochondrial promoter promoter sequence upstream of a restriction enzyme cleavage site (Fig. 1A). Three yeast mitochondrial promoters were used in these experiments: the 14 S rRNA promoter that contains an exact match to the nonanucleotide consensus ATATAAGTA (19), the variant COX2 promoter (20), and the weak tRNA35′ promoter

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### Table I

| Mutation | Phenotype | Core interaction |
|----------|-----------|------------------|
| L53H     | ts petite | Defective        |
| Y54F     | Petite    | Normal           |
| S81N     | Petite    | Normal           |
| E114V    | Petite    | Normal           |
| C192F    | Petite    | Normal           |
| Q219R    | Petite    | Normal           |
| L228S    | Petite    | Normal           |

The in vivo phenotype and ability to interact with Rpo41 are summarized from Clifton et al. (16) for each of the MTF1 petite mutations used in this study.
Promoters like 14 S rRNA and COX2 with a purine at the +2 position are generally strong promoters (22–24), as can be seen in the in vitro transcription reaction using mitochondrial RNAP reconstituted from recombinant His-tagged Rpo41 and GST-tagged wild type Mtf1 (Fig. 1B). Substitution of a pyrimidine for the purine at position +2, as found in the tRNA Cys promoter, results in a much weaker promoter (25) (Fig. 1B). Each of the Mtf1 mutations listed in Table I was expressed in E. coli as a GST fusion protein and purified using glutathione-agarose chromatography (Fig. 1C). We have previously demonstrated that addition of the GST tag to Mtf1 does not interfere with the protein’s ability to interact with Rpo41 or to direct selective transcription (6). Equal amounts of the purified proteins shown in Fig. 1C were used in the in vitro transcription reactions with a purified baculovirus-expressed form of His-tagged Rpo41. Similar results were obtained using untagged Rpo41 purified from yeast (data not shown).

We were surprised to discover that all of the petite and ts petite Mtf1 mutants were capable of transcription from the consensus 14 S rRNA promoter at levels very similar to that observed for wild type Mtf1 (Fig. 2, A and B). Notice that the non-interacting L53H mutation was completely incapable of directing promoter selective transcription by Rpo41. This non-interacting mutation also does not suppress the nonspecific transcription of the DNA template visible as a faint background smear of nucleotide incorporation in this experiment (Fig. 2A; see also below). With transcription by wild type Mtf1 set to 100%, the activity of the mutant Mtf1s ranged from just over 50% to 150%. The less than 2-fold reductions observed with the S81N and C192F mutations seem unlikely to account for the petite and ts petite phenotypes of these mutations.

The C192F Mutation Is Defective for Transcription of the Variant COX2 Promoter—All of the interacting Mtf1 mutations can transcribe the consensus 14 S RNA promoter. However, the yeast mitochondrial genome has multiple promoters with both consensus and non-consensus sequences driving the expression of many RNA and protein coding genes (reviewed in Refs. 26–29). To determine whether any of the mutant Mtf1s had selectively lost the ability to recognize variant mitochondrial promoters, we carried out in vitro transcription reactions using the COX2 promoter. As seen in Fig. 1A, the COX2 promoter varies from the consensus, and the 14 S rRNA promoter, in three positions: T instead of A at –8; A instead of T at –5; and G instead of A at +2. None of these substitutions has a major effect on the ability of this sequence to be recognized as a promoter by the wild type mitochondrial holo-RNAP (20, 23), as shown in a direct comparison with transcription from the 14 S rRNA promoter (Fig. 1B). Most of the interacting mutants...
were capable of nearly wild type levels of transcription with the COX2 promoter (Fig. 3). However, Mtf1 carrying the C192F mutation was incapable of transcription from this variant promoter (Fig. 3). Note that the C192F mutation is still capable of suppressing Rpo41’s nonspecific interaction with DNA (compare transcription by L53H to C192F in Fig. 3A), even though promoter specific transcription is reduced over 10-fold relative to wild type Mtf1 (Fig. 3B).

Both the Y54F and the C192F Mutations Are Defective for Transcription of the Weak tRNA\textunderscore{Cys} Promoter—In addition to the strong COX2 promoter, we also tested the Mtf1 mutations on the weak tRNA\textunderscore{Cys} promoter (21). This promoter varies from the consensus and the 14 S rRNA promoter at two positions: T instead of A at −8 and T instead of A at +2 (Fig. 1A). The substitution of a pyrimidine for a purine at +2 makes the tRNA\textunderscore{Cys} promoter “weak” (30), resulting in 3-fold lower transcription from this template by wild type RNAP than from the strong COX2 promoter and COX2 promoters (Fig. 1B). We found that most of the mutant Mtf1s were still capable of transcribing the tRNA\textunderscore{Cys} promoter at levels indistinguishable from wild type (Fig. 4, A and B). However, the C192F mutation, previously shown to be defective on the COX2 promoter, was also completely defective for transcription from the tRNA\textunderscore{Cys} promoter, with transcript abundance reduced below the limits of detection (Fig. 4B). In addition, the Y54F mutation also demonstrated a significant reduction in transcription, with levels 4- to 5-fold lower than wild type Mtf1 (Fig. 4B). Again, note the ability of both the Y54F and C192F mutations to reduce nonspecific interactions of Rpo41 with DNA relative to the noninteracting mutant L53H (Fig. 4A).

Transcription Defects on the tRNA\textunderscore{Cys} Promoter Can Be Corrected by Dinucleotide Priming or by Supercoiling of the Template DNA—The inability of the Mtf1 mutations to transcribe the variant promoters could be due to defects in DNA binding, promoter melting, open complex formation, nucleotide binding, first bond formation, or promoter escape (31). We first asked whether the defect was at the level of first bond formation by testing the response to addition of dinucleotide primers to the transcription reactions. Biswas (24, 25) has shown that transcription from weak promoters such as tRNA\textunderscore{Cys} is significantly improved when a dinucleotide corresponding to the +1 and +2 positions is provided, whereas transcription from promoter variants with sequence alterations toward the 5′-end is unaffected by the addition of dinucleotides. Consistent with these observations, we found little or no effect of dinucleotide addition on transcription of the COX2 promoter by either wild type or mutant Mtf1 (Fig. 5A). In contrast, we found that addition of the appropriate initiating dinucleotide, AU, to tRNA\textunderscore{Cys} reactions significantly improved transcription both by wild type Mtf1 and by the Y54F, S81N, and C192F mutations (Fig. 5B). Transcription by Y54F was restored to levels indistinguishable from wild type, and although the signal from the C192F transcription reactions was still much lower than wild type, it was now detectable. Addition of an inappropriate dinucleotide, AA, had no effect on transcription. These experiments establish that the Y54F and C192F Mtf1 mutations have specific defects in initiation from a weak mitochondrial promoter that can be partially or fully corrected by the addition of an appropriate dinucleotide.

We also asked whether the mutant specificity factors had defects in promoter melting by comparing transcription from linear templates to that from supercoiled templates, because supercoiling reduces the energy needed for open promoter complex formation (reviewed in Refs. 32 and 33). For these experiments we took advantage of the sequences downstream of the tRNA\textunderscore{Cys} promoter. The first C residue in the tRNA\textunderscore{Cys} transcript is not encountered until the transcript is 38 bases long, so omission of CTP from the reactions produces a 38-nucleotide transcript from both linear and supercoiled circular templates for direct comparison. We found that transcription by wild type Mtf1 and the S81N mutant was relatively unaffected by the
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Biswas (23, 30) determined that a critical determinant of promoter strength is the nucleotide in the +2 position. Strong promoters like 14 S rRNA and COX2 have a purine at +2, whereas the weak tRNA\textsuperscript{Cys} promoter has a pyrimidine at +2. Biswas (25) has also shown that promoters that are weak due to a +2 pyrimidine can be corrected \textit{in vitro} by addition of a dinucleotide corresponding to the +1 and +2 nucleotides of the transcript. Weak promoters that vary from the consensus at positions farther upstream cannot be corrected by dinucleotide addition, possibly due to defects in the initial binding of the RNAP. Biswas (24, 25) has speculated that addition of the dinucleotide either bypasses an energy barrier for first bond formation or stabilizes the initiation complex.

Failure to transcribe even one of the yeast mitochondrial promoters results in a petite phenotype, because every mitochondrially encoded gene product is essential for functional respiration (37). Therefore, the failure of the Y54F mutation to transcribe the tRNA\textsuperscript{Cys} promoter, driving expression of both tRNA\textsuperscript{Cys} and tRNA\textsuperscript{His}, and the decreased ability of the C192F mutation to transcribe either the tRNA\textsuperscript{Cys} or the COX2 promoter, can account for the \textit{in vivo} petite phenotypes of these Mtf1 mutations. However, the \textit{in vitro} results do not exactly mimic the \textit{in vivo} phenotypes. C192F is a ts petite, capable of sustaining mitochondrial function at 30°C but not 37°C (16); whereas \textit{in vitro} it demonstrates drastically reduced function on the variant promoters even at 30°C. In addition, the Y54F mutation has a more severe phenotype \textit{in vivo} than does C192F; it is petite even at 30°C, but it is less impaired in the \textit{in vitro} transcription assays. Clearly our optimized \textit{in vitro} reaction conditions are still not a perfect recreation of the situation inside the mitochondrion, a fact that may also explain why we have not yet identified the defects associated with the remaining petite mutations, S81N, E114V, Q219R, and L228S. Although we have tested a variety of variables, including pH, salt concentration, and temperature (data not shown), we have not uncovered \textit{in vitro} defects for these mutations consistent with their \textit{in vivo} phenotypes. They may be deficient in import into the mitochondrion (the mechanism for import of Mtf1, which lacks a conventional import signal, is still not known (38)), they may have defects on another untested variant promoter, or they may possess defects only revealed by some particular characteristic of the mitochondrial environment.

Another aspect of the mitochondrial milieu probably not replicated in our \textit{in vitro} reactions is the state of the DNA. We found that template supercoiling helps to correct both Y54F and C192F on the tRNA\textsuperscript{Cys} promoter (Fig. 5). Although the genetic map of yeast mitochondrial DNA is circular, the physical state of the DNA is probably linear (39, 40). However, yeast mitochondrial DNA is packaged with DNA binding proteins that include the abundant HMG factor Abf2 (41, 42), and it may be tethered to the inner mitochondrial membrane through protein/protein interactions (43). These associations may create a constrained DNA topology inside the mitochondrion. In an earlier study of site-directed mutations in Mtf1, Shadel and Clayton (44) found several mutations that were functional \textit{in vivo} but not functional \textit{in vitro} on linear templates containing the 14 S rRNA promoter. Two of the five mutations with these properties were corrected by supercoiling the DNA template. It is interesting that these two supercoiling-sensitive mutations (R178A/K179A and H187A/R189A) are located near the C192F mutation that we have analyzed in this work. These mutations all lie in the region between the amino acids predicted to be similar to regions 2 and 3 of the bacterial sigma factors (see Fig. 6A). It may be that this region plays an important role in open complex formation by the mitochondrial RNAP.

The amino acids used by bacterial sigma factors for open
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complex formation have been studied in some detail (reviewed in Refs. 32 and 33). Several aromatic residues in sigma factor region 2.3 are thought to play a critical role in melting the DNA and stabilizing the single-strand region. Based on the alignment proposed by Jang and Jaehning (5, 28), Mtf1 shares several of these critical amino acids, which, when mutated in sigma factor lead to a requirement for supercoiled templates in vitro (33, 45). One of these positions in Mtf1 (Y108) is essential for in vivo and in vitro function (44). In addition, amino acids in region 1 of sigma factor are also important for open complex formation (46); amino acids similar to this region of sigma factor are not shared with Mtf1.

Based on our observation that the Y54F and C192F mutations, and additional mutations near C192F (44), lead to a requirement for a supercoiled template, we propose that Mtf1 plays an important role in open complex formation by the mitochondrial RNA polymerase. Although Y54F and C192F have some similar properties, their relatively distant position from each other on the Mtf1 crystal structure determined by Schubot et al. (17) (see Fig. 6B), makes it unlikely that they define a single region important for promoter opening. It is however possible that, when Mtf1 is in a complex with Rpo41, the relative positions of these amino acids could change significantly, as has been observed for sigma factor when it forms a complex with core RNA polymerase (47, 48).

What contribution does the core RNA polymerase make to this process? The single polypeptide subunit of the core RNAP recognizes and open promoter DNA without an accessory factor. Analysis of the crystal structure of T7 RNAP in an initiation complex revealed that two regions of the RNAP are involved in stabilizing contacts with single-strand DNA (49). First, the amino acids of the specificity loop make base specific contacts with single-strand DNA that define the promoter specificity of the T7 RNAP. In addition, a β-hairpin near the N terminus inserts Val-237 into the duplex DNA at the double-strand/single-strand junction stabilizing the open form. The importance of Val-237 and this β-hairpin region for promoter melting has been confirmed by recent site-directed mutagenesis studies (50).

Although Rpo41 shares significant amino acid similarity with T7 RNAP (10), there is little conservation of the amino acids in either of these promoter melting regions (2), and in fact, these regions in Rpo41 are used as sites of interaction with Mtf1 (51). It is interesting to note that the Mtf1 Y54F mutation identified in this work as critical for promoter recognition is immediately adjacent to the L53H mutation (Fig. 6A) that abolishes interactions with Rpo41 (16). Clearly, amino acids in both RNAP subunits important for protein/protein interactions are in close juxtaposition to those important for DNA contacts. The fact that similar observations have been made for the bacterial RNAP (52, 53) is additional evidence for a conserved mechanism of promoter recognition and transcriptional initiation by mitochondrial and bacterial RNAPs.

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REFERENCES

1. Jaehning, J. A. (1993) Mol. Microbiol. 8, 1–4
2. Cermakian, N., Ikeda, T. M., Miramontes, P., Lang, B. F., Gray, M. W., and Cedergren, R. (1997) J. Mol. Biol. 265, 671–681
3. Kelly, J. L., Greenleaf, A. L., and Lehman, I. R. (1986) J. Biol. Chem. 261, 10494–10531
4. Lysowsky, T. and Michaelis, G. (1988) Mol. Gen. Genet. 214, 218–223
5. Jang, S. H., and Jaehning, J. A. (1991) J. Biol. Chem. 266, 22671–22677
6. Mangus, D. A., Jang, S. H., and Jaehning, J. A. (1994) J. Biol. Chem. 269, 26569–26574
7. Winkley, C. S., Keller, M. J., and Jaehning, J. A. (1985) J. Biol. Chem. 260, 14214–14223
8. Schinkel, A. H., Groot Koerkamp, M. J., and Tabak, H. F. (1988) EMBO J. 7, 3255–3262
9. Schinkel, A. H., Koerkamp, M. J., Touw, E. P., and Tabak, H. F. (1987) J. Biol. Chem. 262, 12765–12770
10. Masters, B. S., Stohl, L. L., and Clayton, D. A. (1987) Cell 51, 89–99
11. Nam, S. C., and Kang, C. (2001) Protein Expr. Purif. 21, 485–491
12. Chang, C. C., Sheen, J., Bligny, M., Niwa, Y., Lerbs-Mache, S., and Stern, D. B. (1999) Plant Cell 11, 911–925
13. Grams, J., Morris, J. C., Drew, M. E., Wang, Z., Englund, P. T., and Hadjuk, S. L. (2002) J. Biol. Chem. 277, 16952–16959
14. Prieto-Martin, A., Montoya, J., and Martinez-Azorin, F. (2001) FEBS Lett. 503, 53–55
15. Lang, B. F., Burger, G., O’Kelly, C. J., Cedergren, R., Goldberg, G. R., Lemieux, C., Sankoff, D., Turnel, M., and Gray, M. W. (1997) Nature 387, 493–497
16. Cifuentes, D. J., Park, J. Y., Davis, B. P., Jang, S. H., and Jaehning, J. A. (1997) Genes Dev. 11, 2897–2909
17. Schubot, P. D., Chen, C. J., Rose, J. P., Dailey, T. A., Dailey, H. A., and Wang, B. C. (1996) J. Biol. Chem. 271, 13391–13394
18. Osinga, K. A., De Haan, M., Christiansen, T., and Tabak, H. F. (1982) Nucleic Acids Res. 10, 7993–8006
19. Cameron, V. L., Fox, T. D., and Poyton, R. O. (1989) J. Biol. Chem. 264, 13890–13896
20. Biswas, T. K. (1996) Genes (Amst.) 170, 23–30
21. Biswas, T. K., Ticho, B., and Getz, G. S. (1987) J. Biol. Chem. 262, 13890–13896
22. Biswas, T. K., and Getz, G. S. (1990) J. Biol. Chem. 265, 19053–19059
23. Biswas, T. K. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 9338–9342
24. Costanzo, M. C., and Fox, T. D. (1996) Annu. Rev. Genet. 24, 91–113
25. Dreschmann, C. L., and Staples, R. R. (1994) Int. Rev. Cytol. 152, 145–181
26. Jang, S. H., and Jaehning, J. A. (1994) in Transcription: Mechanisms and Regulation (Conaway, R. W., and Conaway, J. W., eds) pp. 171–184, Raven Press, Ltd., New York
27. Biswas, T. K. (1998) Gene (Amst.) 212, 305–314
28. Biswas, T. K., and Getz, G. S. (1986) J. Biol. Chem. 261, 3927–3930
29. McClure, W. E. (1986) Proc. Natl. Acad. Sci. U. S. A. 77, 5634–5638
30. deHaas, P. L., and Helmann, J. D. (1995) Mol. Microbiol. 16, 817–824
31. Helmann, J. D., and deHaas, P. L. (1999) Biochemistry 38, 5959–5967
32. Mueller, D. M., and Getz, G. S. (1986) J. Biol. Chem. 261, 11816–11822
33. Christiansen, T., and Rahimzadeh, M. (1983) J. Biol. Chem. 258, 14025–14033
36. Osinga, K. A., De Vries, E., Van der Horst, G. T., and Tabak, H. F. (1984) *Nucleic Acids Res.* **12**, 1889–1900
37. Attardi, G., and Schatz, G. (1988) *Annu. Rev. Cell Biol.* **4**, 289–333
38. Sanyal, A., and Getz, G. S. (1995) *J. Biol. Chem.* **270**, 11970–11976
39. Jacobs, M. A., Payne, S. R., and Bendich, A. J. (1996) *Curr. Genet.* **30**, 3–11
40. Bendich, A. J. (1996) *J. Mol. Biol.* **255**, 564–588
41. Diffley, J. F., and Stillman, B. (1992) *J. Biol. Chem.* **267**, 3368–3374
42. Newman, S. M., Zelenaya-Trotskaya, O., Perlman, P. S., and Butow, R. A. (1996) *Nucleic Acids Res.* **24**, 386–393
43. Cho, J. H., Ha, S. J., Kao, L. R., Megraw, T. L., and Chae, C. B. (1998) *Mol. Cell. Biol.* **18**, 5712–5723
44. Shadel, G. S., and Clayton, D. A. (1995) *Mol. Cell. Biol.* **15**, 2101–2108
45. Juang, Y. L., and Helmann, J. D. (1994) *J. Mol. Biol.* **235**, 1470–1488
46. Wilson, C., and Dombroski, A. J. (1997) *J. Mol. Biol.* **267**, 60–74
47. Callaci, S., Heyduk, E., and Heyduk, T. (1998) *J. Biol. Chem.* **273**, 32995–33001
48. Callaci, S., Heyduk, E., and Heyduk, T. (1999) *Mol. Cell.* **3**, 229–238
49. Cheetham, G. M., Jeruzalmi, D., and Steitz, T. A. (1999) *Nature* **399**, 80–83
50. Stano, N. M., and Patel, S. S. (2002) *J. Mol. Biol.* **315**, 1009–1025
51. Cliften, P. F., Jung, S. H., and Jaehning, J. A. (2000) *Mol. Cell. Biol.* **20**, 7013–7023
52. Gross, C. A., Chan, C., Dombroski, A., Gruber, T., Sharp, M., Tupy, J., and Young, B. (1998) *Cold Spring Harbor Symp. Quant. Biol.* **63**, 141–155
53. Gruber, T. M., Markov, D., Sharp, M. M., Young, B. A., Lu, C. Z., Zhong, H. J., Artsimovitch, I., Geszvain, K. M., Arthur, T. M., Burgess, R. R., Landick, R., Severinov, K., and Gross, C. A. (2001) *Mol. Cell* **8**, 21–31