The Journal of Microbiology, September 1997, p.228-233

Vol. 35, No. 3

# Amino Acid Substitutions Conferring Cold-Sensitive Phenotype on the Yeast MTF1 Gene

**Sei-Heon Jang**

*Department of Molecular Biology, Taegu University, Kyung-San 712-714, Korea*

(Received July 11, 1997 / Accepted August 18, 1997)

**The *MTF1* gene of *Saccharomyces cerevisiae* encodes a 43 kDa mitochondrial RNA polymerase specificity factor which recognizes mitochondrial promoters to initiate correct transcription. To better understand structure-function of the *MTF1* gene as well as the transcription mechanism of mitochondrial RNA polymerase, two cold-sensitive alleles of the *MTF1* mutation were isolated by plasmid shuffling method after PCR-based random mutagenesis of the MTF1 gene. The mutation sites were analyzed by nucleotide sequencing. These *cs* phenotype *mtf1* mutants were respiration competent on the nonfermentible glycerol medium at the permissive temperature, but incompetent at 13°C. The *cs* phenotype allele of the *MTF1*, yJH147, encoded an L146P replacement. The other *cs* allele, yJH148, contained K179E and K214M double replacements. Mutations in both alleles were in a region of Mtf1p which is located between domains with amino acid sequence similarities to conserved regions 2 and 3 of bacterial s factors.**

**Key words: Mitochondrial transcription, MTF1, yeast mitochondrial RNA polymerase**

Transcription of yeast mitochondrial genes is initiated by a nuclear-encoded mitochondrial RNA polymerase composed of two subunits; a core polymerase and a specificity factor. The core polymerase is a 145 kDa polypeptide encoded by the *RPO41* gene (14, 22). This protein shares nine regions of amino acid similarity with T3 and T7 bacteriophage RNA polymerase, including the regions determined to be important for catalysis in T7 RNA polymerase (4, 11, 22). Unlike T3 or T7 RNA polymerase, the mitochondrial RNA polymerase requires a specificity factor to recognize and bind to its simple promoter, 5'-ATATAAGTA-3' (2, 21). This factor is a 43 kDa protein encoded by the nuclear *MTF1* gene (10, 17).

Disruption of the *MTF1* gene in *Saccharomyces cerevisiae* leads to a stable petite phenotype and loss of mitochondrial DNA (33). Inspection of the predicted amino acid sequence of Mtf1p revealed regions that exhibit limited similarity to the conserved regions 2 and 3 of bacterial σ factors (10) and a region rich in basic amino acids (17). Region 2 is the most conserved part of the proteins in the σ family and is divided into 4 subregions (2.1 to 2.4) (7, 18). Subregions 2.2 and 2.3 have been implicated to be involved in the melting of DNA at the initiation site (13, 31), and subregion 2.4 is thought to be involved in -10 promoter sequence recognition (12, 32). Deletions in subregion 2.1 (15) or region 3 (34) of σ factor apparently reduce the affinity of σ factors for the core RNA polymerase, suggesting that these regions are necessary for this interaction.

To further promote work on the analysis of the specificity factor and the mitochondrial transcription mechanism, we tried to isolate mutations of the gene for mitochondrial RNA polymerase specificity factor. In this study we characterized conditional mutations of the *MTF1* gene for the specificity factor which causes cold-sensitive phenotype.

## Materials and Methods

### Media and genetic methods

Rich media such as YP medium (2% peptone, 1% yeast extract) containing 2% of either glucose (YPD) or glycerol (YPG), synthetic complete (SC) medium lacking the appropriate amino acids, and sporulation medium were prepared as previously described (27). 5-FOA medium was prepared by adding 5-fluoroorotic acid to SC medium at concentration of 500 mg/l (3). Yeast cells were transformed using the lithium acetate method (9). Mating, sporulation and dissection were carried out by the usual methods (27). *E. coli* XL1-Blue from Stratagene Co. was used as the host strain for plasmids. Cold-sensitive growth phenotype is expressed as *cs* and denotes distinctively impaired growth on YPG medium at 13°C.

### Plasmid constructions

The low copy vectors YCplac33 (*CEN URA3*)and YCplac111 (*CEN LEU2*) were described elsewhere (5). A genomic clone of *MTF1* in pJH26 (10) was digested with *Dra*I, and the site end-filled with Klenow fragment and changed by inserting an *Eco*RI linker, resulting an 1.5 kb *Eco*RI fragment encompassing the promoter and entire coding sequence of the *MTF1* gene. Plasmids pJH118 and pJH121 were constructed by inserting the wild type *MTF1* gene on an 1.5 kb *Eco*RI fragment into the pUC18 and pUC7, respectively. This same 1.5 kb DNA fragment was inserted at the unique *Eco*RI site of the *URA3* plasmid YCplac33 to create pJH119, and the *LEU2* plasmid YCplac111 to create pJH142. Plasmid pJH133 was generated by inserting a 3.8 kb *Bgl*II/*Bam*HI fragment of the *hisG-URA3-hisG* gene isolated from plasmid pNKY 51 (1) into a *Bgl*II site in the *MTF1* coding region of plasmid pJH121.

## Yeast strains

The *S. cerevisiae* strains used in this study are related to TD28 (6) and listed in Table 1. The diploid strain yJH60 was made by crossing the yJH58 and yJH59. The heterozygous *MTF1/mtf1::hisG-URA3-hisG* of strain yJH61 was constructed by a one-step gene replacement (24) of the 5.3 kb *EcoRI* fragment from plasmid pJH133 into strain yJH60. Diploid yJH64 was generated by excising the *URA3* gene from the heterozygous *MTF1/mtf1::hisG-URA3-hisG* of stain yJH61 on 5-FOA medium. Haploid strain yJH71 used as host in the plasmid shuffle system was constructed as below. Strain yJH64 was transformed into Ura$^+$ with plasmid pJH119 carrying a functional *MTF1* gene, and the resulting transformants were sporulated and dissected to generate *mtf1::hisG* [pJH119] haploid progeny called yJH71. The genotype of yJH71 was confirmed by testing the respiration incompetence on YPG medium after evicting the *URA3* plasmid containing wild-type *MTF1* on 5-FOA medium. All strain constructions were confirmed by Southern blot analysis described previously (29) using a 1040 bp *EcoRI* fragment of pJJ525 (20) as a *MTF1* probe.

**Table 1.** *Saccharomyces cerevisiae* strains

| Strain | Genotype |
|---|---|
| yJH48 | *MATα leu2-Δ1 his3Δ200 ura3-52 mtf1::LEU2* |
| TD28[a] | *MATα ura3-52 ino1-13* |
| yJH58[a] | *MATa leu2-3,112 his4Δ309 ura3-52 ino1-13* |
| yJH59[a] | *MATα leu2-3,112 his4Δ309 ura3-52 ino1-13* |
| yJH60 | *MATa/α leu2-3,112/leu2-3,112 his4Δ309/his4Δ309 ura3-52/ura3-52 ino1-13/ino1-13* |
| yJH61 | *MATa/α leu2-3,112/leu2-3,112 his4Δ309/his4Δ309 ura3-52/ura3-52 ino1-13/ino1-13 MTF1/mtf1::hisG-URA3-hisG* |
| yJH64 | *MATa/α leu2-3,112/leu2-3,112 his4Δ309/his4Δ309 ura3-52/ura3-52 ino1-13/ino1-13 MTF1/mtf1:: hisG* |
| yJH68 | *MATa/Δ leu2-3,112/leu2-3,112 his4Δ309/his4Δ309 ura3-52/ura3-52 ino1-13/ino1-13 MTF1/mtf1::hisG [YCPlac33;MTF1URA3]* |
| yJH71 | *MATα leu2-3,112 his4Δ309 ura3-52 ino1-13 mtf1::hisG [YCplac33;MTF1URA3]* |

[a] Wild type strains were obtained from T.F. Donahue. All other strains are from this laboratory.

## PCR-based mutagenesis and isolation of *mtf1* mutants

Mutagenic PCR was performed essentially as described elsewhere (16) to generate *in vitro* random mutations of *MTF1*. Plasmid pJH118 containing wild-type *MTF1* was used as a DNA template. A 17mer of universal sequencing primer and a 23mer of synthetic oligonucleotide, 5'-CACAGGAAACAGCTATGACCATG-3', encompassing the reverse sequencing primer were used as primers. The PCR reactions contained 30 mM Tricine pH 8.4, 10 ng pJH 118, 0.2 mM dNTP, 7 mM $MgCl_2$, 0.5 mM $MnCl_2$, 20 ρmole of each primer, 5 mM β-mercaptoethanol, 0.01% gelatin, and 1 unit of Taq polymerase from New England Biolab. Reactions were done in 100 ul and preheated to 92°C for an initial denaturation step followed by 30 cycles. The mutagenic PCR-amplified 1.5 kb of DNA fragment was purified by electrophoresis on 0.7% agarose gel and was used for the introduction of mutations into yeast with plasmid pJH142 gapped with *Bam*HI. The gap-duplex recombinant mutagenesis was performed as elsewhere described (23). Plasmid pJH 142 was digested with restriction enzyme *Bam*HI and the gapped vector DNA was purified by agarose gel electrophoresis. Yeast strain yJH71 was transformed into Leu$^+$ with 500 ng of PCR-amplified DNA fragment and 100 ng of plasmid pJH 142 gapped with *Bam*HI. Approximately 5,000 individual Leu$^+$ transformants were plated on 5-FOA medium to select those that lost *URA3* plasmid carrying wild-type *MTF1*. This procedure, known as plasmid shuffling (28), resulted in a set of isogenic strains carrying alleles of *MTF1* mutation on the *LEU2* plasmid. 5-FOA resistant Leu$^+$ transformants were screened for loss of mitochondrial function caused by *MTF1* mutations carried on pJH142 by plating them on both YPD and YPG medium. The replica plates were subsequently incubated at 13°C for 15~20 days and 30°C for 3~4 days. Cells that grew on YPG medium at 30°C but not at 13°C were isolated as cold-sensitive alleles of *MTF1* mutation.

### DNA sequence analysis

Plasmid DNA was recovered from *cs*⁻ *MTF1* allele strains by the technique of Hoffman and Winston (8), and introduced into *E. coli* by the calcium chloride procedure (19). The *MTF1* coding region was sequenced in its entirety for each allele by the Sanger's dideoxy method (25) using universal primer and five *MTF1* anti-sense strand primers (P1, 870~886; P2, 700~716; P3, 507~523; P4, 353~369; P5, 172~188. The A of the start codon is designated +1). To ensure that the *mtf1* mutant alleles were responsible for the conditional growth phenotype, the recovered plasmid were used to transform yJH71 followed by 5-FOA selection.

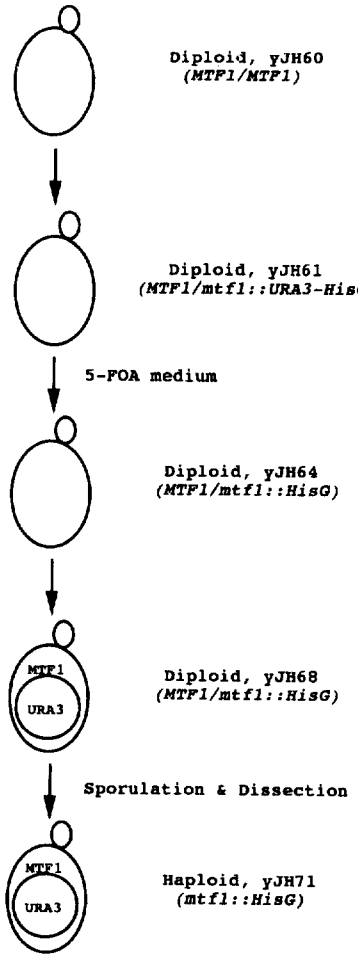


**Fig. 1.** Construction of the recipient strain yJH71. plasmids, strains and protocols are described in Materials and Methods. A haploid strain containing a single functional copy of *MTF1* on a plasmid was created by first disrupting a single copy of *MTF1* in a diploid strain (yJH60). The heterozygous strain was transformed with a plasmid bearing the *URA3* marker and a functional copy of *MTF1*. The resulting strain was sporulated and a *MTF1*, Ura⁺ haploid (yJH71) was identified.

## Results

### Construction of the recipient yeast strain

The haploid yJH71 was constructed as depicted in Fig. 1 for a yeast plasmid shuffling system. Since disruption of the *MTF1* gene causes the loss of yeast mitochondrial DNAs and results in petite phenotype (33), diploid yJH60 whose phenotype is Ura⁻ and Leu⁻ was used to delete the chromosomal *MTF1* locus by replacement with a 3.8 kb DNA fragment containing the yeast *URA3* gene flanked by direct repeats of the *Salmonella* histidine sequence (1). Ura⁺ transformants were patched onto 5-FOA plates to loop out both the *URA3* and one *Salmonella* histidine gene by recombination between the flanking direct repeats. One of the resulting 5-FOA resistants was named yJH64 and retransformed into Ura⁺ with *URA3* plasmid (pJH 119) carrying a functional *MTF1* to create yJH68. The resulting transformant was sporulated and dissected to generate a recipient haploid for a plasmid shuffling system. Tetrad analysis of yJH68 shown in Table 2 indicated that *URA3* plasmids were 2:2 segregated only in tetrads #5 and #9. Spore 5c was able to grow in YPG medium even though it did not carry *URA3* plasmid, indicating that chromosomal *MTF1* gene was not disrupted in this spore, and the *MTF1* gene and vector were not necessarily segregated together. Spores 5a, 5d, 9c, and 9d were able to grow both in -Ura and YPG medium. After evicting *URA3* plasmid on 5-FOA medium, spore 5d was still able to grow in YPG medium but spores 5a, 9c and 9d were not, implying that chromosomal *MTF1* locus was deleted in spores 5a, 9c and 9d. Because the genotypes of those three spores were identical, spore 9c was selected and named yJH71. The physical structures of haploid yJH71 and diploid strains men-

**Table 2.** Tetrad analysis of strain yJH68[a]

| Tetrads \ Spores | YPD | | | | -Ura | | | | YPG | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | a | b | c | d | a | b | c | d | a | b | c | d |
| 1 | +[b] | + | + | | + | + | + | | + | + | + | |
| 2 | + | + | + | + | + | + | + | + | + | + | + | + |
| 3 | + | + | + | | + | + | + | | + | + | + | |
| 3 | + | + | + | | + | + | + | | + | + | + | |
| 5 | + | + | + | + | + | −[c] | − | + | + | − | + | + |
| 6 | + | + | + | | + | + | + | | + | + | + | |
| 7 | + | + | + | | − | + | + | | − | + | + | |
| 8 | + | + | + | + | + | + | + | − | + | + | + | |
| 9 | + | + | + | + | − | − | + | + | + | + | + | + |

[a] Yeast strain yJH68 bearing plasmid pJH119 was sporulated and dissected. Spores from each tetrad were tested for their viability on the indicated media. [b]+, growth; [c]-, no growth.

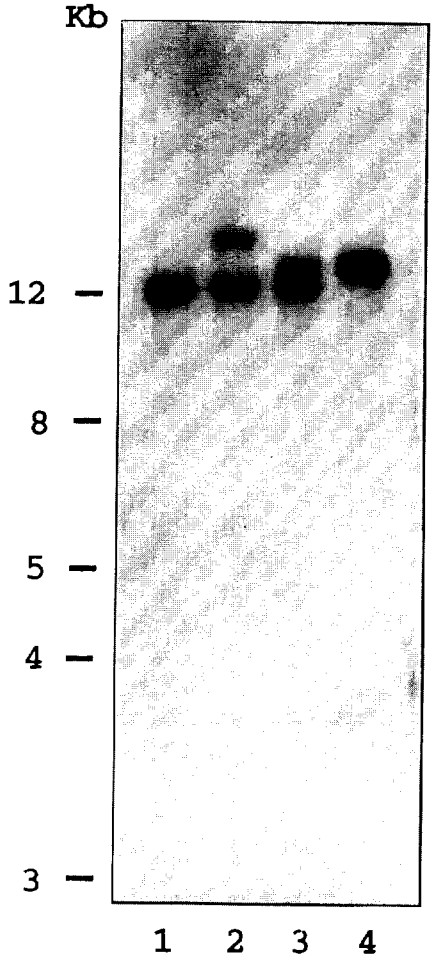


**Fig. 2.** Southern blot analysis of the *MTF1* gene structure during recipient strain construction. Total genomic DNA (10 μg) isolated from indicated strains were digested with *Eco*RI, fractionated in an 1% agarose gel, blotted onto Nytran paper and hybridized with a $^{32}$P-labeled *MTF1* probe. The autoradiograph is shown. Lane 1, yJH60; lane 2, yJH61; lane 3, yJH64; lane 4, yJH71.

tioned in Fig. 1 was confirmed by Southern blot analysis as shown in Fig. 2. Chromosomal DNA from each strain was digested with *Eco*RI, fractionated, blotted onto nytran paper and hybridized with a *MTF1* probe. Southern blotting of yJH60 chromosomal DNA revealed one 12 kb DNA band. In lane 2, Ura$^+$ transformant of yJH60 displayed the pattern of fragments expected for a gene replacement at *MTF1* with the *hisG-URA3-hisG* fragment: 12 and 16 kb bands were detected. Southern blot of 5-FOA resistant yJH64 displayed 12 and 13 kb bands in lane 3. The size of 13 kb DNA band was equal to that expected from a deletion of the *URA3* and one of the *hisG* sequences in 16 kb band. One single band of 13 kb in size was only detected in lane 4, confirming that yJH71 is a haploid containing a disrupted *mtf1::hisG* genotype.

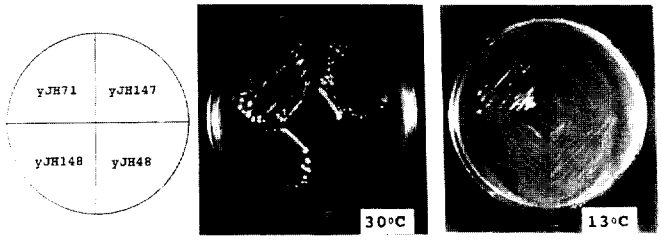


**Fig. 3.** Growth phenotypes associated with *MTF1* mutants. Alleles of *MTF1* mutation were streaked on glycerol medium (YPG) and were incubated for 3 days at the permissive (30°C) or 20 days in the cold (13°C) temperature.

## Isolation of cs *mtf1* mutants

To generate mutant alleles of the *MTF1* gene, PCR-based mutagenesis and gap-duplex recombination were performed as described under "Materials and Methods". A 17mer universal and a 23mer reverse sequencing primers were used for PCR-based mutagenesis reactions. The *LEU2* plasmid pJH142 carrying a wild type *MTF1* was digested with *Bam*HI, electrophoresed, and the gapped-vector DNA was isolated. PCR-amplified 1.5 kb *MTF 1* and gapped-vector DNA were used to transform the plasmid shuffle strain yJH71. Because the *MTF1* gene is single copy and essential for mitochondial functions, yJH71 contains pJH119 (*URA3-MTF1*), a plasmid that contains the *MTF1* gene and thus complements the chromosomal *MTF1* null allele. After growth on -Ura, -Leu medium, transformants were replica plated onto 5-FOA medium to select for cells that have lost the *URA3* plasmid pJH119. 5-FOA resistants were repatched onto both YPG and YPD medium and incubated at 13°C and 30°C to identify cells that fail to grow on YPG at either temperatures relative to control plates (YPD). Both null alleles and cold-sensitive mutations of *MTF1* were isolated from 5,000 transformants screened. However, two *cs mtf1* alleles that fail to grow on YPG at 13°C were analyzed in this study. They exhibited slow growth at 30°C compared to wild types (Fig. 3). The *cs* phenotype associated mutation sites of the *MTF1* gene were determined by the Sanger's sequencing method and shown in Table 3. L146P single amino acid replacement occurred in the one *cs*

**Table 3.** Summary of cold-sensitive mutations[a]

| Mutant yJH | Base changed | Amino acid changed |
|---|---|---|
| 147 | CTT→CCT | $L_{146}$→P |
| 148 | AAA→GAA | $K_{179}$→E |
| | AAG→ATG | $K_{214}$→M |

[a] The nucleotide changed in alleles of *MTF1* mutations is underlined.

*mtf1* allele (yJH147) and K179E, K214M double replacements in the other *cs*⁻ mutant (yJH148). Theses mutation sites are in a region of the protein which is located between domains with amino acid sequence similarities to conserved regions 2 and 3 of bacterial s factors.

## Discussion

The *MTF1* encoded mitochondrial RNA polymerase specificity factor is essential for specific recognition of a yeast mitochondrial promoter. *In vitro*, Mtf1p is the only component other than the core mitochondrial RNA polymerase necessary to achieve specific transcription initiation from a mitochondrial promoter (10). *In vivo*, however, Mtf1p is not the only factor to control transcription of the mitochondrial genes. In fact, the level of Mtf1p is unchanged throughout the cell growth and also unaffected by carbon sources (30), suggesting that additional factors be required *in vivo* to regulate mitochondrial gene transcription. To study the mechanism of yeast mitochondrial transcription, we have isolated cold-sensitive alleles of *MTF1* mutation using plasmid shuffling method.

The *cs mtf1* mutants isolated in this study represented a group of *mtf1* mutants capable of utilizing non-fermentable carbons at both 30°C and 37°C, but incapable at 13°C. The fact that they were able to grow *in vivo* at the permissive temperature, but with slow growth phenotype indicated that functional Mtf1p produced in *cs mtf1* mutants was either in low activity or unstable. Immunoblot analyses are further required to prove whether the cold-sensitive phenotypes observed in the mutant strains were caused as a result of either the rapid degradation or the altered activity of mutant Mtf1p.

The *MTF1* gene product exhibited amino acid sequence similarities with bacterial σ factors in regions 2 and 3 (10). These regions of σ factors have been proposed to be involved in recognition of the bacterial promoter (7), DNA unwinding (12, 13), and interactions between the σ factors and the core RNA polymerase (15). The nucleotide change $T_{437}C$ by mutagenic PCR method results in the replacement of amino acid L146 with P, generating the cold-sensitive *mtf1* mutant yJH147. The amino acid change in this mutant is localized just outside the subregion 2.4 of Mtf1p. The cold-sensitive phenotype observed in a mutant yJH148 results from the replacement of amino acids K179 and K 214 with E and M, respectively. The K179 belongs to the basic region of Mtf1p, and the K214 to the acidic domain defined previously (17). Shadel and Clayton (26) created mutations in sigma-like, basic and acidic regions of the *MTF1* gene by the alanine scanning mutagenesis, and showed that mutants with either R178A and K179A double replacements in the basic or E212A and K214A replacements in the acidic region had wild-type *in vivo* phenotype. In our mutant strain yJH148, the changed amino acid residues are both K179 and K214, implying that both residues are responsible for the cold sensitive phenotype. These amino acid replacements result in conferring more acidity on Mtf1p.

Therefore, identification of the amino acid residues changed in *cs*⁻ alleles of *MTF1* mutation indicates that a region of Mtf1p located between σ factors-like regions 2 and 3 has structural and functional characteristics. Additional studies such as *in vitro* transcription assays, western blotting, and others will provide insight into the structure-function relationships and further into the transcription mechanism of the yeast mitochondrial RNA polymerase. The availability of a readily assayable cold-sensitive phenotype for the *MTF1* gene will also serve as a valuable tool for the future study.

## Acknowledgments

This work was supported by a domestic equipment research grant from the Korea Science and Engineering Foundation to S. H. Jang in 1994. The author thank JEIO TECH Corp. for providing low temperature incubator (model : BI-600M) through the KOSEF, and Jeong-Ae Yoo and Jeong-Eun Yang for making plates and maintaining strains.

## References

1. **Alani, E., L. Cao, and N. Kleckner.** 1987. A method for gene disruption that allows repeated use of *URA3* selection in the construction of multiply disrupted yeast strains. *Genetics* **116**, 541-545.
2. **Biswas, T.K., B. Ticho, and G.S. Getz.** 1987. *In vitro* characterization of the yeast mitochondrial promoter using single-base substitution mutants. *J. Biol. Chem.* **262**, 13690-13696.
3. **Boeke, J.D., F. LaCroute, and G.R. Fink.** 1984. A positive selection for mutants lacking orotidine-5'-phosphate decarboxylase activity in yeast: 5-fluoro-orotic acid resistance. *Mol. Gen. Genet.* **197**, 345-346.
4. **Cermakian, N., T.M. Ikeda, R. Cedergren, and M.W. Gray.** 1996 Sequence homologous to yeast mitochondrial and bacteriophage T3 and T7 RNA polymerases are widespread throughout the eukaryotic lineage. *Nucleic Acids Res.* **24**, 648-954.
5. **Gietz, R.D. and A. Sugino.** 1988. New yeast-*E. coli*

shuttle vectors constructed with *in vitro* mutagenized yeast genes lacking six-base pair restriction sites. *Gene* **74**, 527-534.

6. **Gulyas, K.D. and T.F. Donahue.** 1992. *SSL2*, a suppressor of a stem-loop mutation in the *HIS4* leader encodes the yeast homolog of human ERCC-3. *Cell* **69**, 1031-1042.
7. **Helmann, J.D. and M.J. Chamberlin.** 1988. Structure and function of bacterial sigma factors. *Annu. Rev. Biochem.* **57**, 839-872.
8. **Hoffman, C. and F. Winston.** 1987. A ten-minute DNA preparation from yeast efficiently releases autonomous plasmids for transformation of *Escherichia coli*. *Gene* **57**, 267-272.
9. **Ito, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. *J. Bacteriol.* **153**, 163-168.
10. **Jang, S.H. and J.A. Jaehning.** 1991. The yeast mitochondrial RNA polymerase specificity factor, *MTF1*, is similar to bacterial σ factors. *J. Biol. Chem.* **266**, 22671-22677.
11. **Jang, S.H. and J.A. Jaehning.** 1994. Mechanisms of mitochondrial transcription, p. 171-184. *In* R.C. Conaway and J.W. Conaway (eds.), Transcription: mechanisms and regulation. Raven Press, N. Y.
12. **Jones, C.H., M. Tatti, and C.P. Moran, Jr.** 1992. Effects of amino acid substitutions in the -10 binding region of $\sigma^E$ of *Bacillus subtilis*. *J. Bacteriol.* **174**, 6815-6821.
13. **Juang, Y.L. and J.D. Helmann.** 1994. A promoter melting region in the primary s factor of *Bacillus subtilis*: identification of functionally important aromatic amino acids. *J. Mol. Biol.* **235**, 1470-1488.
14. **Kelly, J.L., A.L. Greenleaf, and I.R. Lehman.** 1986. Isolation of the nuclear gene encoding a subunit of the yeast mitochondrial RNA polymerase. *J. Biol. Chem.* **261**, 10348-10351.
15. **Lesley, S.A. and R.R. Burgess.** 1989. Characterization of the *Escherichia coli* transcription factor $\sigma^{70}$: localization of a region involved in the interaction with core RNA polymerase. *Biochemistry* **28**, 7728-7734.
16. **Leung, D.W., E. Chen, and D.V. Goeddel.** 1989. A method for random mutagenesis of a defined DNA segment using a modified polymerase chain reaction. *J. Cell. Mol. Biol.* **1**, 11-15.
17. **Lisowsky, T. and G. Michaelis.** 1988. A nuclear gene essential for mitochondrial replication suppresses a defect of mitochondrial transcription in *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* **214**, 218-223.
18. **Lonetto, M., M. gribskov, and C.A. Gross.** 1992. The $\sigma^{70}$ family: sequence conservation and evolutionary relationship. *J. Bacteriol.* **174**, 3843-3849.
19. **Mandel, M. and A. Higa.** 1970. Calcium-dependent bacteriophage DNA infection. *J. Mol. Biol.* **53**, 159-162.
20. **Mangus, D.A., S.H. Jang, and J.A. Jaehning.** 1994. Release of the yeast mitochondrial RNA polymerase specificity factors from transcription complexes. *J. Biol. Chem.* **269**, 26568-26574.
21. **Marczynski, G.T., P.W. Schultz, and J.A. Jaehning.** 1989. Use of yeast nuclear DNA sequences to define the mitochondrial RNA polymerase promoter *in vitro*. *Mol. Cell. Biol.* **9**, 3193-3202.
22. **Master, 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.
23. **Muhlrad, D., R. Hunter, and R. Parker.** 1992. A rapid method for localized mutagenesis of yeast genes. *Yeast* **8**, 79-82.
24. **Rothstein, R.J.** 1983. One-step gene disruption in yeast. *Methods Enzymol.* **101**, 202-211.
25. **Sanger, F., S. Nicklen, and A.R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463-5467.
26. **Shadel, G.S. and D.A. Clayton.** 1995. A *Saccharomyces cerevisiae* mitochondrial transcription factor, sc-mtTFB, shares features with sigma factors but is functionally distinct. *Mol. Cell. Biol.* **15**, 2101-2108.
27. **Sherman, F., G. Fink, and J. Hicks.** 1983. Methods in yeast genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
28. **Sikorski, R.S. and J.D. Boeke.** 1991. *In vitro* mutagenesis and plasmid shuffling: from cloned gene to mutant yeast. *Methods Enzymol.* **194**, 302-318.
29. **Southern, E.M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* **98**, 503-517.
30. **Ulery, T.L., S.H. Jang, and J. A. Jaehning.** 1994. Glucose repression of yeast mitochondrial transcription: Kinetics of derepression and role of nuclear genes. *Mol. Cell. Biol.* **14**, 1160-1170.
31. **Waldburger, C. and M.M. Susskind.** 1994. Probing the information content of *Escherichia coli* $\sigma^{70}$ region 2.3 by combinatorial cassette mutagenesis. *J. Mol. Biol.* **235**, 1489-1500.
32. **Waldburger, C., T. Gardella, W. Wong, and M.M. Susskind.** 1990. Changes in conserved region 2 of Escherichia coli $\sigma^{70}$ affecting promoter recognition. *J. Mol. Biol.* **215**, 267-276.
33. **Wilcoxen, S.E., C.R. Peterson, C.S. Winkley, M.J. Keller, and J.A. Jaehning.** 1988. Two forms of *RPO41*-dependent RNA polymerase. *J. Biol. Chem.* **263**, 12346-12351.
34. **Zhou, Y.N., W.A. Walter, and C.A. Gross.** 1992. A mutant $\sigma^{70}$ with a small deletion in conserved region 3 of σ has reduced affinity for core RNA polymerase. *J. Bacteriol.* **174**, 5005-5012.