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Suppressor Analyses of Temperature-Sensitive *cbp1* Strains of *Saccharomyces cerevisiae*: The Product of the Nuclear Gene *SOC1* Affects Mitochondrial Cytochrome *b* mRNA Post-Transcriptionally

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Manuscript received April 28, 1994
Accepted for publication July 9, 1994

ABSTRACT

The induction of mitochondrial function is dependent upon both nuclearly encoded and mitochondrially encoded gene products. To understand nuclear-mitochondrial interactions, we must first understand gene-specific interactions. The accumulation of mitochondrial cytochrome *b* (*COB*) RNA is dependent upon Cbp1p, encoded by the nuclear gene *CBP1*. Thus, respiration is dependent upon Cbp1p. In this study, suppressors of temperature-sensitive *cbp1* (*cbp1^{ts}*) strains were selected for restoration of respiratory capability at the restrictive temperature (*Ts*$^+$). One nuclearly encoded suppressor, extragenic to *CBP1*, is recessive with respect to the wild-type suppressor allele and is unlinked to other known genetic loci whose gene products are necessary for expression of *COB* mRNA. The suppressor, called *soc1* for Suppressor *o*f *c*bp1, suppresses several other *cbp1^{ts}* alleles but does not operate via a bypass mechanism. Molecular analyses indicate that *soc1* allows the steady-state level of *COB* mRNA to increase at high temperature but has little or no effect on the levels of *COB* pre-mRNA. These data have led us to propose that the product of the nuclear gene *SOC1* is required for normal turnover of *COB* mRNA.

EACH step in RNA metabolism, such as transcription, processing and degradation, is a potential regulatory step in gene expression. RNA turnover has been shown to play a key regulatory role in many biological processes [for review see Belasco and Brawerman (1993)]. Although RNA transcription and processing have been studied extensively in the regulation of mitochondrial gene expression in *Saccharomyces cerevisiae* [for review see Costanzo and Fox (1990)], one aspect of yeast mitochondrial gene regulation that remains a mystery is the mechanism and the factors that regulate mRNA turnover. As yet, it is technically difficult to measure mRNA turnover in yeast mitochondria, and how the decay rate of mRNAs affects mitochondrial gene expression remains to be determined. Thus, most research on mRNA stability in yeast mitochondria has focused on nuclearly encoded factors that affect the accumulation of specific mitochondrial transcripts.

Several nuclearly encoded proteins have been described that affect the expression of specific mitochondrial messages. Mutations in these nuclear genes result in a *petite* phenotype (*pet*) in which the strains are viable but not respiratory competent [reviewed in Tzagoloff and Dieckmann (1990)]. *PET* genes affecting the accumulation of specific mitochondrial messages are *AEP2* (Finnegan *et al.* 1991; Payne *et al.* 1991) [also known as *ATP13* (Ackerman *et al.* 1991]) and *NCA1* (Ziaja *et al.* 1993) which affect *ATP9* mRNA, and *CBP1* which affects cytochrome *b* (*COB*) mRNA (Dieckmann *et al.* 1984b). Of these, the effect of *CBP1* on the expression of *COB* has been studied in detail. *cbp1* mutants were shown to be respiratory deficient because they lacked *COB* mRNA, while other mitochondrial transcripts were unaffected (Dieckmann *et al.* 1982, 1984a,b; Dieckmann and Mittelmeier 1987; Mittelmeier and Dieckmann 1990). Therefore, *CBP1* protein (Cbp1p) is required for the accumulation of stable *COB* mRNA.

Cbp1p has been proposed to protect and promote processing of *COB* mRNAs. A model of how Cbp1p acts to affect the expression of *COB* mRNA is shown in Figure 1. This model is supported by the following observations. Mature *COB* mRNA is processed from a polycistronic transcript that includes tRNA^{glu} (Bonitz *et al.* 1982; Christianson *et al.* 1983). Transcription of the *tRNA^{glu}-COB* unit and processing of tRNA^{glu} are not affected in *cbp1* strains (Dieckmann *et al.* 1984b). However, the precursor RNA that is produced following removal of tRNA^{glu} (Chen and Martin 1988; Hollingsworth and Martin 1986) with a 5′ end at −1098 (relative to +1 of the initiating AUG) is dependent upon Cbp1p for its accumulation (Mittelmeier and Dieckmann 1993; Staples and Dieckmann 1993). This transcript is processed further at −954 or −955 to generate the mature 5′ end of *COB* mRNA, cleavages promoted by Cbp1p (Staples and Dieckmann 1993). Most recently, Cbp1p has been shown to be required for the stability of the mature *COB* mRNA following 5′ processing at −954/−955 (Chen and Dieckmann 1994).

In an effort to identify new genetic loci involved in *COB* mRNA accumulation and stability, we have used

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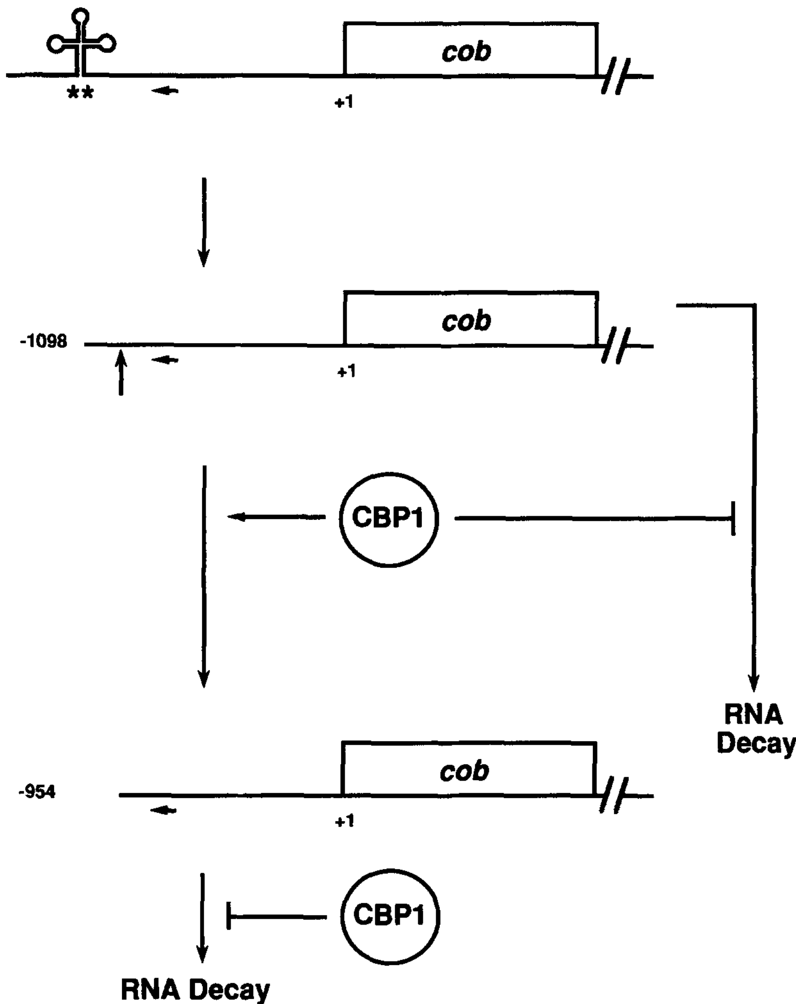


FIGURE 1.—Model of Cbp1p activity on *COB* mRNA and *COB* pre-mRNA. The primary transcript of *COB* contains both *COB* and *tRNA*^{glu}. Processing at the asterisks (*) generates mature tRNA^{glu} and *COB* pre-mRNA with a 5′ end at −1098 (relative to the +1 of the start AUG). The pre-mRNA is processed further (↑) to generate the mature *COB* mRNA with a 5′ end at −954. As described in the text, Cbp1p is required for the stable accumulation of both the mRNA and pre-mRNA and participates in the processing reaction that generates the mature mRNA. As described previously (STAPLES and DIECKMANN 1993) the −1098 molecule observed by primer extension analysis is probably composed of all precursor *COB* RNAs. ← indicates the primer binding site (−854 to −826) for the "cob 5B + 3" primer used in the primer extension assays.

temperature-sensitive *cbp1* (*cbp1*^{ts}) strains (STAPLES and DIECKMANN 1993) to select suppressors of *cbp1*. Here we report on the characterization of a suppressor, *soc1*, that affects the stability of mature *COB* mRNA in *cbp1*^{ts} strains and has little effect on *COB* pre-mRNA. We have proposed that *soc1* encodes a defective degradative nuclease that allows accumulation of the transcript. *soc1* increases the accumulation of the steady-state levels of other mitochondrial transcripts up to 3-fold. This finding supports the hypothesis that *soc1* is involved in mitochondrial mRNA turnover.

MATERIALS AND METHODS

Strains, plasmids and media: The yeast strains used in this study are described in Table 1. RSY1000a was constructed by switching the mating type of RSY1000 with HO endonuclease as described previously (HERSKOWITZ and JENSEN 1991). Plasmids containing *cbp1*^{ts} (pRS415/*cbp1*^{ts}) and *CBP1*$^{+}$ (pRS415/*CBP1*$^{+}$) alleles were described previously (STAPLES and DIECKMANN 1993). Aox1 was constructed by ligation of a 735-bp *Alu*I fragment from DS302 (CORUZZI *et al.* 1981) into the *Sma*I site of pSP65 (Promega, Madison, Wisconsin). EHox3 was constructed by ligation of a 605-bp *Eco*RI/*Hae*III fragment from DS6/A422 (BONITZ *et al.* 1980) into the *Eco*RI/*Sma*I sites of pSP65. Yeast strains were grown in either YPD (1% yeast extract, 2% peptone, 2% glucose), YPG (1% yeast extract, 2% peptone, 3% glycerol) or minimal media (WO; 0.67% yeast nitrogen base without amino acids, 2% glucose) (ROSE *et al.* 1990). Amino acid supplements were added to suggested final concentrations (ROSE *et al.* 1990). Solid media contained 2% agar.

Transformation of yeast strains: *S. cerevisiae* strains were transformed as described previously (GEITZ and SCHIESTL 1991).

RNA preparations and Northern blot analysis: Total cellular RNA was isolated as described (CAPONIGRO *et al.* 1993) from mid-logarithmic cultures grown in YPD or YPG. Approximately 8 μg of RNA were separated by electrophoresis on 1.0% agarose gels containing 6.7% formaldehyde. Following electrophoresis, Northern blot analysis was performed as described previously (MAYER and DIECKMANN 1989; MITTELMEIER and DIECKMANN 1990). *tRNA*^{glu}*-COB, COX3-*, and *ACT1*-specific probes were described previously (STAPLES and DIECKMANN 1993). The *ATP9*-specific probe was generated from pKS/AHol (MITTELMEIER and DIECKMANN 1990) by digestion with *Eco*RI and *Bam*HI (Boehringer Mannheim) to generate a 618-bp fragment spanning *ATP9* from +179 to +797 relative to the +1 of the starting AUG. A *COX2*-specific probe was generated by digestion of Aox1 with *Eco*RI and *Pst*I (Boehringer Mannheim) to generate a 735-bp fragment of *COX2* spanning +542 to +1280. A *COX1*-specific probe was generated by digestion of EHox3 with *Eco*RI and *Pst*I to liberate a 605-bp fragment spanning the recombination junction in DS6/A422 from +9916 to +5051. A *CBP1*-specific probe was generated by digestion of pRS415/*CBP1*$^{+}$ with *Pvu*II generating a 3.2-kb fragment spanning the entire coding region of *CBP1*. Each probe was purified by agarose gel electrophoresis

TABLE 1

Strains and genotypes

Strains	Genotype	Source or reference
S. cerevisiae		
JC3/M9410	*MAT***a** *ade2 lys2 kar* [*rho*$^+$ Δ*cob*]	MITTELMEIER and DIECKMANN (1993)
RSY2000	*MAT*α *ade1 leu2-3,112 ura3-52* Δ*cbp1::LEU2* [*rho*$^+$]	STAPLES and DIECKMANN (1993)
RSY1000	*MAT*α *ade1 leu2-3,112 ura3-52* [*rho*$^+$]	STAPLES and DIECKMANN (1993)
RSY1000a	*MAT***a** *ade1 leu2-3,112 ura3-52* [*rho*$^+$]	This study
RSY1[a]	*MAT*α *ade1 leu2-3,112 ura3-52 cbp1-26(ts)* [*rho*$^+$]	STAPLES and DIECKMANN (1993)
RSY5[a]	*MAT*α *ade1 leu2-3,112 ura3-52 cbp1-27(ts)* [*rho*$^+$]	STAPLES and DIECKMANN (1993)
RSY7[a]	*MAT*α *ade1 leu2-3,112 ura3-52 cbp1-28(ts)* [*rho*$^+$]	STAPLES and DIECKMANN (1993)
RSY8[a]	*MAT*α *ade1 leu2-3,112 ura3-52 cbp1-29(ts)* [*rho*$^+$]	STAPLES and DIECKMANN (1993)
RSY20[a]	*MAT*α *ade1 leu2-3,112 ura3-52 cbp1-30(ts)* [*rho*$^+$]	STAPLES and DIECKMANN (1993)
RSY33[a]	*MAT*α *ade1 leu2-3,112 ura3-52 cbp1-31(ts)* [*rho*$^+$]	STAPLES and DIECKMANN (1993)
RSY11	*MAT*α *ade1 leu2-3,112 ura3-52 cbp1-26(ts) soc2* [*rho*$^+$]	This study
RSY12	*MAT*α *ade1 leu2-3,112 ura3-52 cbp1-26(ts),32* [*rho*$^+$]	This study
RSY13	*MAT*α *ade1 leu2-3,112 ura3-52 cbp1-26(ts),33* [*rho*$^+$]	This study
RSY14	*MAT*α *ade1 leu2-3,112 ura3-52 cbp1-26(ts),34* [*rho*$^+$]	This study
RSY15	*MAT*α *ade1 leu2-3,112 ura3-52 cbp1-26(ts),35* [*rho*$^+$]	This study
RSY16	*MAT*α *ade1 leu2–3,112 ura3-52 cbp1-26(ts),36* [*rho*$^+$]	This study
RSY17[b]	*MAT*α *ade1 leu2-3,112 ura3-52 cbp1-26(ts) soc1-2* [*rho*$^+$]	This study
RSY18[b]	*MAT*α *ade1 leu2-3,112 ura3-52 cbp1-26(ts) soc1-3* [*rho*$^+$]	This study
RSY19[b]	*MAT*α *ade1 leu2-3,112 ura3-52 cbp1-26(ts) soc1-1* [*rho*$^+$]	This study
RSY29[c]	*MAT*α *ade1 leu2-3,112 ura3-52 soc1-1* [*rho*$^+$]	Respiratory competent spore from NPD tetrad of RSY19 × RSY1000a, this study
E67	*MAT*α *met6 cbs2* [*rho*$^+$]	MUROFF and TZAGOLOFF (1990)
N356	*MAT*α *met6 cbs1* [*rho*$^+$]	TZAGOLOFF and DIECKMANN (1990)
E158	*MAT*α *met6 cbp6* [*rho*$^+$]	DIECKMANN and TZAGOLOFF (1985)

[a] Isogenic *MAT***a** strain obtained by mating to RSY1000a.
[b] Isogenic *MAT***a** strain obtained by mating to *MAT***a** parent.
[c] Isogenic *MAT***a** strain obtained from same NPD tetrad as *MAT*α strain.

and radiolabeled with [α^{32}P]dATP (ICN, Costa Mesa, California) using the Random Primed Labeling Kit (Boehringer Mannheim) according to the manufacturer's instructions. An *ATP8/ATP6*-specific probe was generated by end-labeling an *ATP8* mRNA-specific oligo (5′-TGG AAC TAA TTG TGG CAT-3′, kindly provided by N. CAMOUGRAND at Centre National de la Recherche Scientifique, Bordeaux, France) with [γ^{32}P]ATP (ICN) using polynucleotide kinase (Boehringer Mannheim) according to the manufacturer's instructions.

Primer extension analysis of *COB* transcripts: For quantitative analysis by primer extension, 8 μg of total cellular RNA was hybridized to 10 pmol of the "cob5B+3" primer (5′-CAA TTA TTA TTA TTA TTA TTA TAC ATA AA-3′) (MITTELMEIER and DIECKMANN 1993) and carried out as described (MITTELMEIER and DIECKMANN 1993) and modified (STAPLES and DIECKMANN 1993). The primer binding site is shown in Figure 1. JC3/M9410 contains a deletion of *COB* untranslated leader sequence from −975 to −64, and thus the primer binding site is deleted.

Quantitation of Northern blots and primer extension products: Mitochondrial and *ACT1* mRNAs from Northern blots and cDNAs from primer extension gels were quantitated using a Betascope Analyzer (Betagen, Waltham, Massachusetts). For Northern blot analyses of total cellular RNA, mitochondrial mRNA was normalized to *ACT1* mRNA. Primer extension products were normalized to the *ACT1* mRNA detected by Northern analysis.

RESULTS

Selection of spontaneous suppressors of temperature-sensitive *cbp1* (*cbp1*^{ts}) mutations: To identify the factors affecting mRNA stability and accumulation, we chose to isolate suppressors of *cbp1* in the hope that new genetic loci involved in stability of *COB* mRNA might be identified. We used temperature-sensitive *cbp1* strains (*cbp1*^{ts}) that do not respire and lack *cob* mRNA at high temperature (STAPLES and DIECKMANN 1993) to enhance the possibility of acquiring non-bypass suppressors. *cbp1*^{ts} strains RSY1, RSY5, RSY7, RSY8, RSY20 and RSY33 (each of which have different *ts* alleles; Table 1) were grown in YPD broth overnight and plated onto YPD plates for single colonies. An individual colony containing approximately 10^7 viable cells was suspended in sterile water, plated onto a YPG plate supplemented with 0.05% glucose and incubated at 35° for 3–4 weeks to select for revertants (*Ts*$^+$). 125 individual colonies from each strain were plated in this manner, and thus a total of 1.25×10^9 cells from each strain were used to select spontaneous revertants. Individual colonies that appeared on the plates were picked and purified. In the rare event that more than one revertant arose from the same plate, only one revertant was isolated to ensure that each revertant was independent of the others. The total number of revertants from each strain is shown in Table 2.

Genetic analyses of *cbp1*^{ts} suppressors: Our goal was to identify new nuclearly encoded suppressor loci. Previously, Mn^{2+}-induced mutagenesis of *cbp1* null strains yielded mitochondrial genomic rearrangements that were global bypass suppressors of all *cbp1* strains (DIECKMANN *et al.* 1984b). To eliminate from consideration any revertants containing genetically unstable mitochondrial genomic rearrangements, the revertants were grown overnight in YPD to stationary phase and plated for single colonies. These colonies were replica

TABLE 2

Revertants and genetic stability

Strain	No. of independent revertants	No. of stable revertants
RSY1	9	9
RSY5	1	1
RSY7	24	12
RSY8	0	—[a]
RSY20	3	3
RSY33	2	1

[a] Not applicable.

TABLE 3

Results of RSY1 revertants crossed to RSY1000a

Strain	D/R[a]	No. of tetrads	4^+:0^{ts}	2^+:2^{ts}	3^+:1^{ts}	Other
RSY11	R[b]	8	1	3	4	0
RSY12	D	9	9	0	0	0
RSY13	D	5	5	0	0	0
RSY14	D	10	9	0	0	1
RSY15	D	6	6	0	0	0
RSY16	D	8	8	0	0	0
RSY17	R	5	1	0	4	0
RSY18	R	13	7	2	4	0
RSY19	R	10	0	5	5	0

[a] D/R, dominant (D) or recessive (R) with respect to wildtype, scored from diploids of RSY1 revertants crossed to *MATa* RSY1 strain.
[b] Extremely weak suppressor and therefore not analyzed further.

plated to YPG and YPD at 24°, 30° and 35°. Those revertants that exhibited stable retention of the revertant phenotype (*Ts*$^+$) were considered to be either stable mitochondrial revertants or nuclearly encoded revertants (see Table 2). RSY1 and RSY7 yielded the most revertants. However, RSY7 has the weakest *ts* phenotype in our collection (STAPLES and DIECKMANN 1993), which made scoring of suppressors difficult. Thus, we chose to characterize revertants of RSY1 further.

The revertants of RSY1 (RSY11–19, Table 1) were backcrossed to RSY1 (of opposite mating type) and the diploids were tested for respiration at 35° in order to score for recessiveness or dominance of the suppressor mutation with respect to the wild-type suppressor allele (see Table 3). The diploids were sporulated and tetrads were dissected to determine the number of genetic loci involved in suppression. A 2^+:2^{ts} segregation pattern would indicate that the suppressor was encoded by a single nuclear locus. All of the suppressors isolated from RSY1 were shown to be encoded by a single nuclear gene since the segregation pattern was 2^+:2^{ts} in all cases (data not shown).

To determine whether the suppressors were intragenic or extragenic to *CBP1* and whether or not the suppressors had a phenotype other than suppression, the revertants from RSY1 were outcrossed to an isogenic wild-type strain, RSY1000a (wild type at *CBP1* and the suppressor locus, Table 1), and the diploids were sporulated and dissected. If a suppressor was tightly linked or intragenic to the *cbp1*^{ts} locus, then a segregation pattern of 4^+:0^{ts} would be observed for all tetrads. If a suppressor was unlinked to *cbp1*^{ts}, then *ts* spores should be recovered in the tetrad analysis. The results of the outcross of the revertants from RSY1 to RSY1000a are shown in Table 3. All of the dominant suppressors from RSY1 segregated 4^+:0^{ts} indicating that the suppressors were tightly linked or intragenic to *CBP1*. The recessive revertant strains, RSY11, RSY17, RSY18 and RSY19, crossed to RSY1000a segregated 4^+:0^{ts}, 3^+:1^{ts}, and 2^+:2^{ts} indicating that the suppressor was extragenic to the *cbp1*^{ts} gene. We have characterized further the recessive extragenic revertants of RSY1 with the exception of RSY11, which was extremely weak. One such extragenic recessive suppressor strain (RSY19) is shown in Figure 2. We have tentatively named the genetic loci *soc*, for *s*uppressor *o*f *c*bp1^{ts}. From the segregation analysis of the outcross to the wild-type strain, we have concluded that the strains that are *CBP1*$^+$*soc* (for example RSY29, confirmed by segregation analysis of tetrads from diploids of RSY29 crossed to RSY1) were phenotypically indistinguishable from the wild-type strain when grown on YPD or YPG at 24 or 35° medium (Figure 2). In addition, slight differences in growth rate from wild type was observed when grown at 36° and 37° on minimal medium that contained glycerol as a carbon source (data not shown). No other phenotypes were tested.

To determine whether the *soc* mutations in RSY17, RSY18 and RSY19 were in the same complementation group, each revertant was mated to the other revertants, and the diploids were tested for the ability to respire at 35°. As the suppressors are recessive with respect to the wild-type *SOC*$^+$ allele, if they fail to complement each other, the diploids would be respiratory competent at all temperatures (*Ts*$^+$). If they do complement each other, then the diploids would be expected to be *ts* for respiration. The strains all failed to complement each other (*Ts*$^+$) indicating that they are in the same complementation group. To confirm the complementation tests, the diploids were sporulated and tetrads were dissected. An average of 7 tetrads were analyzed and all showed a 4^+:0^{ts} segregation pattern for respiration (data not shown) indicating the suppressors were tightly linked to each other and allelic. We have called the locus defined by the suppressor in these revertants, *SOC1*. As a representative of the three *soc1* mutant strains, RSY19 was analyzed further.

To determine whether *soc1-1* in RSY19 was able to suppress other *cbp1*^{ts} alleles, RSY19 was mated to the other *cbp1*^{ts} strains, RSY5, RSY7, RSY8, RSY20 and RSY33. If *soc1-1* were able to suppress the other *cbp1*^{ts} alleles, then the segregation pattern should be 2^+:2^{ts} in all cases. If it is unable to suppress, then tetratype (TT, 1^+:3^{ts}) and nonparental ditype (NPD, 0^+:4^{ts}) tetrads should be observed also. Only 2^+:2^{ts} tetrads were recovered in the crosses to RSY5, RSY7, RSY8 and RSY20. 2^+:2^{ts}, 0^+:4^{ts} and 1^+:3^{ts} tetrads were recovered in the cross

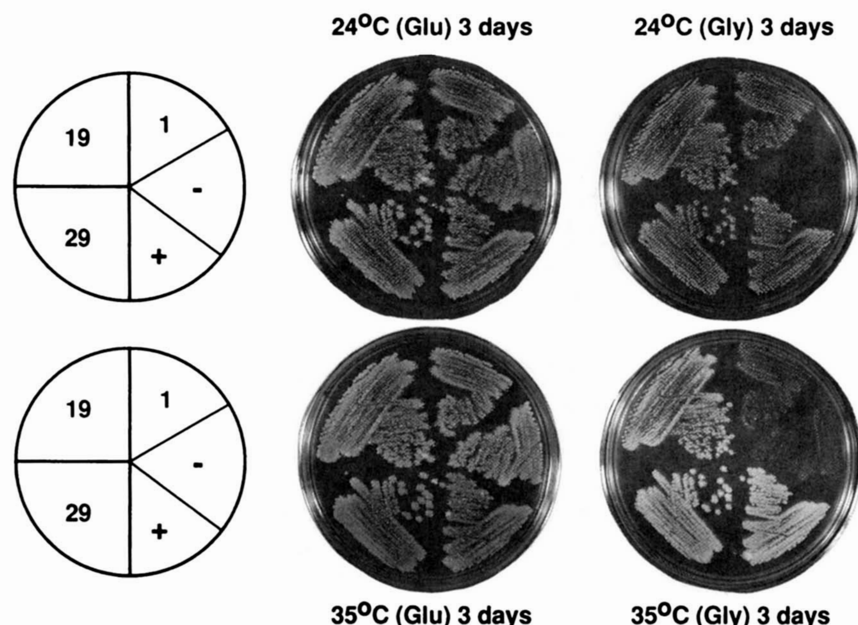


FIGURE 2.—RSY strains grown on fermentable (Glu) and nonfermentable (Gly) media at permissive and restrictive temperatures. RSY strains were streaked for single colonies onto a YPD plate, incubated at 24° and then replica plated to YPD and YPG plates and grown at 24° and 35°. The plates were photographed after 3 days. The plate diagrams to the left indicate the orientation of the strains. −, RSY2000 (Δ*cbp1::LEU2*); +, RSY1000 (isogenic wild type); 1, RSY1 (*cbp1*^{ts} *SOC1*$^+$); 19, RSY19 (*cbp1*^{ts} *soc1-1*); 29, RSY29 (*CBP1*$^+$ *soc1-1*).

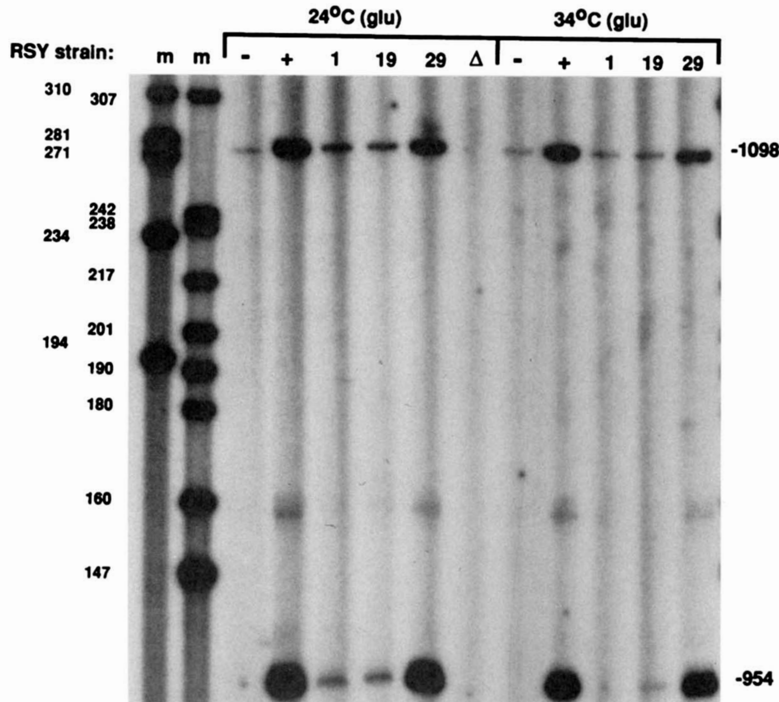
to RSY33. That the segregation pattern was 2^+:2^{ts} for all the strains except RSY33 indicates that *soc1-1* suppresses all the *cbp1*^{ts} alleles except that in RSY33.

To test whether *soc1-1* could suppress a Δ*cbp1* allele, RSY19 was mated to RSY2000 (Δ*cbp1::LEU2*$^+$, and therefore respiratory deficient; Table 1). Diploids were sporulated, and tetrads were dissected. The spores were tested for the ability to respire at all temperatures. If the suppressor was able to suppress the Δ*cbp1* allele, then segregation patterns of 2^+:1^{ts}:1^- (TT), 2^+:2^{ts}:0^- (NPD), and 2^+:0^{ts}:2^- (PD) should be observed. If the suppressor was not able to suppress the Δ*cbp1* allele, then segregation patterns of 1^+:1^{ts}:2^- (TT), 0^+:2^{ts}:2^- (NPD), and 2^+:0^{ts}:2^- (PD) should be observed. The segregation patterns of 42 tetrads were as follows: 30 tetrads segregated 1^+:1^{ts}:2^-, 6 tetrads segregated 0^+:2^{ts}:2^-, and 6 tetrads segregated 2^+:0^{ts}:2^-. These data indicate that *soc1-1* was unable to suppress the Δ*cbp1* allele. As the Δ*cbp1* allele is marked by replacement of *CBP1* with a *LEU2*$^+$ gene, all Leu$^+$ spores should be Δ*cbp1*. In a random spore analysis of the tetrads, 69 Leu$^+$ spores were obtained from a total of 145 spores. Sixty-eight of these were respiratory deficient at all temperatures, and 1 was *ts* for respiration. Presumably, this *ts* spore arose as a product of gene conversion at the *leu2* locus. We conclude that *soc1* does not operate via a mechanism that bypasses the requirement for Cbp1p.

To determine whether *soc1* was linked to previously identified nuclear genes encoding mitochondrial proteins that are required for the expression of *COB* mRNA such as *CBS1*, *CBS2* or *CBP6*, (DIECKMANN and TZAGOLOFF 1985; RÖDEL 1986; RÖDEL and FOX 1987), RSY19 was mated to E67 (*cbs2*), N356 (*cbs1*) and E158 (*cbp6*), nonisogenic strains (Table 1). Diploids were sporulated and tetrads were dissected. The germinating spores had a low frequency of viability, and therefore, complete tetrads could not be scored. However, if *soc1* is unlinked to the other genes, then 1/8 or 12.5% of all the spores should be *ts*. If they are linked, then no *ts* spores should be recovered. The cross to E67 (*cbs2*) yielded 6 *ts* spores out of 92 (7%), N356 (*cbs1*) contained 4 *ts* spores out of 81 (5%), and E158 (*cbp6*) contained 3 *ts* spores out of 31 (10%). We conclude that *soc1* is unlinked to these genetic loci whose gene products are required for expression of *COB* mRNA.

In summary, *soc1-1* in RSY19 is recessive with respect to *SOC1*$^+$. It is encoded by a single nuclear gene and unlinked to known mitochondrial *COB* translation factors. It does not have a phenotype other than suppression and is broadly allele-specific for suppression, although it does not suppress the *cbp1*^{ts} allele [*cbp1-31(ts)*] in RSY33. The mechanism of suppression is not due to a bypass of Cbp1p function.

***soc1-1* acts specifically on mature *COB* mRNA and not *COB* pre-mRNA, tRNA^{glu} or *CBP1* mRNA:** We were interested in determining the mechanism of suppression by *soc1-1* in RSY19. We hypothesized that if *SOC1* encoded a nuclease that turned over *COB* RNAs, then we would expect to observe an increase in the steady-state levels of *COB* RNAs in *soc1–1* strains. As shown previously (STAPLES and DIECKMANN 1993), strain RSY1 is temperature-sensitive for respiration, lacks *COB* mRNA (5′ end at −954) and has decreased steady-state levels of *COB* pre-mRNA (5′ end at −1098) at the restrictive temperature. To test whether *soc1* increased the steady-state levels of *COB* RNAs at high temperature, RNAs from these strains were assayed first by primer extension analysis. This type of analysis has been shown to be sensitive for assaying the levels of both *COB* mRNA and *COB* pre-mRNA (STAPLES and DIECKMANN 1993). As



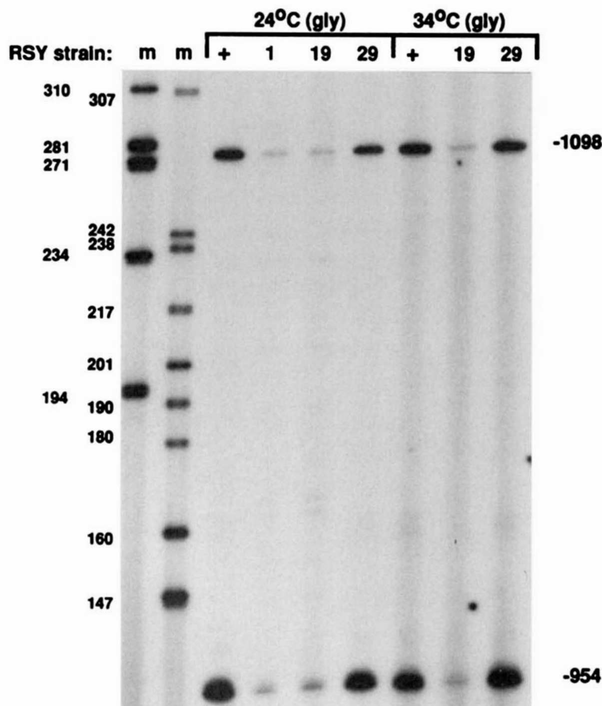


FIGURE 3.—Primer extension analysis of *COB* RNAs from *cbp1*^{ts} and *soc1-1* strains at 24° and 34°. Primer extension analysis was performed as described in MATERIALS AND METHODS. Strains were grown in YPD (glu) shown in (A) or YPG (gly) shown in (B) at 24° and 34°. RSY strains are as described in Figure 2. While RSY19 respires on plates at 35°, it will respire only at 34° or below in liquid YPG medium. m, pBR322 digested with *Msp*I and ϕX174 digested with *Hae*III; markers were labeled as described in MATERIALS AND METHODS. Δ, RNA from JC3/M9410 in which the primer binding site is deleted (Table 1). −1098, *COB* pre-mRNAs with 5′ ends at −1098 (relative to the +1 of the starting AUG); −954, mature *COB* mRNAs with 5′ ends at −954.

shown in Figure 3, RSY1 lacks *COB* mRNA (5′ end at −954) at 34° when grown in YPD (Figure 3A); *COB* mRNA is present in RSY19 at 34° when cells were grown in either YPD (Figure 3A) or YPG (Figure 3B). As shown in Figure 3A, *soc1-1* in RSY19 had little or no effect on *COB* pre-mRNA (5′ end at −1098), but had a dramatic effect on mature *COB* mRNA at the high temperature. The levels of precursor and mature RNAs from the primer extensions were quantitated and normalized to the *ACT1* mRNA levels from Northern blots (not shown) to ensure that approximately equal amounts of total cellular RNA was used in the primer extension

TABLE 4

Levels of mitochondrial RNAs

Strain	Growth temperature (°C)	*COB*	tRNA[E]	*COX1*	*COX2* P[a]	*COX2* M[b]	*COX3*	*ATP9*	*ATP8/6* L[c]	*ATP8/6* S[d]
RSY1000	24	100	100	100	100	100	100	100	100	100
RSY1	24	17 ± 3	84 ± 43	106 ± 31	144 ± 36	168 ± 65	97 ± 19	111 ± 23	117 ± 21	151 ± 38
RSY19	24	25 ± 9	76 ± 50	107 ± 36	140 ± 69	224 ± 102	97 ± 34	133 ± 25	110 ± 37	194 ± 100
RSY29	24	109 ± 24	122 ± 59	127 ± 51	111 ± 36	127 ± 39	107 ± 30	99 ± 22	103 ± 16	139 ± 27
RSY1000	34	100	100	100	100	100	100	100	100	100
RSY19	34	17 ± 7	136 ± 13	112 ± 12	157 ± 20	216 ± 27	171 ± 21	193 ± 37	186 ± 22	301 ± 36
RSY29	34	139 ± 27	101 ± 19	116 ± 33	123 ± 36	135 ± 40	148 ± 35	114 ± 13	130 ± 42	128 ± 37

The RNAs were isolated from 4 cultures of each strain grown in YPG and were subjected to Northern analyses as described in the MATERIALS AND METHODS. The RNAs were quantitated and normalized to *ACT1* mRNA. Wildtype levels were set at 100% for each experiment, and thus the standard deviations for wildtype would be ± 0. The numbers reflect the percentage of RNA compared to that measured in the wildtype strain. Standard deviations are shown also.

[a] Precursor, 3 kb (CORRUZZI *et al.* 1981).

[b] Mature, 0.85 kb (CORRUZZI *et al.* 1981).

[c] Large transcript, 4.5 kb (COBON *et al.* 1982).

[d] Small transcript, 3.9 kb (COBON *et al.* 1982).

analyses. The quantitated levels (not shown) correlated with those observed qualitatively in Figure 3. However, as this method of normalization contains inherent experimental error, and as the levels of precursor RNAs changed little, we chose to quantitate accurately the steady-state levels of the *COB* mRNA using Northern analysis (see below). From these data we conclude that the suppressor *soc1* acts to increase specifically mature *COB* mRNA at 34° with little effect on precursor RNA.

To quantitate accurately the steady-state levels of *COB* mRNA Northern blot analysis was performed. The strains were grown in four separate cultures in YPG at 24° and 34°. Total cellular RNA was isolated and probed for *COB* mRNA (not shown), quantitated, and normalized to *ACT1* mRNA. Wild-type levels were set at 100% for each experiment, and an average of the percent of wild-type for each strain is shown in Table 4. The steady-state level of *COB* mRNA in RSY19 when grown in YPG at 34° was observed to be 17% of wild-type levels (Table 4). *COB* mRNAs from RSY1 grown at 34° in YPD are undetectable when assayed by Northern analysis and primer extension analysis (STAPLES and DIECKMANN 1993) (Figure 3). As we cannot measure *COB* mRNA levels in RSY1 when grown in YPG at 34° (since this strain will not grow in YPG at this temperature), we must estimate the increase in *COB* mRNA due to the suppressor from our limits of detection. Using 1% (our limit of detection) as the highest level possible in RSY1 at 34°, there is approximately a 17-fold increase in the level of *COB* mRNA at 34° due to the suppressor in RSY19. No differences in the amount of *COB* mRNA in RSY29 when grown in YPG at 34° were detected (Table 4).

Since tRNA[glu] is cotranscribed with *COB* mRNA, we can determine by measuring the levels of this tRNA whether there might be an increase in transcription rates. tRNA[glu] levels were quantitated from the blots, normalized to *ACT1* mRNA and are shown in Table 4. tRNA[glu] levels increased 36% in RSY19 at 34° when grown in YPG, while tRNA[glu] did not increase in RSY29 when grown in YPG at 34° (Table 4). That *COB* mRNA levels increased at least 17-fold at 34°, but tRNA[glu] levels did not increase proportionately, is suggestive that the mechanism of suppression is not due to an increase in the transcription rate of *COB* pre-mRNA. In addition, these blots were probed for *CBP1* mRNA. Quantitation of *CBP1* mRNA indicated that the steady-state levels of this RNA in RSY1 were not increased in RSY19 at low or high temperature (data not shown). These data are suggestive that the mechanism of suppression is not due to an increase in expression of *CBP1* mRNA.

There are two possible mechanisms of suppression. Since *soc1-1* is recessive with respect to the wild-type *SOC1*$^{+}$, it is possible that the *SOC1* gene product is a negative regulator of the processing reaction that generates the mature 5′ end of *COB* mRNA. It is also possible that the *SOC1* gene product is a nuclease that specifically degrades *COB* mRNA, but not *COB* pre-mRNA.

The *soc1-1* mutation has a small effect on other mitochondrial transcripts: We hypothesized that if *soc1-1* encoded a defective negative regulator of the *COB* processing reaction, then the steady-state levels of other mitochondrial transcripts would be unaffected. Conversely, if *soc1-1* encoded a defective nuclease, then a more global effect on mitochondrial transcripts might be observed. We measured the steady-state levels of *COX1*, *COX2* (3.0-kb precursor and 0.85 mature transcripts), *COX3*, *ATP9* and the large and small transcripts encoding *ATP8* and *ATP6*. Northern blots of total cellular RNA from four separate cultures each of RSY1000, RSY1, RSY19 and RSY29 grown in YPG at 24° and 34° (with the exception of RSY1) were probed for each transcript, quantitated, and normalized to *ACT1* mRNA as described in the MATERIALS AND METHODS. The average steady-state level expressed as a percentage of wild type is shown in Table 4. The steady-state levels of *COX2*, *COX3*, *ATP9* and *ATP8/ATP6* transcripts in-

creased in RSY19 (*cbp1^ts^ soc1-1*) when grown at 34° in YPG. *ATP9*, *COX2* precursor (3.0 kb) and *COX3* transcripts increased approximately 50% when RSY19 was grown at 34° in YPG, while the mature 0.85 *COX2* and the large and small *ATP8/ATP6* transcripts increased 2- and 3-fold, respectively, when RSY19 was grown in YPG at 34°. However, mitochondrial transcripts from RSY29 (*CBP1*$^+$, *soc1-1*) when grown in YPG at 34° exhibited no increase (Table 4). *COX2* transcripts are thought to have 5′ ends that are products of initiation of transcription and are not thought to be further processed (CORRUZI *et al.* 1981; DE ZAMAROCZY and BERNARDI 1986). Thus, if *SOC1* is a negative regulator of 5′ *COB* processing or even 5′ processing in general, one would predict that *COX2* mRNA levels would not be affected in the *soc1-1* strains. That the steady-state levels of *COX2* transcripts are increased in *soc1-1* strains is suggestive that the *SOC1* gene product is more likely to encode a nuclease that normally degrades *COB* mRNA, and also affects the degradation of other mitochondrial transcripts as well.

DISCUSSION

Little is known about the mechanism and the factors involved in the degradation of mitochondrial mRNAs. Studies of the steady-state levels of individual mitochondrial transcripts have shown that the accumulation of mRNAs is different under conditions of catabolite repression and derepression (MUELLER and GETZ 1986b). These data imply that either the relative rates of transcription are different, or there are differences in the rates at which the RNAs are turned over. It has been reported that different promoter strengths may be partially responsible for the different steady-state levels of mitochondrial transcripts (MUELLER and GETZ 1986a; WETTSTEIN-EDWARDS *et al.* 1986). Our focus has been to understand the role of RNA stability in the regulation of mitochondrial gene expression. As yet, measurement of mitochondrial RNA decay rates is technically very difficult. As a result, we have focused on specific nuclearly encoded factors that affect mRNA stability in yeast mitochondria. The goal of this study was to identify new nuclear loci that affect the stability of mitochondrial mRNAs, in particular *COB* mRNA. In the present study, we have isolated a suppressor (*soc1-1*) of a temperature-sensitive *cbp1* strain (RSY1) that restores respiratory capability to the strains at high temperature (*Ts*$^+$). This suppressor, called *soc1*, is a nuclear gene. *soc1* is unlinked to known *COB*-specific expression factors *CBS1*, *CBS2* and *CBP6* (DIECKMANN and TZAGOLOFF 1985; RÖDEL 1986; RÖDEL and FOX 1987) and does not operate via a bypass of *CBP1* function.

soc1-1 arose spontaneously in RSY1 at a frequency of 4.2×10^{-8}. Thus, the occurrence of spontaneous suppressors of *cbp1-26(ts)* was relatively low. Not all of the *cbp1^ts^* strains yielded spontaneous revertants (for example, RSY8) or extragenic suppressors (for example RSY5 and RSY20, data not shown); however, these *cbp1^ts^* alleles are suppressible by *soc1-1*. Perhaps additional suppressor analysis under mutagenic conditions will yield different nuclear suppressor loci.

In addition to recessive extragenic suppressors, we expected to isolate dominant extragenic suppressors. These types of suppressors might be those whose gene products interact with Cbp1p to allow processing of *COB* pre-mRNA or stabilization of *COB* RNAs. That no such suppressors were isolated may indicate that they do not exist, are essential for growth, or affect mitochondrial DNA stability (FOURY 1989; LISOWSKY and MICHAELIS 1988; MYERS *et al.* 1985). Perhaps a suppressor analysis following mutagenesis of a *cbp1^ts^* homozygous diploid strain would be more productive in yielding dominant extragenic suppressors. This method was used successfully to identify a protein that interacts with actin (ADAMS and BOTSTEIN 1989).

We have attempted to ascertain the mechanism by which *soc1-1* suppresses the temperature-sensitive phenotype of RSY1. Molecular analysis of the suppressor strain indicates that *soc1-1* allows accumulation of *COB* mRNA in *cbp1^ts^* strains at the restrictive temperature. tRNA^{glu} and *CBP1* mRNAs are not increased proportionally to *COB* mRNAs, indicating that the mechanism of suppression is not due to an increase in mitochondrial transcription or increased expression of *CBP1* mRNA. In addition, precursor *COB* mRNA levels change little if at all in *soc1* strains (Figure 3). These data are consistent with two hypotheses: (1) *soc1-1* encodes a defective negative regulator of the processing reaction that generates the mature 5′ end of *COB* mRNA, although we know of no examples of negative regulators of RNA processing reactions and (2) *soc1-1* encodes a defective nuclease that is normally involved in the degradation of *COB* mRNA. *soc1-1* has a smaller effect on the accumulation of mitochondrial transcripts other than *COB*. A 2-fold or greater effect was observed for some transcripts (*COX2* and *ATP8/ATP6*) in RSY19 (*cbp1^ts^ soc1*) but not RSY29 (*CBP1*$^+$ *soc1*) when grown in YPG at 34°. That there are differences in the steady-state levels of RNA between *cbp1^ts^* and *CBP1*$^+$ strains may be indicative of the effect of different respiratory states of the cells. That the two strains respire differently is reflected in that RSY19 and RSY29 have different growth rates on minimal media containing glycerol as the carbon source (data not shown). Since the *COX2* transcript has a 5′ end that is the product of the initiation of transcription rather than processing (CORUZZI *et al.* 1981; DE ZAMAROCZY and BERNARDI 1986), it would not be expected to change if *soc1-1* were involved in 5′ end processing of *COB* mRNA or 5′ processing in general. The increased accumulation of mitochondrial transcripts other than *COB* in the *soc1-1* strains is supportive of the alternative hypothesis that *SOC1* encodes a nuclease that affects the degradation of mitochondrial mRNA.

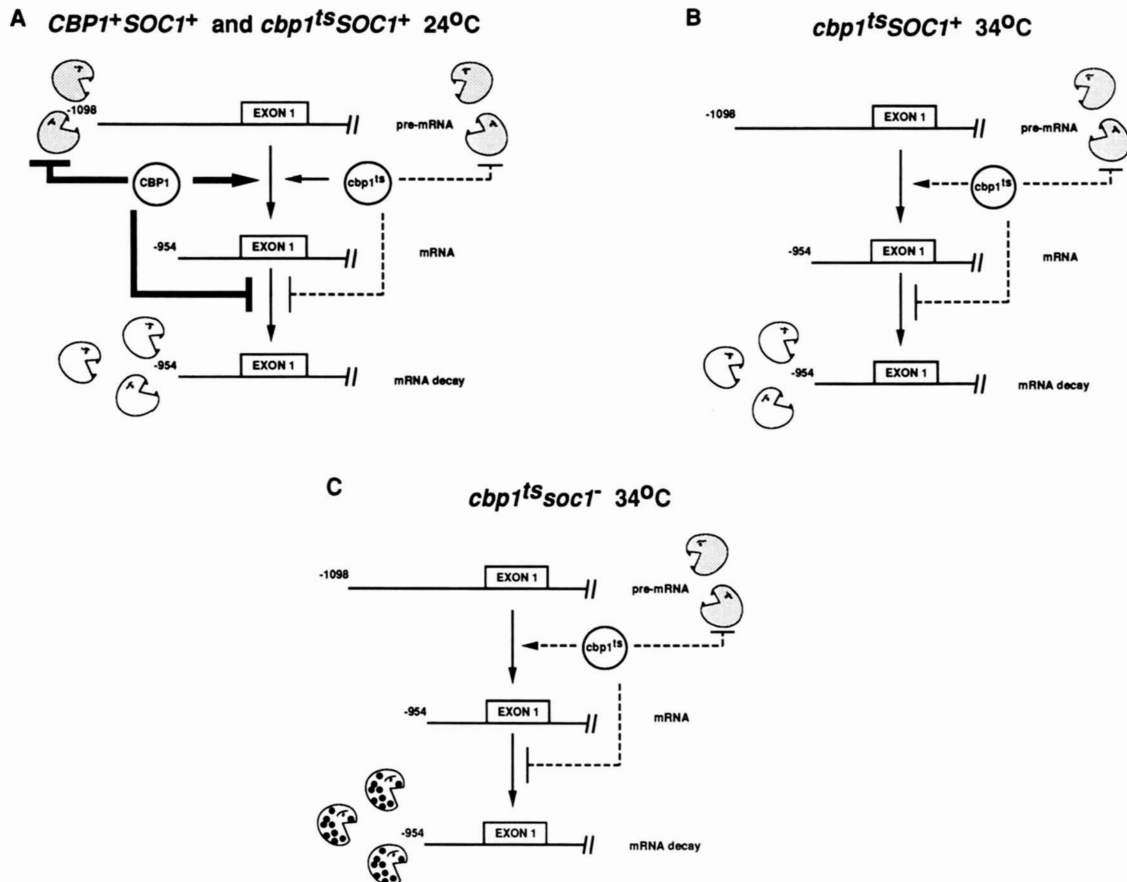


FIGURE 4.—Model of Cbp1p and Soc1p activities and the mechanism by which *soc1-1* allows respiration in *cbp1ts* strains. As described in Figure 1 and in the text, Cbp1p is thought to protect (⊣) both mature *COB* mRNA and *COB* pre-mRNA from degradation by nucleases (cartoon) and to promote (→) processing of pre-mRNA to generate the mature 5′ end of *COB* mRNA (A). As described previously (STAPLES and DIECKMANN 1993), in a *cbp1ts* strain at 24°, Cbp1p marginally protects *COB* pre-mRNA, yet processing still occurs (A). We presume that the binding of Cbp1p to mRNA and pre-mRNA to be similar; as yet we have no method to assess the protection by Cbp1p of mature mRNA following cleavage in these strains. At 34° (B), binding to the pre-mRNA is so weak that processing occurs at levels that are undetectable by primer extension analysis or Northern blot analysis. However, in *cbp1ts soc1-1* strains at 34° (C) the absence of a functional *SOC1* gene product (depicted here as the spotted cartoon), allows any mature mRNA produced to be stabilized further by the loss of such a nuclease. It is possible also that *SOC1* encodes a regulator of such a nuclease. As the phenotype of *soc1-1* mutants have no effect on the pre-mRNA steady-state levels, we presume that the nucleases affecting the two RNAs are different, hence the different colors of the cartoons in panels A and B.

Perhaps if we could find conditions where the steady-state levels of *COX3*, *COX2* or *ATP8/ATP6* were 10% or less of wild type, then a larger increase in these RNAs might be observed in the *soc1* strains.

A working hypothesis for the mechanism of suppression by *soc1-1* is shown in Figure 4. The model indicates that *SOC1* encodes a nuclease that degrades *COB* mRNA with little or no effect on *COB* pre-mRNA. The *cbp1ts* gene product must function at some low undetectable level at high temperature in order for mature *COB* mRNA to be produced. What little *COB* mRNA is present, is then further stabilized by the loss of a nuclease that normally degrades the transcript. That the *cbp1ts* allele in RSY33 and the Δ*cbp1* allele in RSY2000 are not suppressed by *soc1* is consistent with this model, in that Cbp1p in RSY33 may be more dysfunctional than the proteins from other *cbp1ts* strains and absent in RSY2000. We have shown previously that strains with as little as 10% of wild-type *COB* mRNA levels respire normally (STAPLES and DIECKMANN 1993). It is likely that *soc1-1* does not allow *COB* mRNA to accumulate in RSY33 to levels that allow respiration.

Our data also are suggestive that *COB* pre-mRNA and *COB* mRNA may have separate degradative pathways since *COB* mRNA is allowed to accumulate in the *soc1* strain while *COB* pre-mRNA is not. It is not inconceivable that the two RNAs are degraded by different mechanisms. The RNAs differ only at the 5′ end. This difference is suggestive that the 5′ end plays an important role in the degradation of *COB* RNAs. A protein complex from yeast mitochondria has been isolated and shown biochemically to contain a 3′ to 5′ exonucleolytic activity (MIN *et al.* 1993). A second complex which has been shown to bind the 3′ dodecamer sequence of mitochondrial transcripts inhibits the exonucleolytic activity of the 3′ to 5′ exonuclease complex (MIN and ZASSENHAUS

1993). It is possible that the *SOC1* gene product is part of the exonucleolytic complex, but as the genes encoding the components of the exonucleolytic complex have yet to be cloned, we cannot determine linkage. That the exonucleolytic activity acts 3′ to 5′ on mitochondrial RNA is suggestive that it is most likely that the *SOC1* gene product is not a participant in this complex. We would expect such an RNase to affect both the precursor *COB* RNA and the mature RNA equally since their 3′ ends should be the same.

It was shown previously that *NUC1* protein (Nuc1p), a major mitochondrial nuclease, contains RNase and DNase activity (DAKE *et al.* 1988). However, upon deletion of *NUC1* from the yeast genome, no *PET* phenotype was observed (ZASSENHAUS *et al.* 1988). *soc1* is unlinked to *NUC1* since *NUC1* is adjacent to *CBP1* (separated by a few hundred base pairs) (LIU and DIECKMANN 1989; VINCENT *et al.* 1988) and *soc1* is unlinked to *CBP1*. We have measured steady-state levels of mitochondrial transcripts in $NUC1^+$ and $nuc1^-$ strains and detected no differences in mitochondrial transcript levels (R. STAPLES, M. TUCKER and C. DIECKMANN, unpublished data). It remains to be determined whether the *SOC1* gene product Soc1p is related functionally to Nuc1p.

The isolation of *soc1* mutants and the effect of such a mutation on the stability of mitochondrial transcripts is novel. *SUV3-1*, isolated as a dominant suppressor of a deletion of the 3′ dodecamer sequence in *VAR1* (ZHU *et al.* 1989) was shown to increase the steady-state levels of excised group I introns and decrease the steady-state levels of *COX1* and *COB* mRNAs (CONRAD-WEBB *et al.* 1990). Suv3p is thought to play a role in translation of mitochondrial messages (CONRAD-WEBB *et al.* 1990) and in splicing of mitochondrial introns (STEPEIN *et al.* 1992). That the phenotypes of *SUV3-1* and *soc1-1* mutations are different is suggestive that the genes are different. In summary, we propose that *soc1* promotes the stability of *COB* mRNA either by direct loss of a nuclease that turns over the message or indirectly by loss of a regulator of such a nuclease.

We would like to thank ALISON ADAMS, CAROLYN DECKER, TELSA MITTELMEIER, ROY PARKER and KIMBERLY SPARKS for reading the manuscript prior to submission and all of the members of RATC for helpful discussions. We thank N. CAMOUGRAND for supplying us with the oligo for *ATP8/ATP6*. This work was supported by a grant from the National Institutes of Health (34893) (to C.L.D.) and the University of Arizona Cancer Biology Training Grant from the National Cancer Institute (CA09213) (to R.R.S.). C.L.D. is an Established Investigator of The American Heart Association.

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Communicating editor: F. WINSTON