Yeast *CBP1* mRNA 3' End Formation Is Regulated during the Induction of Mitochondrial Function

STEPHEN A. MAYER[1]† AND CAROL L. DIECKMANN[1,2]*

*Department of Biochemistry[1] and Department of Molecular and Cellular Biology,[2]
University of Arizona, Tucson, Arizona 85721*

Alternative mRNA processing is one mechanism for generating two or more polypeptides from a single gene. While many mammalian genes contain multiple mRNA 3' cleavage and polyadenylation signals that change the coding sequence of the mature mRNA when used at different developmental stages or in different tissues, only one yeast gene has been identified with this capacity. The *Saccharomyces cerevisiae* nuclear gene *CBP1* encodes a mitochondrial protein that is required for cytochrome *b* mRNA stability. This 66-kDa protein is encoded by a 2.2-kb mRNA transcribed from *CBP1*. Previously we showed that a second 1.2-kb transcript is initiated at the *CBP1* promoter but has a 3' end near the middle of the coding sequence. Furthermore, it was shown that the ratio of the steady-state level of 2.2-kb *CBP1* message to 1.2-kb message decreases 10-fold during the induction of mitochondrial function, while the combined levels of both messages remain constant. Having proposed that regulation of 3' end formation dictates the amount of each *CBP1* transcript, we now show that a 146-bp fragment from the middle of *CBP1* is sufficient to direct carbon source-regulated production of two transcripts when inserted into the yeast *URA3* gene. This fragment contains seven polyadenylation sites for the wild-type 1.2-kb mRNA, as mapped by sequence analysis of *CBP1* cDNA clones. Deletion mutations upstream of the polyadenylation sites abolished formation of the 1.2-kb transcript, whereas deletion of three of the sites only led to a reduction in abundance of the 1.2-kb mRNA. Our results indicate that regulation of the abundance of both *CBP1* transcripts is controlled by elements in a short segment of the gene that directs 3' end formation of the 1.2-kb transcript, a unique case in yeast cells.

Regulation of alternative mRNA 3' end processing has emerged as an important regulatory mechanism in higher eucaryotic cells (1, 3, 9, 10, 18, 19, 27, 35, 43, 51, 54, 56). Along with 5' end capping and exon splicing, cleavage and polyadenylation of the 3' end are required steps in mRNA maturation (42). In mammalian cells, the consensus sequence AAUAAA located 10 to 30 nucleotides upstream of the cleavage site (20, 41, 66, 70) and a downstream GU-rich element (23, 24, 37, 38, 58, 67; for a review, see reference 30) are required for cleavage and polyadenylation. When multiple polyadenylation signals are located in a single gene, choice of alternative signals in different tissues or at different developmental stages generates structurally diverse transcripts that produce multiple polypeptide isoforms (32). We recently reported that a yeast nuclear gene, *CBP1*, like some mammalian genes, produces two different mRNA molecules via alternative 3' end formation (36). The full-length, 2.2-kb *CBP1* transcript spans the entire open reading frame and encodes a 66-kDa mitochondrial protein required for cytochrome *b* mRNA stability (13, 65). *CBP1* also produces a 1.2-kb transcript that has its 3' end within the coding sequence. As a unicellular organism, the yeast *Saccharomyces cerevisiae* does not have many developmental processes; however, as a facultative anaerobe it undergoes significant changes in mitochondrial structure and function when switched from fermentation to aerobic growth (see reference 25 for a review). Surprisingly, the ratio of the 2.2-kb *CBP1* transcript to 1.2-kb transcript decreases 10-fold during in-

duction of mitochondrial function by growth on a nonfermentable carbon source.

Regulated use of the alternative *CBP1* mRNA 3' processing sites could be influenced by several mechanisms. For instance, factors assembled with RNA polymerase II specifically at the *CBP1* promoter could inhibit procession through pause sites in the middle of the *CBP1* coding sequence and cause 3' end processing to occur at the upstream site. Alternatively, convergent transcription from either of two genes that closely flank *CBP1* could proceed into the *CBP1* coding sequence, occluding elements necessary for the formation of *CBP1* mRNA 3' ends and thus influencing the abundance of the two *CBP1* transcripts. A final model proposes that regulatory factors might act directly at the sites of 3' processing without influence from flanking gene expression or the *CBP1* promoter. Here we report analyses designed to test the hypothesis that the production of two transcripts from *CBP1* is governed by carbon source-dependent alternative 3' end formation. We find that a 146-bp segment of *CBP1* that includes the 1.2-kb mRNA polyadenylation sites contains the elements that are necessary and sufficient for the regulation of both *CBP1* transcripts during the induction of mitochondrial function.

MATERIALS AND METHODS

Strains and media. The yeast and *Escherichia coli* strains used are described in Table 1. The growth conditions for the derepression time course experiments were as described previously (36) except that RNA was extracted at only the 0- and 8-h time points. The conditions for galactose induction of the *GAL10* promoter-*CBP1* transcription fusion strains were also previously described (36). Bacteria were transformed by a standard technique (26). Yeast cells were

---

* Corresponding author.

† Present address: Laboratory of Molecular and Cellular Cardiology, Department of Cardiology, Children's Hospital, Harvard Medical School, Boston, MA 02115.

TABLE 1. Yeast and *E. coli* strains and plasmids

Strain or plasmid	Description	Reference or source
Yeast strains		
LL2	a *leu2-3 leu2-112*	12
B2L	*LEU2* insertion in the *Bam*HI site at +788 of *URF* in LL2	This study
LNUC	*LEU2* insertion at the *Bam*HI site of *NUC1* in LL2	This study
CP1L	*LEU2* insertion at the *Pst*I site of *CBP1* in LL2	This study
CP1L/int	$\rho^{+(int4-35)}$ derivative of CP1L	This study
a70/int	a $\rho^{+(int4-35)}$ *ura3-52 his3-11 his3-15 leu2-3 leu2-112*, with *HIS3* inserted at the *Bgl*II site of *CBP1*	This study
G::-26	a70/int containing YEp52/−26CBP1	This study
ΔHG	a70/int containing pΔHG	This study
ΔGS	a70/int containing pΔGS	This study
ΔGM	a70/int containing pΔGM	This study
ΔC3'	a70/int containing pΔC3'	This study
ΔF3'	a70/int containing pΔF3'	This study
S150-2B	a *ura3-52 Δhis3 leu2-3 leu2-112 trp1-289*	-
Hc-R	S150-2B containing pHc-R	This study
Hf-R	S150-2B containing pHf-R	This study
Hf-L	S150-2B containing pHf-L	This study
Yeast plasmids		
G::-26	Fusion of *GAL10* promoter at position -26 relative to *CBP1* ATG	29
pΔHG	*Hpa*I-*Bgl*II deletion of *CBP1* in YEp52/-26CBP1	This study
pΔGS	*Bgl*II-*Sca*I deletion of *CBP1* in YEp52/-26CBP1	This study
pΔGM	*Bgl*II-*Msc*I deletion of *CBP1* in YEp52/-26CBP1	This study
pΔC3'	Truncation of *CBP1* at *Cla*I in YEp52/-26CBP1	This study
pΔF3'	Truncation of *CBP1* at *Fsp*I in YEp52/-26CBP1	This study
YEp351	Yeast-*E. coli* shuttle vector	28
pURA	*URA3* insertion at *Hin*dIII in polylinker of YEp351	This study
pHc-R	800-bp *Hinc*II *CBP1* fragment inserted at *Stu*I of *URA3* in pURA	This study
pHf-R	146-bp *Hinf*I *CBP1* fragment inserted at *Stu*I of *URA3* in pURA	This study
pHf-L	146-bp *Hinf*I *CBP1* fragment inserted with opposite orientation to pHf-R at *Stu*I of *URA3* in pURA	This study
E. coli strains		
RR1	F$^-$ *hsdS20*(r$_B$ m$_B$) *ara-14 proA2 lacY1 galK2 rpsL20*(Sm^r) *xyl-5 mtl-1 supE44* λ$^-$	5
XL1-Blue	*recA1 endA1 gyrA96 thi hsdR17*(r$_K^-$ m$_K^+$) *supE44 relA1* λ$^-$ *lac* [F' *proAB lacI^{q}Z*ΔM15 Tn*10(tet)*]	6
E. coli plasmids		
pBS+	Plasmid with T3 and T7 phage RNA polymerase promoters flanking pUC19 multiple cloning site	Stratagene
pBS1-2	pBS+ with 700-bp *CBP1 Bam*HI-*Hin*dIII fragment	36
pBS-ACT	pBS+ with 600-bp *ACT1 Cla*I fragment	36
pBS-URA5'	pBS+ with 650-bp *URA3 Hin*dIII-*Stu*I fragment	This study

transformed by the lithium acetate method (31). In experiments in which *cbp1* strains were compared with strains wild type for the *CBP1* gene, the ρ^+ mitochondrial genomes were eliminated from all of the strains by ethidium bromide mutagenesis, and a mitochondrial genome carrying a suppressor of *cbp1* was introduced into each strain via cytoduction from a *kar1* strain harboring the $\rho^{int4-35}$ DNA (11).

Plasmids and probes. The yeast and *E. coli* plasmids used are described in Table 1. Plasmids were constructed by using standard cloning techniques (34). Antisense riboprobes were transcribed in vitro, using T7 or T3 RNA polymerase in the presence of [α-^{32}P]UTP (ICN) as suggested by the enzyme supplier (Boehringer Mannheim).

RNA analysis. Isolation of total yeast RNA and purification of poly(A)$^+$ mRNA were performed exactly as described previously (36). The procedures for RNA fractionation, Northern (RNA) blotting (2), and hybridization were also previously described (36). Quantitation of β decays from Northern blots was performed with a Betascope as recommended by the manufacturer (Betagen, Inc.).

cDNA cloning. cDNA clones specific for the 3' ends of

CBP1 mRNA were isolated precisely as described in the rapid amplification of cDNA ends (RACE) protocol (21), with an annealing temperature of 54°C in the polymerase chain reaction (PCR). The reverse transcriptase used was supplied by Boehringer Mannheim, and *Taq* polymerase was supplied by U.S. Biochemicals. The first-strand cDNA synthesis primer contained sequences directing the incorporation of *Sal*I, *Xba*I, and *Eco*RI linkers into the cDNA. The sequence of that oligonucleotide was 5'-GCGTCGACTCTA-GAGAATTC(T)$_{17}$-3'. The *CBP1*-specific PCR primer was a 22-mer that matched the sequence of *CBP1* at position +700 relative to the *CBP1* ATG: 5'-AAGGCCCTCGGCTTAAG-TAATG-3'. The second PCR primer contained the *Sal*I-*Xba*I-*Eco*RI linker sequence of the first-strand cDNA primer but lacked the T$_{17}$ sequence. PCR products were digested with *Eco*RI and either *Hin*dIII for 1.2-kb mRNA 3' end fragments or *Pst*I for 2.2-kb mRNA 3' end fragments. Following agarose gel purification, the fragments were subcloned into pBS+ (Table 1). Miniprep DNA was sequenced by using a T7 promoter primer (Stratagene) and the dideoxy

sequencing method (57) as modified by U.S. Biochemicals for the use of Sequenase.

RESULTS

CBP1 mRNA regulation is not influenced by expression of convergently transcribed genes. We previously reported that the yeast nuclear *CBP1* gene encodes two transcripts that are reciprocally regulated during the induction of mitochondrial development (36). A full-length 2.2-kb transcript encodes the 66-kDa mitochondrial CBP1 protein (65) that is required for mitochondrial cytochrome *b* mRNA stability (13). A shorter 1.2-kb transcript shares transcription initiation sites with the full-length message but has a 3' end within the *CBP1* coding sequence (36). The ratio of 1.2-kb transcripts to 2.2-kb transcripts increases 10-fold when mitochondrial function is induced by replacing glucose medium with one containing a nonfermentable carbon source such as glycerol (36). The reciprocal change in the level of these two transcripts has led us to begin to identify the *cis* elements that are required for (i) the 3' end formation of each of the *CBP1* transcripts and (ii) the regulation of alternative use of these 3' ends to dictate *CBP1* mRNA levels.

During the determination of the structure of the *CBP1* gene, we learned that two genes closely flank the *CBP1* locus (33, 35a). These genes are convergently transcribed toward *CBP1*, and we wondered whether the expression of these genes could influence the regulation of *CBP1* mRNA levels. To determine whether *CBP1* mRNA regulation was affected by convergent transcription, disruption mutations were constructed in each of the genes flanking *CBP1*. The first mutant (B2L) has a disruption in the 3' end of an open reading frame (*URF*, unknown function) which ends 260 bp upstream of the *CBP1* translational start codon and is transcribed from the same strand as *CBP1* (36). A second mutant, LNUC (33), has a disruption in the *NUC1* open reading frame located downstream of *CBP1* on the opposite strand. *NUC1* encodes a nonspecific mitochondrial nuclease (64), and the reading frame ends just 244 bp downstream of *CBP1*. Both *URF* and *NUC1* encode transcripts that are regulated during the induction of respiration (36a); however, neither the *urf* nor *nucl* disruption mutations affect the ability of the cells to respire.

To assess the effect of these disruptions on the regulation of *CBP1* mRNA abundance, we compared *CBP1* mRNA levels in repressed and derepressed cultures of B2L and LNUC with that of the wild-type parent, LL2 (Fig. 1). Poly(A)$^+$ RNA was isolated from wild-type and mutant cells grown to early log phase on glucose (0-h) medium and from cells switched to glycerol medium for 8 h to induce mitochondrial function. The RNA was analyzed on duplicate Northern blots hybridized to either a *CBP1* probe or an actin (*ACT1*) probe, which served to normalize for the amount of poly(A)$^+$ RNA loaded in each lane. The ratio of 1.2-kb *CBP1* transcripts to 2.2-kb *CBP1* transcripts increased in strains harboring the B2L (Fig. 1, lanes 3 and 4) and LNUC (lanes 5 and 6) disruption mutations in amounts similar to those observed in the wild-type strain (lanes 1 and 2). Since these mutants formed transcripts that terminated within the *LEU2* sequence inserted into either *URF* or *NUC1* (data not shown) but illustrated the wild-type pattern of *CBP1* mRNA regulation, we conclude that the regulation of *CBP1* mRNA levels is not affected by expression of the *URF* and *NUC1* genes.

We previously demonstrated that a disruption mutation in the 3' end of the *CBP1* coding sequence allowed proper
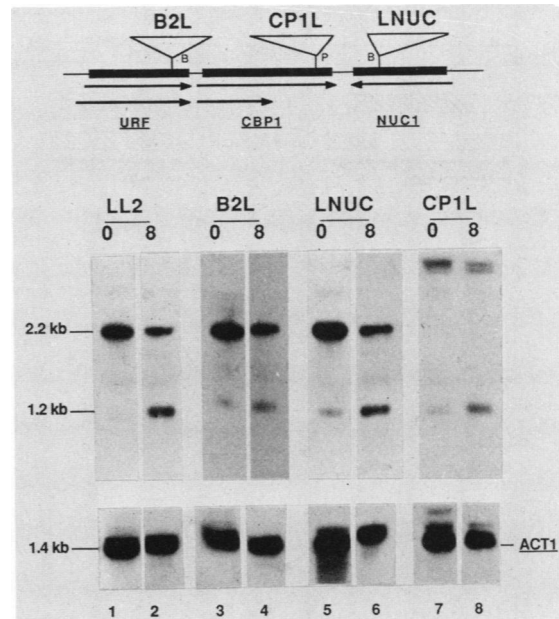

FIG. 1. Proper formation of flanking gene transcripts and the 2.2-kb *CBP1* transcript are not required for *CBP1* mRNA regulation. Disruption mutations B2L and LNUC were constructed by inserting the yeast *LEU2* gene into the 3' ends of the *URF* and *NUC1* coding regions. CP1L contains *LEU2* inserted into *CBP1* downstream of the 1.2-kb mRNA 3' end (36). The disrupted genes were used to replace the wild-type chromosomal copies in yeast strain LL2 by homologous recombination (53). Poly(A)$^+$ RNA (12 μg) isolated from repressed (0-h) and derepressed (8-h) cultures of each strain was characterized by Northern analysis. Northern blots were hybridized with cRNA probes complementary either to the 5' half of the *CBP1* coding sequence (pBS1-2) or to *ACT1* (pBS-ACT) and were then washed and exposed as indicated in Materials and Methods. Restriction sites: B, *Bam*HI; P, *Pst*I.

production of the 1.2-kb message even though the full-length transcript was not correctly formed (36). This mutant, CP1L, does not respire because it lacks stable cytochrome *b* mRNA, a phenotype equivalent to that of *cbp1* point mutants (35a). To determine whether this mutation affected the regulation of 1.2-kb mRNA levels, *CBP1* mRNA extracted from repressed and derepressed cultures of CP1L was analyzed on Northern blots. To allow respiratory growth of CP1L on glycerol, the *cbp1* mutation was suppressed by a rearranged mitochondrial genome ($\rho^{int4-35}$) that expresses cytochrome *b* in the absence of *CBP1* function (11). The abundance of the 1.2-kb *CBP1* transcript increased during derepression in CP1L (Fig. 1, lanes 7 and 8). Thus, we conclude that proper formation of the full-length *CBP1* transcript is not required for regulation of *CBP1* mRNA levels. This result implies that the sequences responsible for *CBP1* mRNA regulation are contained within the *CBP1* gene, upstream of the site of the disruption in CP1L. Since we had previously shown that *CBP1* transcripts were properly formed when the gene was driven by a heterologous promoter (36), the simplest model that would account for the increase in 1.2-kb mRNA in CP1L and the reciprocal switch in *CBP1* expression in wild-type strains would be one in which regulation of 1.2-kb mRNA 3' end formation would dictate the level of both transcripts.

Localization of sequences required to regulate CBP1 mRNA formation. To more precisely locate the region of *CBP1* that
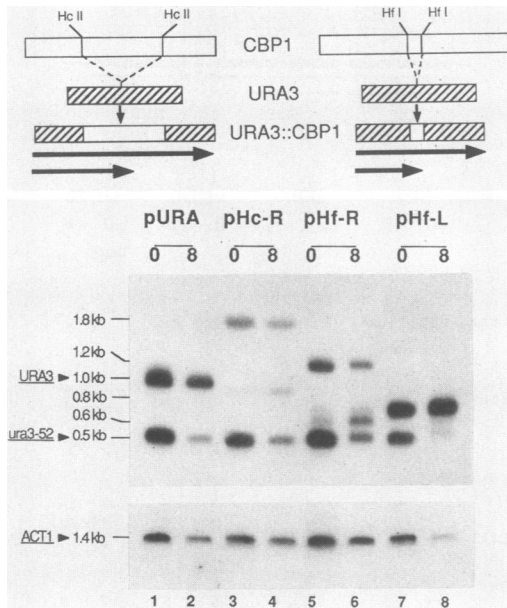
FIG. 2. A 146-bp *CBP1* fragment directs carbon source-regulated 3′ end formation in the middle of *URA3*. pURA contains the yeast *URA3* inserted into the polylinker of the multicopy plasmid YEp351. Either the 800-bp *Hinc*II or the 146-bp *Hinf*I *CBP1* fragment was inserted into the unique *Stu*I site of *URA3* in pURA. pHf-R and pHf-L contain the *Hinf*I fragment inserted in the *CBP1* sense and antisense orientation, respectively. Plasmids bearing either the wild-type or disrupted *URA3* genes were transformed into the yeast strain S150, which contains a Ty element disruption of the chromosomal *URA3* gene called *ura3-52* (52). Expression of the *ura3-52* transcript is not related to expression of the plasmid-borne wild-type *URA3* copy in YpURA. Poly(A)+ RNA (12 μg) extracted from repressed (0-h) and derepressed (8-h) yeast cultures was analyzed on Northern blots by hybridization to the pBS-URA5′ and pBS-ACT cRNA probes as indicated. Restriction sites: HcII, *Hinc*II; Hfl, *Hinf*I.

directs the regulated production of two transcripts from this gene, we sought to identify segments of *CBP1* that were sufficient to confer regulated alternative mRNA 3′ end formation on a heterologous gene. On the basis of Northern analyses with various probes subcloned from *CBP1*, the location of the 1.2-kb mRNA 3′ end had previously been assigned to a region near a *Bgl*II site located near the middle of the *CBP1* coding sequence (position +977 relative to the translational start codon; 36). An 800-bp *Hinc*II and a 146-bp *Hinf*I fragment of *CBP1* each contain this *Bgl*II site and were inserted into a *Stu*I restriction site in the middle of the yeast *URA3* gene, which encodes a single 1.0-kb transcript and is not regulated by glycerol derepression (Fig. 2, lanes 1 and 2). Poly(A)+ mRNA was extracted from a yeast strain containing a multicopy plasmid with the *URA3* gene alone or a *URA3* gene that had been disrupted by the *Hinc*II fragment (pHc-R) or the *Hinf*I fragment inserted in either orientation (pHf-R and pHf-L).

Transcripts initiated at the *URA3* promoter in pHc-R should be approximately 800 nucleotides long if they utilize the upstream *CBP1* mRNA polyadenylation site and 1800 nucleotides long if they end in *URA3*. A *URA3* cRNA probe detected both long (1.8-kb) and short (0.9-kb) transcripts on a Northern blot of RNA extracted from repressed and derepressed cultures of this strain (Fig. 2, lanes 3 and 4).

Moreover, the abundance of the two transcripts changed reciprocally during derepression in the manner expected for the wild-type *CBP1* transcripts. Even the small 146-bp *CBP1* fragment in pHf-R was competent in directing the respiration-regulated production of two appropriately sized, 1,200- and 600-nucleotide-long transcripts (lanes 5 and 6). If this small fragment functions in an orientation opposite to that found in the *CBP1* gene, a full-length 1.2-kb message and a truncated 0.6-kb message would be observed. If the reverse orientation prevented 3′ end formation, only the full-length 1.2 kb message would be observed. In fact, only one 700-nucleotide-long transcript was observed (pHf-L; Fig. 2, lanes 7 and 8). This truncated *URA3* transcript observed in pHf-L may result from activation of a cryptic 3′ end site near the *URA3*-*CBP1* boundary. Alternatively, since longer transcripts were not observed in pHf-L RNA, the 3′-end-forming element in the inserted *CBP1* segment might be more efficient in the wrong orientation. These results rule out the involvement of the *CBP1* promoter in regulation of *CBP1* mRNA production and support the hypothesis that regulation of 1.2-kb *CBP1* mRNA 3′ end formation dictates the levels of both *CBP1* transcripts during derepression.

Location of *CBP1* mRNA 3′ ends. To ensure that the 146-bp *Hinf*I fragment inserted into *URA3* contained the sequences directing production of the wild-type *CBP1* 1.2-kb mRNA 3′ end, it was first necessary to precisely map the position of the polyadenylation site within the *CBP1* coding sequence. As we were unable to map the position of that 3′ end by nuclease protection experiments (36), we decided to sequence cDNA clones of *CBP1* mRNA 3′ ends. *CBP1*-specific clones from a library of oligo(dT)-primed cDNA copied from 8-h derepressed poly(A)+ mRNA were selectively amplified by the PCR-RACE protocol (21). Sequence data from 23 independently isolated clones identified seven different polyadenylation sites for the 1.2-kb *CBP1* message (Fig. 3). The sites were located in three clusters: cluster I, sites at positions +1003, +1007, and +1010; cluster II, sites at +1020 and +1021; and cluster III, sites at +1043 and +1049. All of the 1.2-kb mRNA polyadenylation sites were located near the middle of the 146-bp *Hinf*I fragment from *CBP1* shown to be sufficient for carbon source-dependent regulation of alternative 3′ end formation of *URA3* mRNA. Polyadenylation sites at positions +1003 (cluster I) and +1049 (cluster III) were each represented by more than 25% of the clones sequenced. Five of the seven polyadenylation sites identified were located at pyrimidine-A dinucleotides, the other two were at C-U dinucleotides, with polyadenylate being added to the C. Interestingly, while only one of the seven polyadenylation sites (+1021) creates an in-frame UAA translational stop codon upon polyadenylation, both *CBP1* transcripts are associated with polyribosomes in the same ratio as that observed on Northern blots of polyadenylated mRNA (35a).

The sequences of eight cDNA clones of the 3′ end of the 2.2-kb *CBP1* transcript amplified according to the RACE protocol identified a single U-A dinucleotide at position +2059 as the polyadenylation site, a position previously mapped by S1 nuclease protection assays (36). The analysis of the *CBP1* cDNA clones refined our earlier estimation of the position of the 3′ end of the 1.2-kb mRNA and ascertained that the 146-bp *Hinf*I fragment inserted in *URA3* contains the 1.2-kb *CBP1* mRNA polyadenylation sites of the wild-type gene.

The region sufficient for *CBP1* mRNA regulation contains sequences that form the 1.2-kb mRNA 3′ end. To complement our analysis of *CBP1* sequences that were sufficient to

I   II   III

TAGATCTATATGTAGCGAAATGTTTTGGCAATTAACTCCAGAGTACTATTGTAATAACCCTTTGATATTACCGGCA

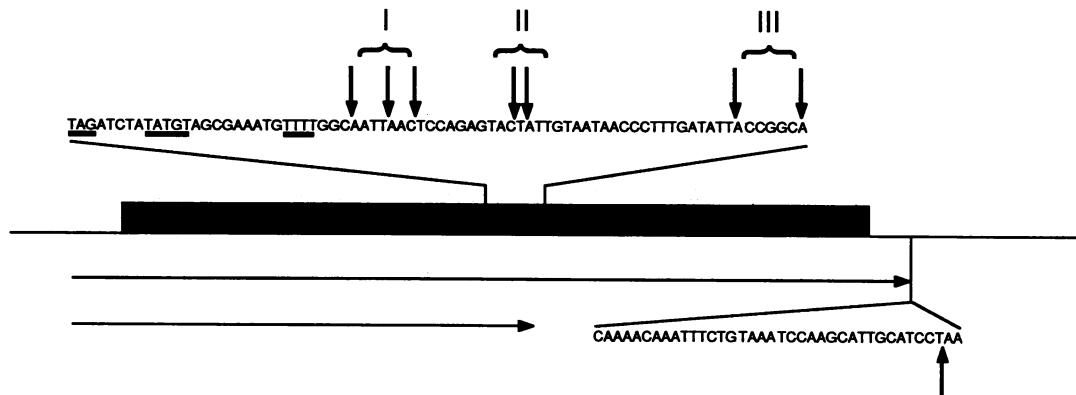CAAAACAAATTTCTGTAAATCCAAGCATTGCATCCTAA

FIG. 3. Sequences of PCR-amplified cDNA clones that define the *CBP1* mRNA polyadenylation sites. A cDNA library copied from oligo(dT)-primed, 8-h-derepressed, poly(A)$^+$ RNA was amplified according to the RACE protocol (21). Seven poly(A) sites utilized by the 1.2-kb mRNA were localized to regions designated clusters I, II, and III (top). A single poly(A) site corresponding to the 2.2-kb mRNA 3' end was identified (bottom right). The underlined nucleotides are homologous to a sequence upstream of the yeast *CYC1* gene poly(A) sites (69).

regulate 3' end formation of the 1.2-kb mRNA, we wanted to identify sequences required to produce the 3' end of that transcript. As *CBP1* is expressed at very low levels in wild-type strains, deletion mutations in the region of the 1.2-kb mRNA 3' end were constructed in a plasmid-borne *GAL10* promoter-*CBP1* transcription fusion which overexpresses both *CBP1* transcripts several hundred-fold when induced by growth on galactose (36). Each *CBP1* deletion was expressed in a strain which has a *cbp1* disruption mutation in the chromosome and produces no *CBP1* transcripts. To allow respiratory growth of these strains, the *cbp1* mutations were suppressed by the $\rho^{int4-35}$ mitochondrial genome (11). Since the unique *Bgl*II site at position +977 of the *CBP1* coding sequence is conveniently located 25 bp upstream of the 1.2-kb mRNA polyadenylation sites, we chose this restriction site as a central point for deletions. The mutation in ΔHG (Fig. 4) removed 311 bp of *CBP1* coding sequence between a *Hpa*I site at position +667 and the downstream *Bgl*II site. The mutation in ΔGS was constructed by deleting 28 bp of coding sequence containing the cluster I polyadenylation sites between the *Bgl*II site and a downstream *Sca*I site. A third mutation extended the deleted sequences in ΔGS another 62 bp beyond the *Sca*I site to an *Msc*I site at position +1087. The mutation in this strain, ΔGM, deleted all three polyadenylation clusters plus an additional 40 bp of *CBP1* coding sequence. ΔC3' and ΔF3' have truncations of *CBP1* at positions +1602 and +1803, respectively, in the coding sequence. Both truncations delete the 2.2-kb *CBP1* mRNA polyadenylation site.

The *CBP1* transcripts produced by these five mutant constructs were compared with those of the parent *GAL10*-*CBP1* fusion plasmid, G::-26. Following galactose induction of these strains, total RNA was isolated and analyzed on Northern blots for transcripts that hybridized to a *CBP1* cRNA probe (Fig. 4). The abundance of all *CBP1* transcripts was determined by directly quantitating the β decays from the Northern blot (see legend to Fig. 4). This probe detected both *CBP1* transcripts which were overexpressed from the G::-26 plasmid (Fig. 4, lane 1). The mutation in ΔHG (lane 2) prevented the formation of the 1.2-kb transcript yet allowed the generation of a transcript equivalent to the 2.2-kb mRNA with a 300-nucleotide internal deletion. This result suggests that sequences more than 30 bp upstream of the most 5' 1.2-kb mRNA polyadenylation site are required to produce
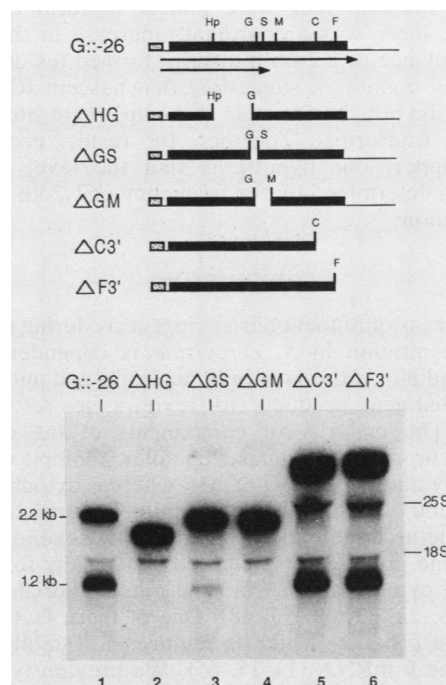

FIG. 4. Regions of *CBP1* necessary for production of the short transcript. Deletion mutations were constructed by removing the indicated restriction fragments from a *GAL10* promoter-*CBP1* transcription fusion gene (G::-26; 36). Total RNA (12 μg) isolated from galactose-induced cells was analyzed on Northern blots for *CBP1* transcripts with the pBS1-2 probe as described in Materials and Methods. The bands in each lane were quantitated by direct counting of the β decays from the filter. After subtraction of background levels from each lane, the counts for the full-length and truncated transcripts, respectively, were as follows: G::-26, 178 and 112 cpm; ΔHG, 316 and 0 cpm; ΔGS, 441 and 23 cpm; ΔGM, 421 and 0 cpm; ΔC3', 290 and 200 cpm; ΔF3', 321 and 171 cpm. The stippled box represents the *GAL10* promoter fused to *CBP1* at position -26 relative to the *CBP1* coding sequence, which is represented by the filled box. Restriction sites: C, *Cla*I; F, *Fsp*I; G, *Bgl*II blunt-ended by Klenow fragment; H, *Hpa*I; M, *Msc*I; S, *Sca*I.

the 3' end of that transcript. Since the 1.2-kb mRNA polyadenylation sites were left intact by the mutation in ΔHG, these sites were necessary but not sufficient to direct 3' end formation of the transcript. Interestingly, the mutation in ΔGS (lane 3), which deleted three of the seven 1.2-kb mRNA polyadenylation sites, allowed accumulation of 1/10 the amount of the 1.2-kb message measured for the G::-26 control strain (lane 1). Production of the 1.2-kb transcript was completely abolished when the deletion endpoint was extended downstream to the MscI site, removing all of the 1.2-kb mRNA polyadenylation sites (lane 4). Separation of RNA isolated from the G::-26 and the deletion mutant strains into poly(A)$^+$ and poly(A)$^-$ fractions indicated that no poly(A)$^-$ CBP1 transcripts could be detected by Northern analysis (data not shown).

The truncation mutations in ΔC3' and ΔF3' allowed formation of the 1.2-kb mRNA but prevented proper 3' end formation of the 2.2-kb message (Fig. 4, lanes 5 and 6). Instead, we observed much longer transcripts that ended in vector sequences, presumably because the normal 2.2-kb mRNA 3' end formation signals were missing in those constructs. That the 1.2-kb mRNA is properly formed in ΔC3' and ΔF3' is consistent with our earlier observations (Fig. 1; 36) that formation of the 1.2-kb message is not dependent on proper formation of the 2.2-kb transcript. In the three mutants that were unable to form the 1.2-kb transcript, there was a coordinate increase in the steady-state abundance of 2.2-kb transcript formed (as determined by β-decay counting), suggesting that nascent RNA molecules that did not acquire ends at the upstream sites became full-length transcripts. Together, the results presented in Fig. 4 support our hypothesis that the level of 2.2-kb message is determined by the frequency of 1.2-kb mRNA 3' end formation.

DISCUSSION

The process of mitochondrial biogenesis during the induction of respiration in *S. cerevisiae* is dependent on the coordinated biosynthesis of both nuclearly and mitochondrially encoded gene products (for reviews, see references 25 and 61). The majority of components of the coenzyme QH$_2$-cytochrome *c* reductase complex (complex III) are encoded by nuclear genes (62, 63), whereas cytochrome *b* is the only subunit synthesized in the mitochondria. In addition to the structural subunits of complex III, several auxiliary nuclear gene products are required specifically for the production of cytochrome *b* from the mitochondrial *cob* gene (12, 13, 16, 22, 39, 49, 50, 68). One of these factors is the *CBP1* gene product, which is required for stabilization of cytochrome *b* mRNA (13–15, 65). We previously reported that the *CBP1* gene encodes two transcripts that have different 3' ends and that the steady-state levels of the two transcripts are reciprocally regulated during the induction of mitochondrial function (36). Our observations on *CBP1* mRNA abundance were made while testing the hypothesis that regulation of *CBP1* expression might coordinate nuclear and mitochondrial contributions to complex III. We found that the abundance of the 2.2-kb *CBP1* mRNA decreases during derepression, whereas the abundance of the 1.2-kb message rises reciprocally. Knowing that the 66-kDa product of the 2.2-kb mRNA is required for respiratory growth (36, 65), we found it odd that the level of the full-length message would drop when cells were switched from fermenting on glucose medium to respiring on glycerol medium. Though we have not solved this mystery, we have investi-

gated the nature of the reciprocal regulation of *CBP1* mRNA 3' end formation. Here we have presented evidence that regulated production of the 1.2-kb *CBP1* mRNA 3' end dictates the steady-state levels of both *CBP1* transcripts during the course of derepression on glycerol medium.

The discovery that regulation of the short *CBP1* transcript was not dependent on proper formation of the full-length, 2.2-kb transcript (Fig. 1) focused our attention on the region encompassing the 3' end of the 1.2-kb mRNA. Northern blot analyses of strains carrying *cbp1* deletion mutations in this region (Fig. 4) suggested that formation of the 1.2-kb transcript is dependent on the presence of sequences upstream of the multiple polyadenylation sites (Fig. 3) and of the region including the sites themselves. As blockage of 1.2-kb mRNA formation by these mutations was concomitant with an increase in the amount of full-length mRNA observed, we conclude that the level of the 2.2-kb mRNA is dictated by the frequency of 3' end formation in the middle of the *CBP1* coding sequence. Furthermore, our results indicated that a small 146-bp segment of *CBP1* DNA containing the 1.2-kb mRNA polyadenylation sites is sufficient to confer regulated alternative mRNA 3' end formation on another yeast gene, *URA3*, in an orientation-dependent fashion (Fig. 2). These results suggest that regulation of *CBP1* 1.2-kb mRNA 3' end formation is directed by the region very near the sites of 3' end processing.

During the induction of mitochondrial development by a switch to a nonfermentable carbon source, *trans*-acting factors stimulate transcriptional initiation of several yeast nuclear genes that encode mitochondrial proteins (17, 44). Though it also encodes a mitochondrial polypeptide, *CBP1* is the only yeast gene described to date that is regulated via differential mRNA 3' end formation rather than transcriptional initiation. However, in higher eucaryotes, alternative 3' end processing, splicing, and transcription termination have been demonstrated to play a significant role in the developmental and tissue-specific regulation of complex transcription units (for reviews, see references 4, 32, 47, and 59). Experimental evidence suggests that differential use of multiple mRNA 3' end processing sites in a single gene generates structurally diverse polypeptides by altering the coding sequences included in the mature message. In mammalian cells, for example, infection with herpes simplex virus induces the production of a nuclear factor that regulates utilization of alternative polyadenylation sites in the viral genome (40). Regulation of alternative polyadenylation signals has also been suggested to control diversity of protein expression from adenovirus, simian virus 40, and hepatitis B virus genomes (9, 19, 54, 71) as well as immunoglobulin μ genes (1, 18) and the human nonmuscle myosin heavy-chain gene (56). Regulation of transcriptional termination between immunoglobulin μ exons has been suggested to determine use of alternative 3' processing sites, ultimately directing the production of either the membrane-bound or secreted form of this protein in plasma cells (35). Interestingly, regulation of polyadenylation site utilization in several viral genomes reportedly involves sequences upstream of those sites (9, 10, 54). It is clear that sequence elements upstream of the 1.2-kb *CBP1* mRNA polyadenylation sites are required to form that transcript (Fig. 4). Signals in this region may modulate the efficiency of the polyadenylation sites located just downstream. The observation that *CBP1* encodes two transcripts that are differentially regulated by 3' end formation suggests that, like higher eucaryotes, yeasts may also exploit alternative mRNA processing to express different proteins from a single gene.

The work described here has determined that the short region of *CBP1* which directs formation of the 1.2-kb message 3' end is sufficient to regulate the production of both *CBP1* transcripts. However, we do not know whether transcription termination or cleavage and polyadenylation is the regulated step in formation of this 3' end. Comparison of the 3' untranslated regions of many yeast genes has failed to locate a strictly conserved signal such as AAUAAA that is required for mammalian mRNA 3' processing. Other studies have indicated that unlike higher eucaryotic genes, yeast genes have closely juxtaposed 3' processing and transcription termination sites. For example, an 82-bp segment of the yeast iso-1-cytochrome *c* gene (*CYC1*) acts as an efficient transcription terminator in a variety of assays (45, 46, 55, 60) and also contains the signal for cleavage and polyadenylation of precursor RNA in vitro (7). Regulation of 1.2-kb *CBP1* mRNA 3' end formation could be achieved by influencing transcriptional termination between the 1.2-kb and 2.2-kb mRNA polyadenylation sites, in a manner similar to the regulation of antitermination at NUT sites on lambda phage genes in *E. coli* by the lambda N protein (48). Termination at a site upstream of the 2.2-kb *CBP1* mRNA polyadenylation sites would force processing to occur at the 1.2-kb mRNA polyadenylation sites. Alternatively, a full-length *CBP1* precursor RNA could be cleaved at either the upstream or downstream polyadenylation sites to form the truncated or full-length messages, respectively. Either the termination or processing steps could be influenced by factors responsive to the respiratory status of the cell.

As the results of our experiments on sequences required to form the 1.2-kb *CBP1* mRNA parallel those showing the juxtaposition of the *CYC1* mRNA termination and processing signals, we suggest that in addition to polyadenylation signals there may be a transcriptional terminator or pause site in the middle of *CBP1*. The phenotype of the ΔGS mutant (Fig. 4) is very similar to that of the *cyc1-512* mutant originally described by Zaret and Sherman (69) in which both transcriptional termination (55) and the cleavage-polyadenylation activities are inhibited (7). Both mutations are small deletions (<40 bp) upstream of the respective polyadenylation sites, and they each decrease the amount of properly formed transcript by at least 90%. Prevention of 3' end formation by both *cbp1* and *cyc1* alleles results in use of the next downstream 3' end formation site (Fig. 3; 69). For example, transcripts initiated at the *GAL10* promoter in the ΔGS gene end at the 2.2-kb polyadenylation site, as evidenced by the increase in levels of 2.2-kb message compared with the wild-type gene level (Fig. 4). Finally, there is a high degree of sequence similarity between the region just upstream of the 1.2-kb *CBP1* mRNA 3' end and the region of *CYC1* deleted in the *cyc1-512* allele (Fig. 3; 36), suggesting that formation of the 1.2-kb mRNA may involve both termination and cleavage-polyadenylation reactions.

Whether the purpose for switching the level of expression of the two *CBP1* transcripts is to induce the level of expression of a protein encoded by the truncated transcript or to down-regulate the level of CBP1 protein encoded by the full-length transcript (64) is unknown, as it is yet to be determined whether the 1.2-kb mRNA encodes a stable protein. However, on the basis of the information presented here, we propose that pausing of polymerase near the mid-gene polyadenylation sites in concert with cleavage and polyadenylation forms the mature 3' end of the 1.2-kb *CBP1* message. Conversely, transcription through the pause site to the end of the coding sequence would allow formation of structures which inhibit cleavage at the 1.2-kb mRNA poly-

adenylation site and ensure proper formation of the full-length transcript. Factors sensitive to the carbon source in the growth media could regulate *CBP1* expression by altering the frequency or duration of a transcriptional pause or the efficiency of the cleavage and polyadenylation reactions.

REFERENCES

1. **Alt, F. W., A. L. M. Bothwell, M. Knapp, E. Siden, E. Mather, M. Koshland, and D. Baltimore.** 1980. Synthesis of secreted and membrane-bound immunoglobulin Mu heavy chain is directed by mRNAs that differ at their 3' ends. Cell **20**:293–301.
2. **Alwine, J. C., D. J. Kemp, and G. R. Stark.** 1977. Method for detection of specific RNAs in agarose gels by transfer to diazobenzyloxymethyl paper and hybridization with DNA probes. Proc. Natl. Acad. Sci. USA **74**:5350–5354.
3. **Amara, S. G., R. M. Evans, and M. G. Rosenfeld.** 1984. Calcitonin/calcitonin gene-related peptide transcription unit: tissue-specific expression involves selective use of alternative polyadenylation sites. Mol. Cell. Biol. **4**:2151–2160.
4. **Birnstiel, M. L., M. Busslinger, and K. Strub.** 1985. Transcription termination and 3' processing: the end is in site! Cell **41**:349–359.
5. **Bolivar, F., R. L. Rodriguez, P. J. Green, M. C. Betlach, H. L. Heyneker, and H. W. Boyer.** 1977. Construction and characterization of new cloning vehicles II. A multi-purpose cloning system. Gene **2**:95–113.
6. **Bullock, W. O., J. M. Fernandez, and J. M. Short.** 1987. XL1-Blue: a high efficiency plasmid transforming recA *Escherichia coli* strain with beta-galactosidase selection. Biotechniques **5**:376–378.
7. **Butler, J. S., and T. Platt.** 1988. RNA processing generates the mature 3' end of yeast *CYC1* messenger RNA in vitro. Science **242**:1270–1274.
8. **Butler, J. S., P. Sadhale, and T. Platt.** 1990. RNA processing in vitro produces mature 3' ends of a variety of *Saccharomyces cerevisiae* mRNAs. Mol. Cell. Biol. **10**:2599–2605.
9. **Carswell, S., and J. C. Alwine.** 1989. Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. Mol. Cell. Biol. **9**:4248–4258.
10. **DeZazzo, J. D., and M. J. Imperiale.** 1989. Sequences upstream of AAUAAA influence poly(A) site selection in a complex transcription unit. Mol. Cell. Biol. **9**:4951–4961.
11. **Dieckmann, C. L., and B. Gandy.** 1987. Preferential recombination between GC clusters in yeast mitochondrial DNA. EMBO J. **6**:4197–4203.
12. **Dieckmann, C. L., G. Homison, and A. Tzagoloff.** 1984. Assembly of the mitochondrial membrane system: nucleotide sequence of a yeast nuclear gene (*CBP1*) involved in 5' end processing of cytochrome *b* pre-mRNA. J. Biol. Chem. **259**:4732–4738.
13. **Dieckmann, C. L., T. J. Koerner, and A. Tzagoloff.** 1984. Assembly of the mitochondrial membrane system: *CBP1*, a yeast nuclear gene involved in 5' end processing of cytochrome *b* pre-mRNA. J. Biol. Chem. **259**:4722–4731.
14. **Dieckmann, C. L., and T. M. Mittelmeier.** 1987. Nuclearly-encoded *CBP1* interacts with the 5' end of mitochondrial cytochrome *b* pre-mRNA. Curr. Genet. **12**:391–397.
15. **Dieckmann, C. L., L. K. Pape, and A. Tzagoloff.** 1982. Identification and cloning of a yeast nuclear gene (*CBP1*) involved in expression of mitochondrial cytochrome *b*. Proc. Natl. Acad. Sci. USA **79**:1805–1809.
16. **Dieckmann, C. L., and A. Tzagoloff.** 1985. Assembly of the mitochondrial membrane system: *CBP6*, a yeast nuclear gene necessary for synthesis of cytochrome *b*. J. Biol. Chem. **260**:

1513–1520.

17. **Dorsman, J. C., W. C. van Heeswijk, and L. A. Grivell.** 1988. Identification of two factors which bind to the upstream sequences of a number of nuclear genes coding for mitochondrial proteins and to genetic elements important for cell division in yeast. Nucleic Acids Res. **16:**7287–7301.

18. **Early, P., J. Rogers, M. Davis, K. Calame, M. Bond, R. Wall, and L. Hood.** 1980. Two mRNAs can be produced from a single immunoglobulin μ gene by alternative RNA processing pathways. Cell **20:**313–319.

19. **Falck-Pederson, E., and J. Logan.** 1989. Regulation of poly(A) site selection in adenovirus. J. Virol. **63:**532–541.

20. **Fitzgerald, M., and T. Shenk.** 1981. The sequence 5'-AAUAAA-3' forms part of the recognition site for polyadenylation of late SV40 mRNAs. Cell **24:**251–260.

21. **Frohman, M. A., M. K. Dush, and G. R. Martin.** 1988. Rapid production of full-length cDNAs from rare transcripts: amplification using a single gene-specific oligonucleotide primer. Proc. Natl. Acad. Sci. USA **85:**8998–9002.

22. **Gampel, A., M. Nishikimi, and A. Tzagoloff.** 1989. CBP2 protein promotes in vitro excision of a yeast mitochondrial group 1 intron. Mol. Cell. Biol. **9:**5424–5433.

23. **Gil, A., and N. J. Proudfoot.** 1984. A sequence downstream of AAUAAA is required for rabbit β-globin mRNA 3' end formation. Nature (London) **312:**473–474.

24. **Gil, A., and N. J. Proudfoot.** 1987. Position-dependent sequence elements downstream of AAUAAA are required for efficient rabbit β-globin mRNA 3' end formation. Cell **49:**399–406.

25. **Grivell, L. A.** 1989. Nucleo-mitochondrial interactions in yeast mitochondrial biogenesis. Eur. J. Biochem. **182:**477–493.

26. **Hanahan, D.** 1985. Techniques for transformation of E. coli, p. 109–135. In D. M Glover (ed.), DNA cloning, vol. I. IRL Press, Oxford.

27. **Helfmann, D. M., S. Cheley, E. Kuismanen, L. A. Finn, and Y. Yamawaki-Kataoka.** 1986. Nonmuscle and muscle tropomyosin isoforms are expressed from a single gene by alternative RNA splicing and polyadenylation. Mol. Cell. Biol. **6:**3582–3595.

28. **Hill, J. E., A. M. Myers, T. J. Koerner, and A. Tzagoloff.** 1986. Yeast/E. coli shuttle vectors with multiple unique restriction sites. Yeast **2:**163–167.

29. **Homison, G.** 1984. The identification, isolation and characterization of a nuclear encoded gene product in yeast which is involved in the processing of mitochondrial RNA. Ph.D. dissertation. Columbia University, New York, N.Y.

30. **Humphrey, T., and N. J. Proudfoot.** 1988. A beginning to the biochemistry of polyadenylation. Trends Genet. **4:**243–245.

31. **Ito, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153:**163–168.

32. **Leff, S. E., M. G. Rosenfeld, and R. M. Evans.** 1986. Complex transcriptional units: diversity in gene expression by alternative RNA processing. Annu. Rev. Biochem. **55:**1091–1117.

33. **Liu, Y, and C. L. Dieckmann.** 1989. Overproduction of yeast viruslike particles by strains deficient in a mitochondrial nuclease. Mol. Cell. Biol. **9:**3323–3331.

34. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

35. **Mather, E. L., K. J. Nelson, J. Haimovich, and R. P. Perry.** 1984. Mode of regulation of immunoglobulin μ- and δ-chain expression varies during B-lymphocyte maturation. Cell **36:**329–338.

35a.**Mayer, S. A.** Unpublished data.

36. **Mayer, S. A., and C. L. Dieckmann.** 1989. The yeast CBP1 gene produces two differentially regulated transcripts by alternative 3'-end formation. Mol. Cell. Biol. **9:**4161–4169.

36a.**Mayer, S. A., and C. L. Dieckmann.** Unpublished data.

37. **McDevitt, M. A., R. P. Hart, W. W. Wong, and J. R. Nevins.** 1986. Sequences capable of restoring poly(A) site function define two distinct downstream elements. EMBO J. **5:**2907–2913.

38. **McDevitt, M. A., M. J. Imperiale, H. Ali, and J. R. Nevins.** 1984. Requirement of a downstream sequence for generation of a

poly(A) addition site. Cell **37:**993–999.

39. **McGraw, P., and A. Tzagoloff.** 1983. Assembly of the mitochondrial membrane system: characterization of a yeast nuclear gene involved in the processing of the cytochrome b pre-mRNA. J. Biol. Chem. **258:**9459–9468.

40. **McLauchlan, J., S. Simpson, and J. B. Clements.** 1989. Herpes simplex virus induces a processing factor that stimulates poly(A) site usage. Cell **59:**1093–1105.

41. **Montell, C., E. F. Fisher, M. H. Caruthers, and A. J. Berk.** 1983. Inhibition of RNA cleavage but not polyadenylation by a point mutation in mRNA 3' consensus sequence AAUAAA. Nature (London) **305:**600–605.

42. **Nevins, J. R.** 1983. The pathway of eukaryotic mRNA formation. Annu. Rev. Biochem. **52:**441–466.

43. **Nevins, J. R., and M. C. Wilson.** 1981. Regulation of adenovirus-2 gene expression at the level of transcriptional termination and RNA processing. Nature (London) **290:**113–118.

44. **Olesen J., S. Hahn, and L. Guarente.** 1987. Yeast HAP2 and HAP3 activators both bind to the CYC1 upstream activation site, UAS2, in an interdependent manner. Cell **51:**953–961.

45. **Osborne, B. I., and L. Guarente.** 1988. Transcription by RNA polymerase II induces changes of DNA topology in yeast. Genes Dev. **2:**766–772.

46. **Osborne, B. I., and L. Guarente.** 1989. Mutational analysis of a yeast transcriptional terminator. Proc. Natl. Acad. Sci. USA **86:**4097–4101.

47. **Proudfoot, N. J.** 1989. How RNA polymerase II terminates transcription in higher eukaryotes. Trends Biochem. Sci. **14:**105–110.

48. **Roberts, J. W.** 1988. Phage lambda and the regulation of transcription termination. Cell **52:**5–6.

49. **Rödel, G., and T. D. Fox.** 1987. The yeast nuclear gene CBS1 is required for translation of mitochondrial mRNAs bearing the cob 5' untranslated leader. Mol. Gen. Genet. **206:**45–50.

50. **Rödel, G., A. Körte, and F. Kaudewitz.** 1985. Mitochondrial suppression of a yeast nuclear mutation which affects the translation of the mitochondrial apo-cytochrome b transcript. Curr. Genet. **9:**641–648.

51. **Rogers, J., P. Early, C. Carter, K. Calame, M. Bond, L. Hood, and R. Wall.** 1980. Two mRNAs with different 3' ends encode membrane-bound and secreted forms of immunoglobulin μ chain. Cell **20:**303–312.

52. **Rose, M., and F. Winston.** 1984. Identification of a Ty insertion within the coding sequence of S. cerevisiae URA3 gene. Mol. Gen. Genet. **193:**557–560.

53. **Rothstein, R. J.** 1983. One-step gene disruption in yeast. Methods Enzymol. **101C:**202–210.

54. **Russnak, R., and D. Ganem.** 1990. Sequences 5' to the polyadenylation signal mediate differential poly(A) site use in hepatitis B virus. Genes Dev. **4:**764–776.

55. **Russo, P., and F. Sherman.** 1989. Transcription terminates near the poly(A) site in the CYC1 gene of the yeast Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA **86:**8348–8352.

56. **Saez, C. G., J. C. Myers, T. B. Shows, and L. A. Leinwand.** 1990. Human nonmuscle myosin heavy chain mRNA: generation of diversity through alternative polyadenylation. Proc. Natl. Acad. Sci. USA **87:**1164–1168.

57. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.

58. **Simonsen, C. C., and A. D. Levinson.** 1983. Analysis of processing and polyadenylation signals of the hepatitis B virus surface antigen gene by using simian virus 40-hepatitis B virus chimeric plasmids. Mol. Cell. Biol. **3:**2250–2258.

59. **Smith, C. W. J., J. G. Patton, and B. Nadal-Ginard.** 1989. Alternative splicing in the control of gene expression. Annu. Rev. Genet. **23:**527–577.

60. **Snyder, M., R. J. Sapolsky, and R. W. Davis.** 1988. Transcription interferes with elements important for chromosome maintenance in Saccharomyces cerevisiae. Mol. Cell. Biol. **8:**2184–2194.

61. **Tzagoloff, A., and A. M. Myers.** 1986. Genetics of mitochondrial biogenesis. Annu. Rev. Biochem. **55:**249–285.

62. van Loon, A. P. G. M., R. J. de Groot, E. van Eyk, G. T. J. van der Horst, and L. A. Grivell. 1982. Isolation and characterization of nuclear genes coding for subunits of the yeast ubiquinol-cytochrome c reductase complex. Gene 20:323–337.

63. van Loon, A. P. G. M., E. van Eyk, and L. A. Grivell. 1983. Biosynthesis of the ubiquinol-cytochrome c reductase complex in yeast: discoordinate synthesis of the 11-kd subunit in response to increased gene copy number. EMBO J. 2:1765–1770.

64. Vincent, R. D., T. J. Hofmann, and H. P. Zassenhaus. 1988. Sequence and expression of NUC1, the gene encoding the mitochondrial nuclease in Saccharomyces cerevisiae. Nucleic Acids Res. 16:3297–3312.

65. Weber, E. R., and C. L. Dieckmann. 1990. Identification of the CBP1 polypeptide in mitochondrial extracts from Saccharomyces cerevisiae. J. Biol. Chem. 265:1594–1600.

66. Wickens, M., and P. Stephenson. 1984. Role of the conserved AAUAAA sequence: four AAUAAA point mutants prevent messenger RNA 3' end formation. Science 226:1045–1051.

67. Woychik, R. P., R. H. Lyons, L. Post, and F. M. Rottman. 1984. Requirement for the 3' flanking region of the bovine growth hormone gene for accurate polyadenylation. Proc. Natl. Acad. Sci. USA 81:3944–3948.

68. Wu, M., and A. Tzagoloff. 1989. Identification and characterization of a new gene (CBP3) required for the expression of yeast coenzyme QH_2-cytochrome c reductase. J. Biol. Chem. 264:11122–11130.

69. Zaret, K. S., and F. Sherman. 1982. DNA sequence required for efficient transcription termination in yeast. Cell 28:563–573.

70. Zarkower, D., P. Stephenson, M. Sheets, and M. Wickens. 1986. The AAUAAA sequence is required both for cleavage and for polyadenylation of simian virus 40 pre-mRNA in vitro. Mol. Cell. Biol. 6:2317–2323.

71. Ziff, E. B. 1980. Transcription and RNA processing by the DNA tumour viruses. Nature (London) 287:491–499.