DNA sequences required for yeast actin gene transcription do not include conserved CCAAT motifs

Janet M.Munholland, John K.Kelly and Alan G.Wildeman*
Department of Molecular Biology and Genetics, University of Guelph, Guelph, Ontario N1G 2W1, Canada

Received June 14, 1990; Revised and Accepted September 19, 1990

ABSTRACT

Sequences required for *Saccharomyces cerevisiae* actin gene transcription were mapped and compared to the regulatory region of the actin gene from a thermophilic fungus, *Thermomyces lanuginosus*. Two CCAAT motifs conserved in position in these two species could be mutated without affecting promoter activity, regardless of whether the yeast were grown in fermentable or non-fermentable carbon sources. Two TATA-like sequences and an upstream activation sequence (UAS) composed of multiple elements were identified. The contribution of sequence motifs within these elements to UAS activity varied depending on the carbon source. The *Thermomyces* gene contains sequences highly homologous to this UAS, but in the opposite orientation.

INTRODUCTION

Constitutively expressed genes in multicellular organisms could be regulated by DNA sequence elements that function in all cell types, or by a mosaic of elements, with each individual element exhibiting some cell or tissue specificity but expression in all cell types nevertheless being ensured. The cytoplasmic β and γ actin proteins are encoded by genes whose regulation can be studied in this regard. These proteins are a major component of microfilaments, and play a role in many functions, including cell motility, cell division, and cytoplasmic streaming (1, 2). Their importance is underlined by the observation in the yeast *Saccharomyces cerevisiae* that disruption of the single copy β-actin gene is lethal (3).

Mammalian β and γ-actin genes are inducible with serum and with inhibitors of protein synthesis, such as cycloheximide (4,5,6,19). These results have been interpreted to mean that these genes are regulated by both positive and negative control mechanisms, with the inhibitors possibly acting by preventing synthesis of a labile repressor(s) of transcription. Both serum and cycloheximide effects may be mediated through a sequence, termed the serum responsive element (SRE; 8, 19). The sequences flanking both of the mammalian cytoplasmic actin genes also contain, in addition to the reported SRE, TATA and CCAAT motifs typical of many eukaryotic promoters. A GC-rich region containing binding sites for the transcription factor Sp1 is found upstream of the gene encoding γ actin (8). However, it is not known if this or any other as yet unidentified promoter elements contribute to differential expression of the β and γ actin genes.

The conserved structure and functions of cytoplasmic actin proteins might be anticipated to be reflected in similarities in regulation of their genes in diverse species. We have begun therefore to use *S. cerevisiae* as a model for the study of actin genes. Numerous observations have highlighted the similarity of transcription initiation mechanisms in yeast and in mammalian cells. For example, factors which bind the TATA box in yeast can substitute for their mammalian counterpart (20,21), and a number of mammalian transcription factors such as JUN (22) and FOS (23) function in yeast. One yeast factor, MCM1, has been identified that binds to yeast and mammalian SRE sequences (24,25). In addition, yeast contains a single actin gene, encoding a β-actin protein, and provides a simpler genetic system than mammalian cells to study.

Unicellular yeast cannot be used to ask whether eukaryotic actin genes contain promoter elements that exhibit tissue or isoform specificity. However, we have asked whether the promoter of the *S. cerevisiae* gene contains an element(s) that is active under all growth conditions, or multiple and distinct regulatory sequences that each exhibit specificity for particular conditions.

A starting point for this study was the isolation of an actin gene from another fungal species, the thermophile *Thermomyces lanuginosus* (9). Initial comparisons of the promoters indicated that the genes of both species shared, in similar positions, a TATA-like element and two CCAAT motifs (9). These motifs were of particular interest, in light of recent reports that the *S. cerevisiae* proteins HAP2, HAP3, and HAP4 form a complex which binds to CCAAT elements found in the upstream activation sequences (UASs) of genes encoding respiratory proteins (10, 18). The gene encoding CYC1 (iso-1-cytochrome c) has two UASs; only one, UAS2, has a CCAAT-like element (CCAAC), and it is this UAS that enables the CYC1 gene to be active during growth on non-fermentable carbon sources (11,12). The HAP2/HAP3/HAP4 complex is functionally homologous to the mammalian CCAAT-binding protein CP1 (13, 18). The occurrence of two conserved CCAAT motifs in the *S. cerevisiae* and *Thermomyces* actin genes suggested that the actin genes may also have promoter elements that ensure maintained production

* To whom correspondence should be addressed

of this essential protein during growth on various carbon sources.

In this paper we report and discuss some of the results of an ongoing analysis of the *S. cerevisiae* actin promoter. Surprisingly, in either fermentable or non-fermentable carbon sources the conserved CCAAT elements appear to contribute very little to promoter function. The regulatory elements are, however, multiple, with two TATA-like sequences, and further upstream a complex 100 base pair region containing interspersed and reiterated elements that do exhibit different specificities for a fermentable or non-fermentable carbon source.

MATERIALS AND METHODS

Plasmid constructions

Two vectors, pABO and pAYBO (Figure 1) were used to analyse the *S. cerevisiae* actin gene promoter. Both have the 5′ flanking region of the actin gene fused to a promoterless rabbit β-globin coding sequence. Details of their construction have previously been given (31). Both contain the LEU2 allele for selection and 2μ plasmid sequences for autonomous replication. In pABO, actin promoter sequences from −479 to +3 (relative to the ATG codon, with the A being position 0) are present. The plasmid pAYBO is similar, except that additional upstream sequences are present. These include the gene found upstream of the actin gene on chromosome VI, the ras-related YPT1 gene (14), as well as a portion of the next gene, encoding β-tubulin (see Figure 1). A frameshift mutation at the Eco R1 site within the YPT1 gene (Figure 1), prevents overexpression of YPT1 protein in cells carrying this plasmid.

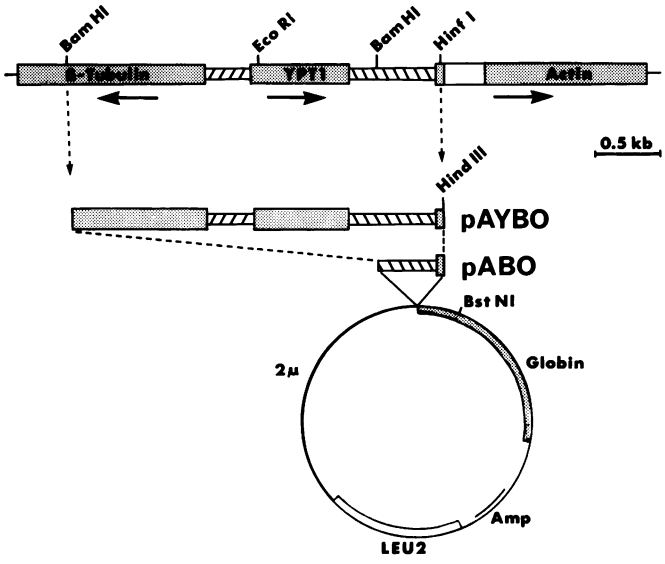


Figure 1. Recombinant plasmids used to study the yeast actin promoter. At the top is shown the arrangement of the actin, YPT1, and tubulin genes on chromosome VI. Descriptions of these plasmids have been given (see Materials and Methods). The 2 μm sequences for autonomous replication in yeast and the LEU2 gene for selection are indicated. The probe for nuclease S1 mapping was 5′-end labeled at the Bst NI site indicated.

Deletion mutations in the actin promoter were made by inserting the Bam HI (position −479) to Hind III (linker at +3) promoter fragment from pABO into the polylinker region of the phagemid pTZ18U (15). Bal 31 digestions (16) were carried out from either the 5′ (Bam HI) or 3′ (Hind III) end of the promoter; deletion end-points were repaired with Bam HI or Bgl II linkers, respectively, and their positions determined by sequence analysis. Deletions internal to the promoter region were created by taking Bam HI-Bgl II fragments from the 3′ deletion series and inserting them in the correct orientation into the Bam HI site of 5′ deletion mutants. This restored a unique Bam HI site at the upstream side. All 5′ and internal deletion mutants were transferred into pABO using Bam HI and Hind III. Some were also examined in the pAYBO vector. Site-directed mutagenesis (15) was used to alter the sequences containing the CCAAT motif centered at position −221 from CC*CA*ATC to CC*AG*ATC. This double point mutation was inserted into both pABO and pAYBO. It was also assessed in combination with some of the deletions by transferring the mutation from the pTZ18U vector in which it was constructed into the appropriate deletion mutant using the unique Mlu I (position −368 in the actin promoter; Figure 1) and Hind III sites.

Yeast strains, transformation, and media

The haploid *S cerevisiae* strain AH22 (MATa, *leu 2−3, 112, his 4−519, can 1*) was used in this study. Transformations were carried out as previously described (17), and transformants grown in minimal medium (0.67% Gibco yeast nitrogen base without amino acids) supplemented with histidine (20 μg/ml) and either 2% glucose, or 2% glycerol plus 2% sodium lactate.

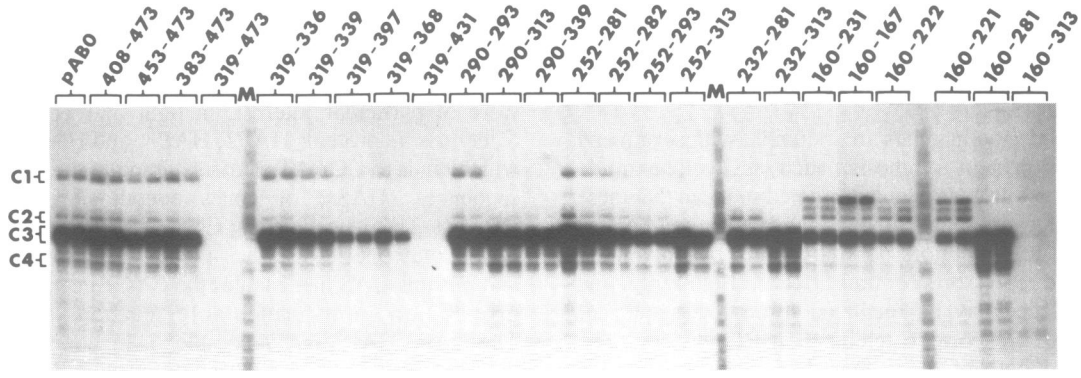


Figure 2. Effect of deletion mutations on actin promoter function during growth in glucose. This autoradiogram shows a representative set of the mutations analysed; these and others are summarized in Figure 3. RNA samples prepared from yeast transformants carrying either pABO or derivatives with deletions of the nucleotides indicated at the top of each pair of lanes (refer to Figure 3), were analysed by nuclease S1 mapping with a probe labeled at the Bst NI site in the globin gene. For each mutant two different transformants were analysed. Four sets of cap sites (C1, C2, C3, and C4) are seen. The marker lanes (M) contain an A+G chemical sequencing reaction of the probe (16).

RNA extraction and S_1 nuclease mapping

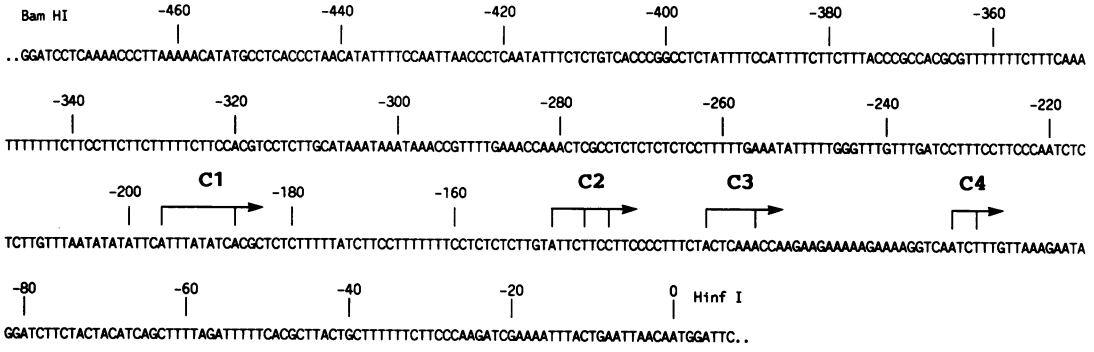
Transformants were harvested at mid-log. Total RNA was extracted by resuspending cells from 10 ml of culture in 2 ml cold 50 mM Tris, pH 7.9, 1 mM EDTA, 0.1 M NaCl, and vortexing in the presence of glass beads. SDS was added to 0.5%, and the lysate extracted with phenol and chloroform. After ethanol precipitation, the RNA samples were resuspended in water, and the concentration and quality of each verified by running 5 μg aliquots on 8% polyacrylamide/8.3 M urea gels, followed by visualization with methylene blue staining.

Nuclease S_1 mapping was carried out with 20 μg of RNA per sample. The probe was prepared by end-labeling with polynucleotide kinase and γ-^{32}P ATP a DNA fragment from pABO extending from Bst NI in the globin sequence to Bam HI upstream of the promoter region (Figure 1). The appropriate strand was resolved on a 5% strand separation gel (16). Hybridization of the probe and RNA samples was carried out at 42°C in 50% formamide, 0.4 M NaCl, 10 mM Pipes, pH 6.4, 1 mM EDTA. Digestion with S_1 nuclease was performed at 25°C for two hours, using 100 units of enzyme (Boehringer Mannheim), and the samples were analysed on 6% polyacrylamide/8.3M urea sequencing gels. Densitometric analysis of the autoradiograms was used to quantitate the amount of actin-globin fusion mRNA in each sample.

RESULTS

Effect of deletion mutants on actin promoter function during growth on glucose

A series of 5′ and 3′ deletion mutants spanning a region of approximately 300 base pairs upstream of the transcription



pABO	UAS	GLUCOSE C1	C2	C3	C4	TOTAL	GLYCEROL/LACTATE C1	C2	C3	C4	TOTAL
		100	100	100	100	100	100	100	100	100	100
		(3%)	(2%)	(93%)	(2%)		(27%)	(15%)	(57%)	(1%)	
453-473	--[ ]----------	100	100	100	100	100	-	-	-	-	-
408-473	--[ ]----------	130	114	120	116	120	98	98	118	100	108
383-473	--[ ]----------	100	90	94	95	94	46	58	19	10	32
319-473	--[ ]----------	1	1	1	1	1	10	10	2	2	9
408-431	---------[ ]----------	113	105	113	120	113	101	103	178	167	146
383-397	----------[ ]----------	101	87	86	93	87	81	76	86	83	83
383-431	---------[ ]----------	50	35	60	28	59	61	52	19	10	35
319-336	----------[ ]----------	113	90	101	90	101	220	204	192	160	201
319-339	----------[ ]----------	61	60	57	60	57	196	168	176	150	180
319-368	----------[ ]----------	20	20	27	40	27	59	54	20	20	36
319-397	----------[ ]----------	15	25	21	30	21	60	55	21	25	37
319-431	---------[ ]----------	1	1	1	1	1	10	10	2	2	9
290-293	----------[]----------	100	100	100	100	100	288	286	296	560	295
290-313	----------[ ]----------	5	50	120	173	117	49	259	353	600	259
290-339	----------[ ]----------	5	50	120	112	116	-	-	-	-	-
252-281	----------[ ]----------	80	100	122	121	119	-	-	-	-	-
252-282	----------[ ]----------	80	100	109	100	104	-	-	-	-	-
252-293	----------[ ]----------	5	70	69	60	67	-	-	-	-	-
252-313	----------[ ]----------	1	1	90	110	86	-	-	-	-	-
232-281	----------[ ]----------	5	116	98	88	95	-	-	-	-	-
232-313	----------[ ]----------	1	1	154	195	147	-	-	-	-	-
160-167	----------[ ]-	240*	170	54	23	61	-	-	-	-	-
160-221	----------[ ]-	100*	210	27	10	36	37*	534	106	87	151
160-222	----------[ ]-	60*	210	24	10	27	51*	629	178	183	212
160-231	----------[ ]-	60*	238	46	32	49	-	-	-	-	-
160-281	----------[ ]-	1*	1	113	259	110	10*	79	359	2133	241
160-313	----------[ ]-	1*	1	1	1	1	71*	114	46	187	71

Figure 3. Summary of the effects of deletion mutants on actin promoter function during growth on glucose or on glycerol/lactate. The promoter sequence present in pABO is shown at the top, and the positions of the four sets of cap sites are indicated. The boundaries of all deletion mutants analysed are shown, and on pABO are marked the positions of the upstream activation sequence (UAS) and two TATA boxes (▼) mapped in this study. In the table on the right are the phenotypes of each of the recombinants tested. The data in the table were derived by densitometric scanning of S1 mapping autoradiograms. The effects of the mutation on each set of cap sites and on total transcription are given, relative to pABO which was assigned a value of 100%. The contribution of each cap site to total pABO transcription is given as a percentage in brackets. Not all deletion mutations were analysed in glycerol/lactate. Every entry in the table is an average of two separate transformants, and most mutants were analysed independently in at least two separate experiments. Within an experiment, duplicate transformants routinely gave results within 10% of each other.

initiation sites of the *S. cerevisiae* actin gene were examined in the vector pABO. For all mutants, two separate transformants were analysed, and most transformants were grown on more than one occasion, to confirm the quantitation of the effect of the mutations. Southern blot analysis of DNA extracted from transformants was done to verify that the deletion mutants did not affect copy number of the plasmid (data not shown).

A representative S_1 mapping gel of a series of deletion mutants is shown in Figure 2. There are four sets of cap sites, designated C1, C2, C3, and C4, with transcription initiating predominantly (93%) from one set, C3. Figure 3 summarizes the effects of these and other deletions on transcription from each set of cap sites, and on total transcription.

The mutations revealed the presence of an upstream activation sequence (UAS) region bounded by positions −336 to -408 (relative to the AUG codon), or nearly 200 bp from C3 at its 3′ side. From the Bam HI site, progressive deletions to position −383 reduced total transcription slightly from wild type levels (to 94%). Removal of additional sequences from −383 to −319 abolished transcription. Deletions proceeding upstream from position −319 confirmed that sequences beyond −397 can maintain some transcription (almost 20% of the wild type level). Other mutants (Δ383−397, Δ383−431 and Δ408−431) revealed that residues between −397 and −408 are responsible for this activity. Two deletions (Δ408−473 and Δ408−431) indicated that between positions −408 and -431, there may be a sequence that has a slight negative effect on promoter function.

The 3′ border of the UAS was identified by comparing Δ319−336 and Δ319−339; the latter, which removed only three more base pairs, began to show a loss of function. A larger deletion which removed more of the active region of the UAS, Δ319−368, reduced activity to little more than a quarter of wild type (27%), and removal of residues to −397 (Δ319−397) caused an additional drop in activity, to 21%.

Downstream of the UAS other sequences important for transcription show some specificity for particular sets of cap sites. Deletion of sequences from −290 to −313 removed a TATA-like element (TAAATAAATAAA), and resulted in an almost complete loss of activity from C1 and, to a much lesser extent, from C2. There was a concurrent small increase in transcription from C3 and C4. A larger deletion (mutant Δ290−339) resulted in no further loss of promoter function, supporting the indication that the TATA-like element is the important motif in this region. The slightly elevated levels of activity from C3 and C4 in the Δ290−339 mutant may be because the UAS has been moved closer to these cap sites.

Downstream of this TATA region, deletion of nucleotides from −252 to −282 had little effect, but the removal of additional residues in either direction resulted in a loss of C1 function. One such deletion, Δ232−281, affected C1 and not C2, indicating that even though most of the sequences controlling transcription from these two minor cap sites overlap, some subtle differences exist. The only residues between positions −232 and -336 that had an appreciable effect on total transcription (by lowering C3 activity) are between −282 and -290 (compare Δ252−282, Δ252−293, and Δ290−293). The largest deletion in this region (Δ232−313) resulted in slightly elevated activity from C3 and C4, most likely because the UAS was moved nearer to these initiation sites.

A final series of mutants analysed extended upstream from position −160. The smallest of these deletions (Δ160−167) lowered somewhat activity from C3 and C4, and resulted in elevated activity from C1 and C2. This deletion is actually a substitution mutation that does not alter spacing between C1 and C2, since the Bam HI and Bgl II linkers used in generating and cloning the deletions were 8 nucleotides long. A larger deletion (Δ160−221) removes a conspicuous TATA-like element near −200 (TAATATATATT) and further diminishes transcription from C3 and C4; C2 continues to be elevated in activity. Deletions of increasing size (Δ160−231 and Δ160−281) move the UAS nearer to the cap sites and restore activity from C3 and C4. However, C3 and C4 abruptly cease to be active when sequences from −281 to −313 are removed (compare Δ160−281 and Δ160−313). This region contains the TATA-like element that was noted to be required for C1 and C2 function, as described earlier.

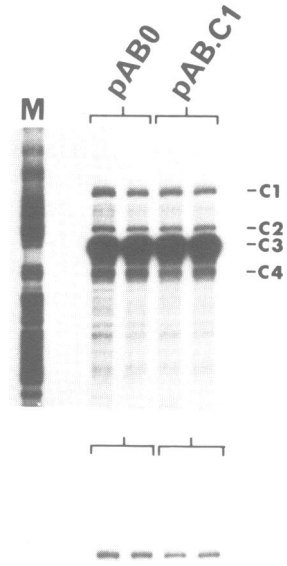


Figure 4. Analysis of CCAAT box mutation. Site directed mutagenesis was used to change the CCAAT motif centered at position −221 to CAGAT, as described in the Materials and Methods. The resulting plasmid, pAB.C1, was compared to pABO during growth on glucose. Two separate transformants of each were analysed. The lower portion of the figure shows a lighter exposure of the upper autoradiogram, to show that C3 is unaffected by the mutation. The marker lane (M) is as described in Figure 2.

When transcription activity of pABO and pAYBO was compared, no differences were found (gels not shown). Since pAYBO contains the entire YPT1 gene and YPT1-actin intergenic region (see Figure 1), during growth in glucose it is unlikely that sequences upstream of the BamHI site play any role in transcription.

Conserved CCAAT motifs are not required for promoter activity

The *S. cerevisiae* actin gene promoter contains two CCAAT boxes, one centered around position −430 and the other around position −221. Deletion mutations which removed the one at −430 did not exhibit reduced transcription (Figure 3). Site-directed mutagenesis was used to alter the CCAAT at −221 to CAGAT, and subsequent S_1 mapping analysis showed that compared to pABO this change also had no effect on promoter function (Figure 4). We also examined whether the CCAAT box at −430 might compensate for the disruption at −261 by testing

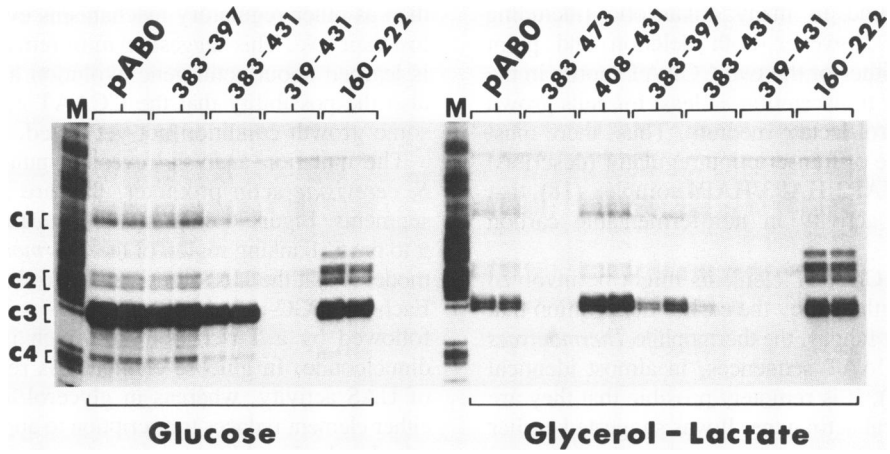


Figure 5. Effect of deletion mutations on actin promoter function during growth on glycerol/lactate or glucose. This autoradiogram shows a representative sampling of mutations that were analysed in both carbon sources. As in Figure 2, two separate transformants for each mutation were analysed. The marker lanes (M) and nucleotides deleted are labeled as in Figure 2.

the double point mutation in combination with the Δ453−473 and Δ408−473 deletions, and again found that there was no effect on transcription (data not shown). Thus, under the experimental conditions used this conserved motif is not required for actin gene expression.

Growth in a non-fermentable carbon source alters promoter element utilisation

In the *S. cerevisiae* CYC1 gene, a CCAAT-like element in one of the UAS regions flanking the gene (UAS2) enables CYC1 expression on non-fermentable carbon sources. However, as was found during growth in glucose, on a glycerol/lactate substrate the disruption of either one or both CCAAT boxes in the actin promoter had no effect on transcription (gels not shown). To determine whether any promoter sequences had activity that varied depending upon the carbon source, a number of the transformants carrying deletion mutant recombinants were grown on glycerol/lactate. A representative S_1 mapping gel of some of the mutants used in these carbon source comparisons is shown in Figure 5, and the data from a number of mutants are summarized in Figure 3.

Several differences between the carbon sources were seen. First, less globin RNA was produced in glycerol/lactate than in glucose (compare pABO in the two carbon sources in Figure 5). Second, much of this difference resulted because C3, the major cap site, was particularly less active, contributing only 57 percent of the total transcription from pABO, in contrast to 93 percent in glucose. Third, the sequence requirements for transcription in glycerol/lactate were not identical to those noted in glucose. Specifically, the deletion of −383 to −473 resulted in a large drop in activity, particularly from C3 (see Figure 5), whereas in glucose its effect was minimal. This and other deletions (Δ408−431, Δ383−397, Δ383−431) led to the conclusion that the UAS region between positions −383 and −408 detected in the studies on glucose played a much greater role in the non-fermentable carbon source. The inhibition of transcription by sequences from −408 to −431 was more evident in glycerol/lactate, where their removal led to an approximate 50% increase in promoter activity (Figures 3 and 4). Interestingly, sequences upstream of position −431 have some effect on UAS activity in glycerol/lactate (compare Δ408−431 and Δ408−473).

In addition, Δ319−336 and Δ319−339 resulted in an elevation of transcription, on the non-fermentable carbon source, whereas in glucose the latter caused expression to drop to approximately half of wild type levels (Figure 3). The next deletion, Δ319−368, led to a major loss of activity in glycerol/lactate; compared to Δ319−339, there was a five-fold drop in total transcription, and an eight-fold drop in transcription from C3. In glucose, this deletion resulted in only a two-fold decrease compared to Δ319−339. Extending the deletion from −319 to −397 resulted in no further loss of transcription in glycerol/lactate, but did in glucose. The deletion of all UAS sequences (Δ319−Δ431) resulted in basal transcription levels similar to those seen in Δ319−473.

The effect of removing the TATA box centered at −300 was also assessed in glycerol/lactate with Δ290−293 and Δ290−313. Both deletions caused a several-fold increase in total transcription, whereas in glucose the Δ290−293 mutation had no effect, and Δ290−313 gave a slight increase (117%). In both carbon sources the removal of the TATA region greatly reduced C1 activity; in glycerol/lactate this reduction was about six-fold (288% to 49% of wild type C1 activity).

Finally, deletions near the cap sites again showed differences due to carbon source. Most notably, removal of nucleotides −160 to −221, a region containing a second putative TATA box, caused a greater than five-fold increase in C2 activity, while C3 remained essentially unchanged. In glucose this deletion caused only a two-fold increase in C2 and a four-fold drop in C3. A slightly larger deletion, Δ160−222, led to an even further increase on glycerol/lactate, and a further decrease on glucose. Removal of residues from −160 to −281 elevated C3 activity on both carbon sources, and lessened transcription from C2. Finally, extending the deletion to include the TATA box near −300 (Δ160−313) caused nearly a ten-fold drop in C3 and C4 function in glycerol/lactate, but did not abolish promoter activity as completely as it did in glucose (Figure 3).

DISCUSSION

The data presented here provide some insight into the organization of the *S. cerevisiae* actin promoter. First, it has been cited as an example of a yeast promoter that contains a CCAAT motif,

a regulatory element found in many eukaryotic (including mammalian) genes (26). However, both deletion and point mutations indicate that neither of the two CCAAT motifs in the actin gene are required for transcription, at least for cells grown in either glucose or glycerol/lactate medium. Thus, there must exist in yeast a second type of transcription regulator (described below) in addition to the HAP2/HAP3/HAP4 complex (18), that is responsible for UAS activity in non-fermentable carbon sources.

The prediction that the CCAAT elements might be involved in transcription was strengthened by the earlier observation that the actin gene from another fungus, the thermophile *Thermomyces lanuginosus*, also had CCAAT sequences, in almost identical positions (9; see Figure 6). It is remotely possible that they are merely relics of an ancestral actin gene. It was suggested earlier that the *Thermomyces* gene may be representative of an ancestral actin gene, based on the observation that the gene has multiple introns in positions that previously were thought to be unique to either vertebrate, invertebrate, or yeast actin genes (9). Considering that CCAAT binding proteins are also conserved in evolution, an ancestral actin gene may have used them, but then as other regulatory mechanisms evolved for the gene they lost their role. This suggestion must remain speculative until more is learned about actin gene evolution and regulation. There is also the possibility that the CCAAT elements function during some growth condition not yet tested.

The mutation analysis revealed multiple components in the *S. cerevisiae* actin promoter, that are a reiteration of smaller segments. Figure 6 diagrams the yeast promoter and compares it to the 5′ flanking region of the *Thermomyces* gene. Our current model is that the UAS consists of two similar elements, I and II. Each has a GC-rich domain (concensus sequence -GNCACNC-) followed by a T-rich domain which is interrupted by a CA dinucleotide. In glucose element I is responsible for 70–80% of UAS activity, whereas in glycerol/lactate the disruption of either element reduces transcription to approximately 20% of wild type levels. In addition, in glucose the T-rich domain provides most of the activity to the element, while in glycerol/lactate the GC domain is crucial. This can be seen from the deletions that removed positions −397 to −408, which corresponds to the GC domain of element II (compare Δ408−431, Δ383−431, and Δ383−397). Deletions in element I give a similar result, as seen

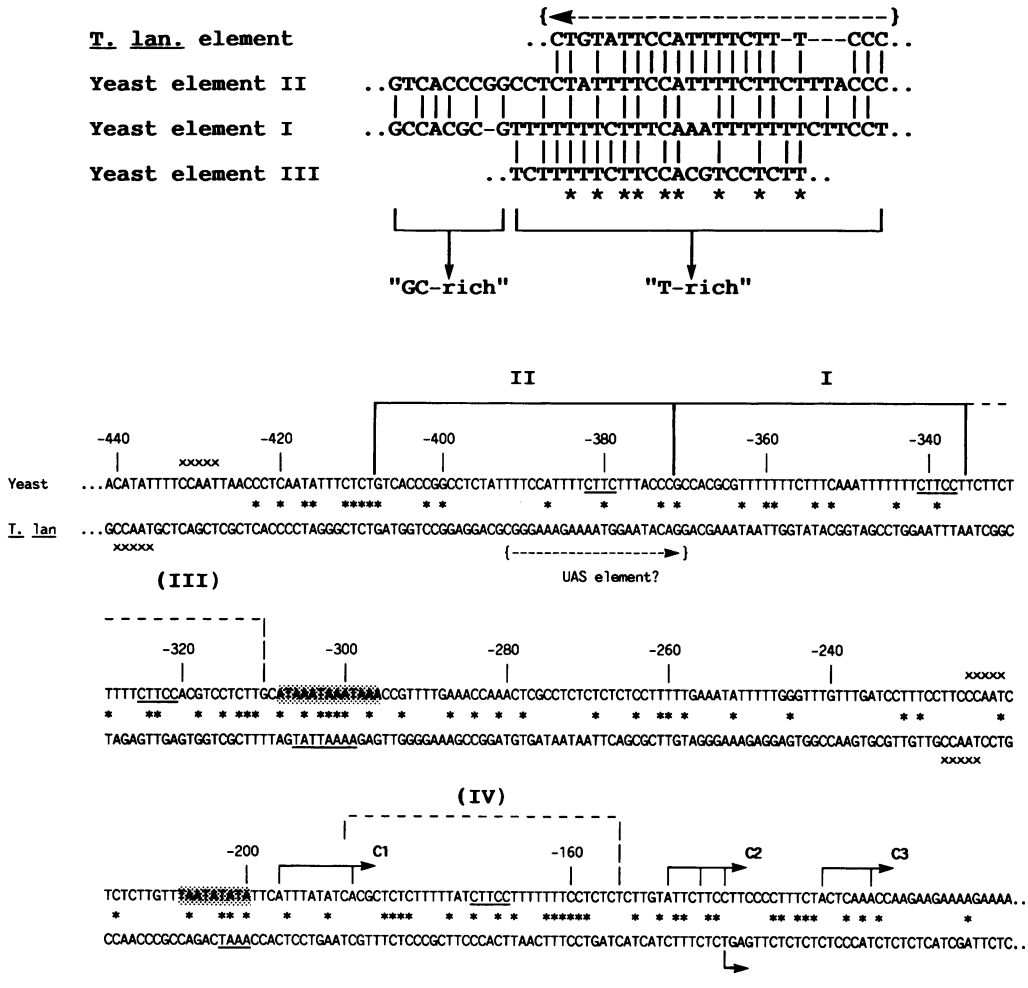


Figure 6. Model for the structure of the *S. cerevisiae* actin promoter, and comparison to the 5′ flanking region of the *Thermomyces lanuginosus* actin gene. The UAS is proposed to consist of two similar elements, I and II. Related sequences are indicated as elements (III) and (IV). The two TATA boxes are shaded, and the CTTCC in the elements underlined (see text for discussion). The *Thermomyces* sequence, and transcription initiation site, are from Ref. 9. The two CCAAT boxes that are present in each species are indicated by xxxxx., and the possible TATA boxes in the *Thermomyces* gene are underlined. The *Thermomyces* gene has a putative UAS element, shown by brackets, that has extensive sequence homology to the yeast elements, but in the opposite orientation. The upper part of the diagram shows an alignment of the yeast elements I, II, and (III), and the inverted *Thermomyces* element.

by comparison of Δ319−339 and Δ319−368. The latter removes the GC domain of the element I and affects transcription much more in glycerol/lactate than in glucose. The former removes part of the T domain and lowers activity in glucose but not in glycerol/lactate. In the absence of element I, removal of the T domain of element II (Δ319−397) lowers its activity in glucose but not in glycerol/lactate. The remaining activity in glucose may in fact result because the deletion juxtaposes the GC domain of element II to a region (identified as element III in Figure 6) with some homology to the T domain, thus regenerating a complete element. A factor, GRF2, that binds to a region between the GAL1 and GAL10 genes, has recently been shown to have binding sites in a number of yeast promoters including actin (32). Its binding site overlaps the GC domains revealed by our mutational analysis, and it will be of interest to determine whether this protein plays a role on non-fermentable carbon sources.

The data also indicate that tracts of T's in the vicinity of the TATA boxes can contribute to promoter activity; Δ160−167 removes a block of seven A/T base pairs and lowers transcription by almost 50%. The sequence surrounding these T's shows some homology to the UAS elements (Figure 6), again pointing to the suggestion that the actin promoter consists of a reiteration of elements. It was also observed that removal of some T tracts increased transcription in glycerol/lactate. This was most noticeable with Δ319−336 and Δ290−293. Further evidence for differences in the mechanisms whereby T-rich and GC-rich domains activate transcription comes from two observations. One is that there is a greater distance dependence of UAS activity in glycerol/lactate. Deletions extending upstream from position −160 demonstrate this. The second is that Δ160−313, which removes both TATA boxes and positions the UAS near the cap sites, remains transcriptionally active in the non-fermentable carbon source but not in glucose. Thus, the potential TATA boxes in the −160 to −313 region are not absolutely required for the activity of the GC-domains.

It is known that poly dA/dT tracts can function as UAS elements for constitutively expressed genes in yeast (27). The actin UAS elements, and particularly element I (which is more T-rich than element II), clearly represent another example, at least in glucose. The T rich UAS region of the HIS3 gene is strikingly similar, having seventeen T residues interrupted by a CA dinucleotide (27). Data from *in vitro* transcription studies suggest that these tracts function by binding specific cellular proteins (e.g. ref. 28). Alternatively, it has been proposed that they may act by excluding nucleosomes and thereby providing the transcription machinery with easy access to other promoter elements (27, 28). Finally, it may be significant that within elements I (near −340) and II (near −380), and related elements III (near −320), and IV (near −170) there are pentanucleotides with the concensus sequence CTTCC. This is a functional motif in the UAS of the phosphoglycerate kinase gene, and is thought to interact specifically with a nuclear protein (29). The deletion Δ319−339, compared to Δ319−336, removes the CTTCC block from the end of the T domain of element I, and does indeed weaken UAS activity. There remains a need for further dissection and analysis of T-rich UAS sequences in yeast.

There are two potential TATA box regions, near positions −200 (T1) and −300 (T2). In the presence of T1, T2 functions primarily for transcription from C1, and to a much lesser extent C2. In the absence of T1, T2 can direct transcription from all cap sites. The two TATA boxes do not appear to exhibit specificities for the carbon sources tested here. This is in contrast to the HIS3 promoter, which also contains two TATA elements, termed Tc and Tr. Transcription occurs from two cap sites, and Tr responds to GCN4 binding during amino acid starvation to specifically increase transcription from one of the cap sites (28, and references therein).

Two other sequence motifs in the actin promoter resemble known elements. One is the TGAAACC tract centered near −285. It is almost identical to the concensus sequence TGAAACA identified as being important for stimulation by the mating pheromone α-factor. The second motif ACCCTACAT, located between −449 and −438, is very similar to the 'RPG-box' (ACCCATACATT) located upstream of yeast ribosomal protein genes (30). A comparison of Δ408−431 with Δ408−473 in glycerol/lactate suggests that a sequence between −431 and −473 does contribute slightly to UAS activity, but we have not investigated this further. It is noteable that none of the promoter elements identified contained a recognizable MCM1 binding site ($CC^A/T^A/T^A/T^A/T^A/TGG$). The MCM1 factor is related to mammalian factors that bind to serum responsive elements (24, 25). Considering that SRE's mediate the induction by serum and cycloheximide of cytoplasmic actin genes, one might have predicted that MCM1 would have a role in regulation of the *S. cerevisiae* actin gene. We have observed that the yeast gene is inducible with cycloheximide (31), but have not identified the sequences responsible.

In addition to the CCAAT sequences, the *Thermomyces* gene has TATA-like elements in positions analagous to those in *S. cerevisiae* (Figure 6). While at first glance it did not appear to have a UAS similar to the yeast gene, closer inspection revealed that in the same region there is a sequence that when read in the opposite orientation is remarkably similar to the yeast elements. While this could be coincidence, it seems to underscore the evolutionary link between these two species.

In conclusion, our data indicate that the *S. cerevisiae* actin promoter contains a mosaic of regulatory elements. The identification of their carbon source specificities is probably only the first of what may prove to be many subtleties in how the expression of this essential gene is regulated.

ACKNOWLEDGEMENTS

We thank Michael McLean for useful discussions and a critical review of the manuscript. This work was supported by the Natural Sciences and Engineering Research Council of Canada.

REFERENCES

1. Pollard, T.D. and Cooper, J.A. (1986) *Ann. Rev. Biochem.* **55**, 987−1035.
2. Huffaker, T.C., Hoyt, M.A. and Botstein, D. (1987) *Ann. Rev. Genet.* **21**, 259−284.
3. Shortle, D., Haber, J. and Botstein, D. (1982) *Science* **287**, 371−373.
4. Elder, P.K., Schmidt, L.J., Ono, T. and Getz, M.J. (1984) *Proc. Natl. Acad. Sci.*, U.S.A. **81**, 7476−7480.
5. Elder, P.K., French, C.L., Subramanian, M., Schmidt, L.J. and Getz, M.J. (1988) *Mol. Cell. Biol.* **8**, 480−485.
6. Greenberg, M.E., Hermanowski, A.L. and Ziff, E.B. (1986) *Mol. Cell. Biol.* **6**, 1050−1057.
7. Orita, S., Makino, K., Kawamoto, T., Niwa, H., Sugiyama, H. and Kakunaga, T. (1989) *Gene* **75**, 13−19.
8. Erba, H.P., Eddy, R., Shows, T., Kedes, L. and Gunning, P. (1988) *Mol. Cell. Biol.* **8**, 1775−1789.
9. Wildeman, A.G. (1988) *Nucleic Acids Res.* **16**, 2553−2564.
10. Forsburg, S.L. and Guarente, L. (1988) *Mol. Cell. Biol.* **8**, 647−654.
11. Guarente, L., Lalonde, B., Gifford, P. and Alani, E. (1984) *Cell* **36**, 503−511.
12. Oleson, J., Hahn, S. and Guarente, L. (1987) *Cell* **51**, 953−961.
13. Chodosh, L.A., Oleson, J., Hahn, S., Baldwin, A.S., Guarente, L. and Sharp, P.A. (1988) *Cell* **53**, 25−35.

14. Gallwitz, D., Donath, C. and Sander, C. (1983) *Nature* (London) **306**, 704–707.
15. Mead, D.A., Szczesna-Skorupa, E. and Kemper, B. (1986) *Protein Engineering* **1**, 67–74.
16. Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
17. Hinnen, A., Hicks, J.B. and Fink, G.R. (1978) *Proc. Natl. Acad. Sci.*, U.S.A. **75**, 1929–1933.
18. Forsburg, S.L. and Guarente, L. (1989) *Genes and Development* **3**, 1166–1178.
19. Subramaniam, M., Schmidt, L.J., Crutchfield, C.E. and Getz, M. (1989) *Nature* (London) **340**, 64–66.
20. Cavallini, B., Huet, J., Plassat, J.-L., Sentenac, A., Egly, J.-M. and Chambon, P. (1988) *Nature* (London) **334**, 77–80.
21. Buratowski, S., Hahn, S., Sharp, P. and Guarente, L. (1988) *Nature* (London) **334**, 37–42.
22. Struhl, K. (1988) *Nature* (London) **332**, 649–650.
23. Lech, K., Anderson, K. and Brent, R. (1988) *Cell* **52**, 179–184.
24. Passmore, S., Elble, R. and Tye, B.-K. (1989) *Genes and Development* **3**, 921–935.
25. Jarvis, E.E., Clark, K.L. and Sprague, G.F. (1989) *Genes and Development* **3**, 936–945.
26. Gallwitz, D., Perrin, F. and Seidel, R. (1981) *Nucleic Acids Res.* **9**, 6339–6350.
27. Struhl, K. (1985) *Proc. Natl. Acad. Sci.*, U.S.A. **82**, 8419–8423.
28. Ponticelli, A.S. and Struhl, K. (1990) *Mol. Cell. Biol.* **10**, 2832–2839.
29. Chambers, A., Stanway, C., Kingsman, A.J. and Kingsman, S.M. (1988) *Nucleic Acids Res.* **16**, 8245–8260.
30. Huet, J., Cottrelle, P., Cool, M., Vignais, M., Thiele, D., Marck, C., Buhler, J., Sentenac, A. and Fromageot, P. (1985) *EMBO J.* **4**, 3539–3547.
31. Munholland, J.M. and Wildeman, A.G. *Gene*, in press.
32. Chasman, D.I., Lue, N.F., Buchman, A.R., LaPointe, J.W., Lorch, Y. and Kornberg, R.D. (1990) *Genes and Development* **4**, 503–514.