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Three GCN4 responsive elements act synergistically as upstream and as TATA-like elements in the yeast *TRP4* promoter

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The yeast *TRP4* promoter contains three responsive elements (GCREs) for the 'general control' transcriptional activator GCN4, which are arranged in two upstream elements, UAS1 (GCRE1) and UAS2 (GCRE2 and GCRE3). A point mutation analysis of these elements revealed that all three GCREs are required for GCN4-dependent transcription, but none are involved in basal transcription. Basal transcription and GCN4-dependent transcription use completely different initiator elements in the *TRP4* promoter. UAS1 acts synergistically with UAS2 to activate the GCN4-dependent transcription of *TRP4*. A consensus TATA box can functionally replace the UAS2 element to allow normal GCN4-dependent transcription, suggesting that UAS2 is analogous to the TATA element of other promoters. GCN4 might therefore activate transcription by exhibiting two alternative functions within the natural *TRP4* promoter.

Key words: amino acid biosynthesis/GCN4/initiation of transcription/*Saccharomyces cerevisiae*/TATA element

Introduction

Transcriptional regulation of a yeast RNA polymerase II promoter requires three kinds of *cis*-acting sequences, namely upstream, TATA and initiator elements (reviewed by Struhl, 1989). Upstream elements are target sites for various activator proteins; they work in a distance- and orientation-independent manner ~100–600 bp upstream of the transcription initiation site. TATA elements are located close to mRNA initiation sites and mediate the first step in the pathway of transcription initiation by binding the general transcription factor TFIID (Van Dyke *et al.*, 1988; Buratowski *et al.*, 1989). Two possible models have been proposed to explain how specific activator proteins could interact with the basic transcription machinery. In one model, the specific activator recruits one or more of the general transcription factors to facilitate assembly of a preinitiation complex. The general transcription factor TFIID has been proposed to be such a candidate. In another model, the activator enhances some step following prior assembly of the general factors into a preinitiation complex (Buratowski *et al.*, 1989), for example by direct interaction of the activator with RNA polymerase II (Allison *et al.*, 1988; Bartolomei *et al.*, 1988; Brandl and Struhl, 1989).

The regulator protein GCN4 of *Saccharomyces cerevisiae* is required for response to amino acid starvation. GCN4 shares homology to the jun oncoprotein and the human *trans*-activator protein AP-1 (Struhl, 1987; Bohmann *et al.*, 1987) and contains the 'leucine zipper' structure responsible for its specific DNA-binding properties (Agre *et al.*, 1989). GCN4 activates transcription of numerous amino acid biosynthetic genes in a system called the 'general control' of amino acid biosynthesis of yeast (Hinnebusch and Fink, 1983). 'General control' promoters can be regulated by two control systems: general (GCN4-dependent) and basal. Basal transcription factors include the two proteins BAS1 and BAS2/PHO2 that regulate the basal expression of the yeast *HIS4* gene independently of GCN4 (Arndt *et al.*, 1987; Tice-Baldwin *et al.*, 1989). The GCN4 protein can, in specific cases, also regulate the basal expression as shown for the yeast *ARO3* gene (Paravicini *et al.*, 1989). The optimal promoter binding site for the GCN4 protein is the well characterized palindrome 5′ ATGA(C/G)TCAT 3′ (Hope and Struhl, 1985, 1987; Hill *et al.*, 1986; Arndt and Fink, 1986; Oliphant *et al.*, 1989). Such GCN4 recognition elements (GCREs) have been found repeated upstream of every structural gene under general control examined thus far (reviewed by Hinnebusch, 1988). The naturally occuring sites analyzed so far are not identical to the consensus sequence, but they differ by 1–2 bp (Struhl, 1989). The GCN4 protein binds general control promoters at all GCRE sequences (Arndt and Fink, 1986). Deletion analysis of a number of these promoters has demonstrated that GCRE sequences are both necessary and sufficient for general control mediated regulation of transcription *in vivo* (Donahue *et al.*, 1983; Struhl, 1982). Little is known, however, about the interplay of multiple GCREs in a naturally occurring general control promoter *in vivo*.

The transcription initiator element is the primary determinant of where transcription begins in yeast (for review see Struhl, 1989). Yeast mRNA initiation sites are determined primarily by specific sequences, not by the distance from the TATA element as in higher eukaryotes (Chen and Struhl, 1985). Two types of start site selection patterns have been found in yeast in GCN4-controlled genes when transcription start sites of the basal expression have been compared to the start sites of the GCN4-driven transcription. Only a single start site of transcription can be found in the *HIS4* promoter region when the 5′ ends of basal controlled transcripts, as well as of GCN4-controlled transcripts are determined (Nagawa and Fink, 1985). The *HIS3* promoter initiates transcription equally from two sites at +1 and +12, during basal expression. The GCN4-driven transcription of this promoter then preferentially initiates at the basal initiation site at +12 (Chen and Struhl, 1985).

The promoter of the *TRP4* gene of *Saccharomyces cerevisiae*, coding for the enzyme anthranilate phophoribosyl transferase (PR transferase, EC 2.4.2.18), contains two putative UAS elements for the GCN4 protein: UAS1 comprises a single GCN4 binding site, ATGACTAAT (from −246 relative to the translational start site to −238),

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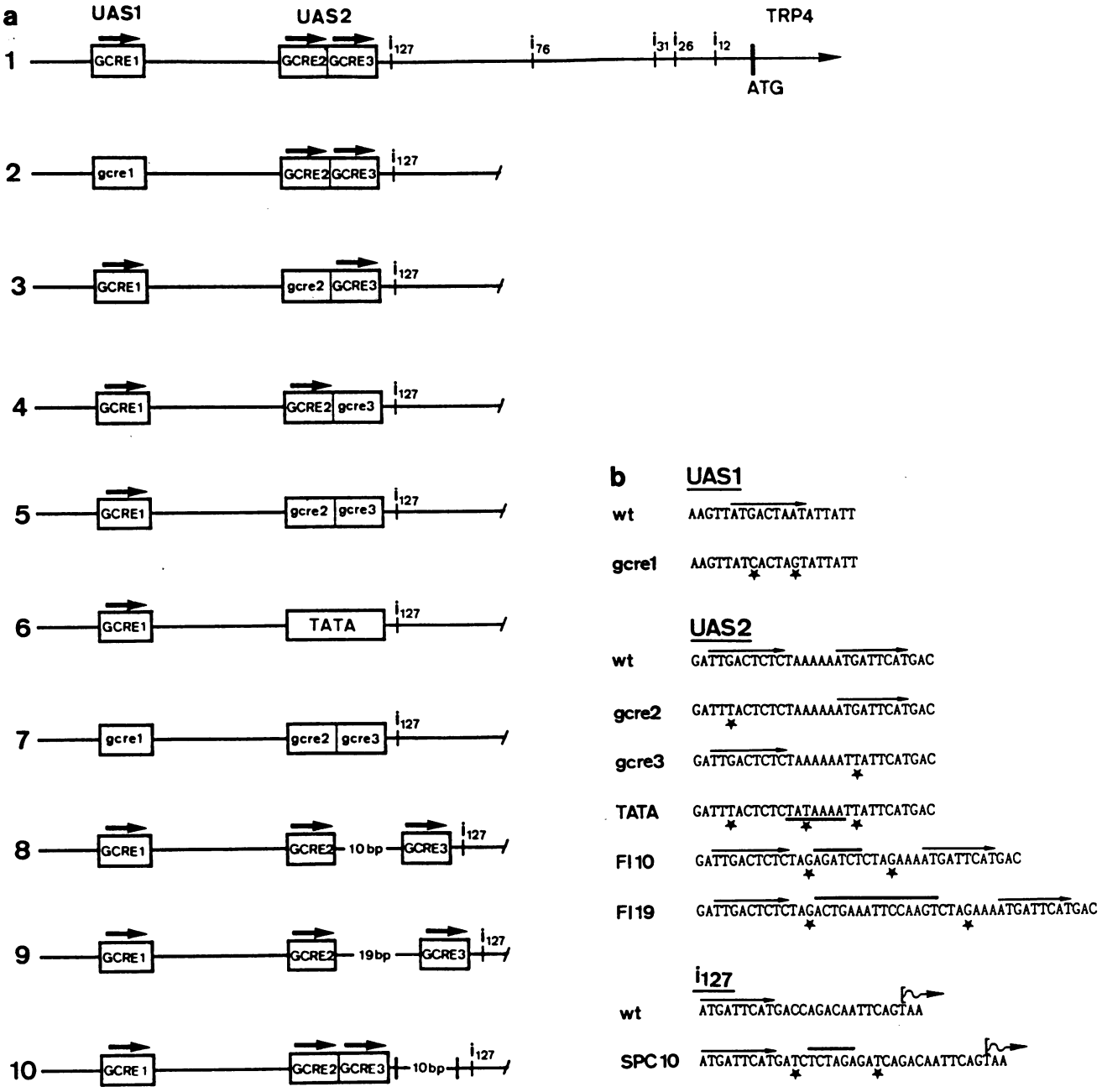

Fig. 1. *TRP4* promoter region of wild-type and mutant strains. (**a**) Schematic illustration of *TRP4* promoter mutant alleles. **1**: 280 bp wild-type *TRP4* promoter region; UAS elements are represented as boxes; GCN4 recognition elements (GCRE), binding GCN4 protein *in vitro*, are indicated by an arrow; start sites for transcription are designated as i (subscribed numbers indicate their position relative to the translational start site at +1); ATG marks the translational start site (Furter *et al.*, 1986). **2–7**: mutant alleles were constructed by site-directed mutagenesis; mutated GCN4 recognition elements are designated as gcre (for sequences see b: gcre1, gcre2 and gcre3); TATA indicates the newly created TATA box substituted for GCRE2 and GCRE3 (for sequence see b: TATA). **8** and **9**: mutant alleles were constructed by introducing *Xba*I restriction site (at position −155) between GCRE2 and GCRE3 followed by insertion of synthetic linker DNAs of 6 bp and 15 bp respectively into the blunt-ended *Xba*I site (for sequences see b: FI 10 and FI 19). **10**: mutant allele was constructed by introducing a *Bcl*I restriction site (at position −140) and subsequent insertion of a synthetic 6 bp linker into the blunt-ended *Bcl*I site (for sequence see b: SPC 10). (**b**) DNA sequences of wild-type (wt) and mutated *TRP4* promoter regions: UAS1 region, sequence −252 to −232; UAS2 region, sequence −108 to −140; i127 region, sequence −151 to −125; point mutations are indicated by asterisks; arrows represent functional GCRE sequences, the consensus TATA box is underlined; sequences of synthetic spacer DNA linkers are overlined; the transcription start site i127 is indicated by a wavy arrow.

designated as GCRE1, and UAS2 comprises two adjacent repeats, TTGACTCTC (−166 to −158) and ATGATTCAT (−151 to −143), designated as GCRE2 and GCRE3, respectively (Figure 1). UAS1 and UAS2 are both able to bind the activator protein GCN4 specifically *in vitro*. UAS1 is also known to be essential for the GCN4-dependent activation of the *TRP4* gene *in vivo* (Braus *et al.*, 1989). GCN4 has been shown to compete at the UAS1 site with the transcriptional regulator PHO2/BAS2, a protein containing a homeo box. The PHO2 protein presumably represses GCN4-binding when yeast cells are simultaneously starved for phosphate and amino acids and thereby prevents the

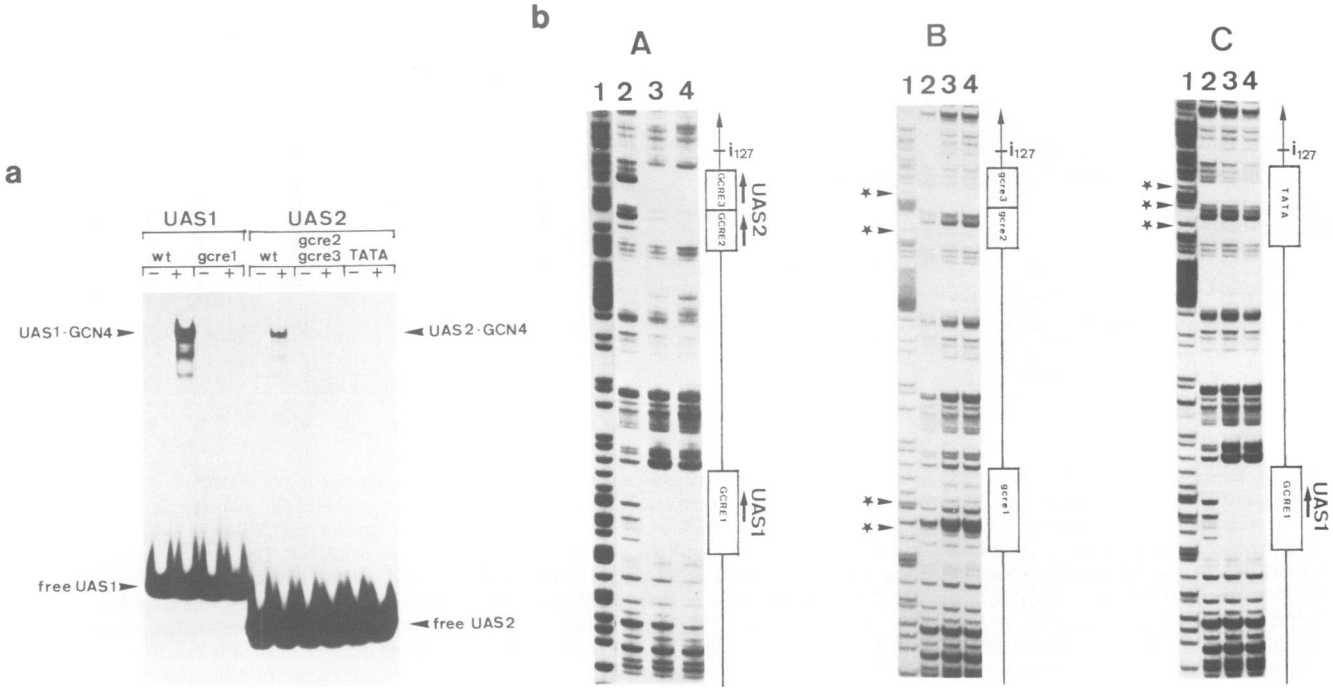

Fig. 2. Effects of site-directed point mutations on binding of GCN4 protein *in vitro*. (**a**) Gel retardation assays. Radiolabeled *TRP4* promoter fragments were analyzed for complex formation with (+) or without (−) GCN4 protein produced in *E.coli*. UAS1 fragments are 53 bp *Mlu*I−*Hha*I fragments (from positions −279 to −226) containing either a wild-type or a mutated GCRE1 element, indicated as wt or gcrel, respectively (for sequence details see Figure 1). UAS2 fragments (36 bp from position −172 to −137) were synthesized and contain wild-type (wt) or mutated GCRE2 and GCRE3 elements (gcre2, gcre3) or a consensus TATA box instead of GCRE2 and GCRE3 (TATA). (**b**) DNase I footprint analysis. DNase I protection with GCN4 protein was performed using different *TRP4* promoter fragments (A: wild-type sequence; **B**: construct No. 7; **C**: construct No. 6 as referred to in Figure 1) that were radiolabeled at position −448 (relative to the translational start site). The DNA probes were incubated with *E.coli* extracts containing GCN4 protein (5 μg in **lane 3**, 10 μg in **lane 4**) or without GCN4 protein (**lane 2**). After treatment with DNase I, the samples were separated on a standard sequencing gel. UAS elements are represented as boxes; GCN4 recognition elements (GCRE), protected by GCN4, are indicated by an arrow. Positions of point mutations in GCRE elements (designated as gcre) are marked by asterisks. An A/G sequence ladder (**lane 1**) was used as a size marker.

GCN4 protein from activating the *TRP4* gene. The role of the UAS2 element in the *TRP4* promoter is not known.

In this paper, we investigated the roles or each of the three GCRE sequences in the *TRP4* promoter *in vivo* by performing site-directed point mutagenesis on all three GCREs in different combinations and measuring the effects on basal and GCN4-mediated transcription of the *TRP4* gene. Our results show that (i) all three GCREs are required for a normal GCN4-dependent transcription activation but do not affect basal transcription; (ii) basal transcription and GCN4-mediated transcription initiate at different start sites; (iii) GCN4-mediated transcription can initiate at the basal start sites when the natural configuration of UAS2 is altered; (iv) UAS1 (GCRE1) acts synergistically together with UAS2 (GCRE2 and GCRE3) to mediate the GCN4-dependent transcription activation; (v) a consensus TATA box is able to replace the UAS2 element functionally suggesting that UAS2 has a function *in vivo* comparable to that of a TATA element. Taken together, these results suggest that GCN4 activates transcription by exhibiting two alternative functions within one natural promoter.

Results

Two GCN4-dependent cis-elements act synergistically in the TRP4 promoter

We determined the roles of UAS1 and UAS2 *in vivo* by creating point mutations in all GCRE sequences in the *TRP4* promoter (Figure 1a and b). The effects of all base pair exchanges on GCN4 binding were initially tested by *in vitro* binding studies with GCN4 protein, overproduced in *E.coli* (Arndt and Fink, 1986; Hill *et al.*, 1986; Oliphant *et al.*, 1989). Specific DNA−protein complexes were completely lacking in a gel retardation assay when GCN4 protein was incubated with either a radiolabeled promoter fragment (53 bp *Mlu*I−*Hha*I fragment from positions −279 to −226) containing a mutated GCRE1 (gcre1) in UAS1, or with a synthetic UAS2 fragment (36 bp, from positions −172 to −137) containing the mutated GCRE2 (gcre2) and GCRE3 (gcre3), as shown in Figure 2A. DNase I footprint analysis with a mutated *TRP4* promoter fragment (Figure 1A, construct 7), lacking all three functional GCREs, confirmed these results. No binding of GCN4 protein to the mutated *TRP4* promoter was detected even at high concentration of the protein (Figure 2B). The different mutant promoter alleles were substituted for the wild-type *TRP4* promoter (Figure 1, constructs 2, 3, 4, 5 and 7) by gene replacement in the genomic *TRP4* locus.

TRP4 expression of all promoter alleles was studied in yeast cells containing either repressed amounts of GCN4 protein or derepressed amounts of GCN4 protein using a *gcd2-1* mutation or containing no GCN4 protein. Figure 3 summarizes the data of the *TRP4* transcript analysis as well as the different specific enzyme activities of the *TRP4* gene product PR transferase. Deleting any one of the three GCRE sequences severely reduced the GCN4-mediated transcrip-

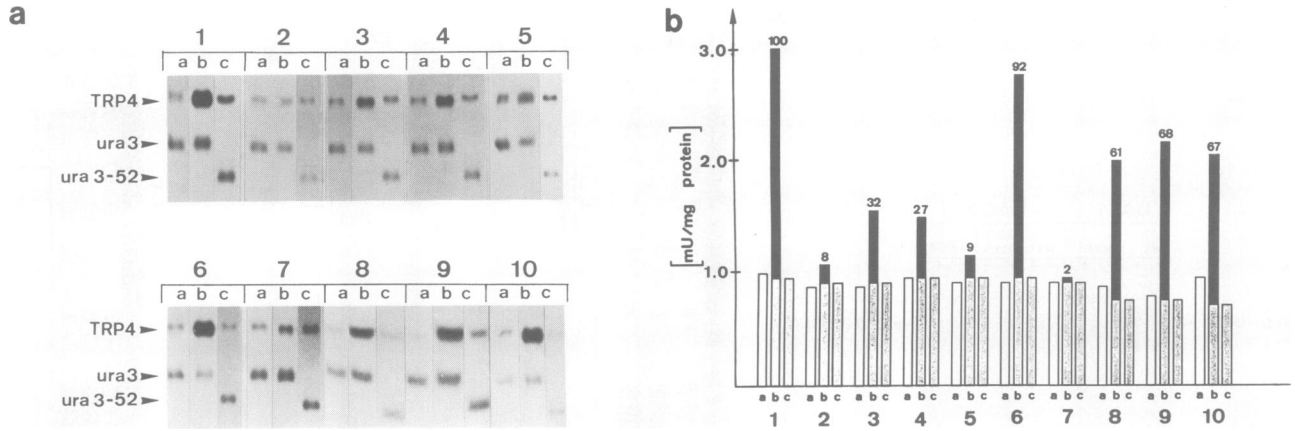

Fig. 3. Expression of the *TRP4* gene under control of different promoter mutants and GCN4. (**a**) *TRP4* transcript levels. Poly(A)$^+$ RNA isolated from yeast strains (a: Δ*ura3*; b: Δ*ura3*, *gcd2-1*; c: *ura3-52*, Δ*gcn4*) carrying different *TRP4* promoter mutant alleles (1, wild-type; 2–10, numbering of the constructs 2–10 according to Figure 1) at the chromosomal *TRP4* locus were cohybridized against radiolabeled *TRP4* and *URA3* probes. The *URA3* transcript was chosen as an internal standard for the amount of mRNA as it is not under the control of GCN4. The *gcd2-1* mutation (all b lanes) causes constitutively derepressed expression levels of all genes under control of GCN4 (Hinnebusch, 1988). Transcript sizes are: *TRP4*, 1.4 kb; Δ*ura3*, *0.9 kb*; *ura3-52*, 0.6 kb. (**b**) *TRP4* gene product enzyme activities. Specific PR transferase enzyme activities were assayed *in situ* in identical yeast strains as described in Figure 3A. The given values (in mU/mg protein) are the means of four independent cultivations each measured twice (standard deviation did not exceed 20%); GCN4-dependent expression was determined by subtracting basal expression (c lanes) from constitutively derepressed expression (b lanes) and is indicated by black bars (given values are relative to a wild-type *TRP4* situation defined as 100).

tion. A promoter containing a mutation of either UAS1 (gcre1) or UAS2 (gcre2 + gcre3) was no longer inducible by the GCN4 protein (Figure 3, lanes 2, 5 and 7). The use of the *TRP4* promoter by GCN4 was reduced to ~30% when either GCRE2 or GCRE3 was mutated (Figure 3, lanes 3 and 4). This evidence shows that all three GCRE sequences in the *TRP4* promoter are required for the GCN4-mediated part of the promoter. Both upstream elements, UAS1 and UAS2, are essential for a GCN4-mediated part of the promoter and act together in a synergistic manner *in vivo*.

The basal expression of the *TRP4* gene remained unaffected by mutating any one of the GCRE sequences, demonstrating that none of these elements forms part of the basal promoter of the gene.

Basal and GCN4-mediated transcription select different initiator elements

We determined the 5′ start sites of *TRP4* transcripts generated in cells containing either basal levels of GCN4 protein (a), derepressed levels of GCN4 protein (b) or no GCN4 (c) (Figure 4). A basal level of *TRP4* transcription resulted in transcripts starting at two sites at positions −127 (i127) and −76 (i76) relative to the translational start site (Figure 4, lanes 1a and 1c). The basal transcripts remained unchanged, but three additional signals for mRNA start sites appeared at positions −31 (i31), −26 (i26) and −12 (i12) in the presence of high amounts of the GCN4 regulator in the cell, which corresponds to the situation of amino acid starvation *in vivo* (Figure 4, lane 1b). These additional transcripts correspond to the increase in transcription initiation as measured at the mRNA (Figure 3A) and enzyme (Figure 3B) levels and therefore represent the product of the GCN4-driven part of the *TRP4* promoter. These GCN4-dependent start sites were lacking when the GCN4 regulator was missing from the cell (Figure 4, all c lanes) as well as when the GCN4-driven transcription of the *TRP4* gene was abolished by mutations in UAS1 or UAS2 or both (Figure 4; lanes 2b, 5b and 7b). The usage of the initiator elements i31, i26 and i12 by the transcription machinery is therefore solely dependent on the presence of the regulator protein GCN4 or its recognition elements in the *TRP4* promoter. These results are different from any other previously described GCN4-dependent yeast promoter and show that basal transcription and GCN4-driven transcription of the *TRP4* gene are distinct events, even in respect to their transcription sites.

GCN4-driven transcripts initiate at the basal start sites when the normal configuration of UAS2 is altered

Mutating either GCRE2 or GCRE3 in the UAS2 region results in a *TRP4* promoter that can be induced only partially by GCN4 when compared to an intact promoter (Figure 3, lanes 3 and 4). The transcript start site selection pattern for these two constructs, however, was identical in the absence or presence of GCN4 protein in the cell (Figure 4, lanes 3 and 4). A partial deletion of UAS2 resulted in a promoter that could still be driven to 30% efficiency by GCN4 but that now initiated transcription at the same sites as the basal *TRP4* promoter. The same effect was found when the configuration of UAS2 was disrupted by introducing spacer DNA of 10 or 19 bp between GCRE2 and GCRE3 instead of mutating either repeat of UAS2 (Figure 1A constructs 8 and 9): A GCN4-dependent transcription of both of these constructs was still possible up to ~65% efficiency when compared to the wild-type (Figure 3, lanes 8 and 9). These transcripts initiated at the same start sites as the GCN4-independent transcription. An increased spacing of 10 bp between GCRE2 and GCRE3 abolished the usage of the normally employed GCN4-dependent start sites (i31, i26 and i12) and the GCN4-controlled transcription now initiated at the basal start sites at −127 and −76 (Figure 4, lane 8). An increased spacing of 19 bp between the same GCRE sequences resulted in a similar situation in that the basal transcription now started not only at −127 and −76 but additionally at a new start site at position −139. The same

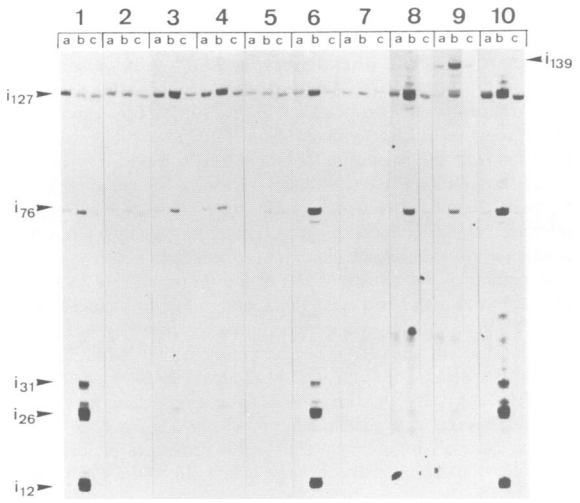

Fig. 4. Transcription start site selection patterns. 100 μg of poly(A)$^+$ RNA from identical preparations as used for quantitative Northern hybridizations in Figure 3 were hybridized to completion with an excess of a ^{32}P-labeled *TRP4* primer and subsequently elongated using AMV reverse transcriptase. Elongation products were analyzed on a standard sequencing gel. Initiation sites of *TRP4* transcription are designated as i (subscripts indicate their position relative to the translational start site at +1) and are marked by arrows.

basal start sites were also used when the promoter was transcribed under the control of GCN4 (Figure 4, lane 9). A large spacing of 157 bp between GCRE2 and GCRE3 abolished both basal and GCN4-dependent transcription and resulted in a *trp4* phenotype (data not shown).

A mutant allele, in which the spacing between UAS2 and the basal transcription start site i127 was increased from 15 bp to 25 bp by introducing 10 bp (Figure 1, construct 10), was created in order to test whether this distance is critical for normal start site selection of the GCN4-driven promoter. This spacing caused a drop in the GCN4-driven transcription to 67% (compared to the wild-type situation) but did not severely affect the start site selection pattern of the GCN4-dependent transcription (Figure 4, lane 10).

A change in the normal configuration of UAS2 by deleting either one of its two GCRE sequences or by increasing the natural spacing between these two repeats from 6 bp to 16 bp (+10) or 25 bp (+19) thus prevents the usage of the GCN4-dependent transcription start sites (i31, i26, and i12) and causes GCN4-dependent transcription to start at the same sites as the basal promoter. This situation is similarly found in other GCN4-dependent promoters such as *HIS3* and *HIS4*. Increasing the distance between UAS2 and the basal initiator element i127 does not, however, affect the start site selection pattern of the *TRP4* promoter.

A consensus TATA box can replace the UAS2 element functionally

Upstream activator proteins like GCN4 or GAL4 normally stimulate transcription when bound upstream of a TATA element (for review see Struhl, 1989). No functional consensus TATA box (like TATAAA, TATATA or TATCTA, Chen and Struhl, 1988) can be found in the *TRP4* promoter between UAS2 and the transcription initiation sites of the GCN4-mediated transcription (Furter *et al.*, 1988). This observation led us to ask the question of whether UAS2 could be the analogue of a TATA box for the GCN4-dependent *TRP4* promoter. We therefore tested whether a consensus TATA box could substitute functionally for the UAS2 element.

We exchanged the *TRP4*-UAS2 element for a consensus TATA box as shown in Figure 1B. Such a construct can be made by exchanging one base pair in a mutant *TRP4* promoter where GCRE2 and GCRE3 of UAS2 have been deleted by point mutations. The UAS2 region of this construct (Figure 1A, construct 6) no longer contains functional GCRE sequences but instead has a perfect TATAAA sequence identical to the GCN4-dependent TATA element in the *HIS3* promoter (Chen and Struhl, 1988). This newly created *TRP4* promoter sequence, **TCTCTATAAAATT**, also shows a high similarity to the *CYC1* −52 TATA element (**TGTATATAAAACT**) that binds to the transcription factor TFIID *in vitro* (Hahn *et al.*, 1989). We performed gel retardation assays and a DNase I footprint analysis to rule out the possibility that the newly created TATA element in the UAS2 region of the *TRP4* promoter had a restored affinity for binding the GCN4 protein *in vitro*. A synthetic UAS2 region consisting of a TATA box instead of the GCRE2 and GCRE3 elements was unable to form any specific DNA−protein complex with GCN4 protein in the gel retardation assay (Figure 2A). The GCN4 protein also failed to bind this TATA sequence in a DNase I footprint analysis (Figure 2B) when the corresponding mutant allele (construct 6) of the *TRP4* promoter was used.

Expression studies revealed that the newly introduced TATA box was able to restore the GCN4-driven transcription of a *TRP4* promoter with a mutated UAS2 (Figure 3). The basal level of *TRP4* transcription was unaffected by the other mutant promoter alleles. A primer extension analysis is shown in Figure 4. Transcription of mutant *TRP4* promoter no. 6 started mainly at i127 at repressed levels or in the absence of GCN4 protein, as found for the wild-type promoter (Figure 4, lanes 6a and 6c). Transcription initiated again at i31, i26 and i12 at high levels of GCN4 protein in the cell (Figure 4, lane 6B). We also found an induced initiation at i76 in contrast to a wild-type *TRP4* promoter.

The regulated initiator elements i31, i26 and i12 can, therefore, be used in two possible ways: (i) when transcription is driven by GCN4 acting synergistically via UAS1 and UAS2 (wild-type situation) or (ii) when transcription is dependent on GCN4 binding at UAS1 and on a TATA factor (presumably TFIID) binding to a TATA box situated at the position of UAS2 (mutant allele no. 6). These results show that a consensus TATA box can functionally replace the UAS2 element in the GCN4-dependent *TRP4* promoter, suggesting that the UAS2 element has a function *in vivo* which is analogous to that of a TATA element in other eukaryotic promoters.

Discussion

The *TRP4* promoter contains three GCN4 responsive elements which are arranged in two upstream elements, UAS1 (GCRE1) and UAS2 (GCRE2 and GCRE3). Both transcriptional activation by GCN4 and repression of this GCN4-mediated activation by PHO2/BAS2 act via UAS1 (Braus *et al.*, 1989). Here, we investigated the crucial role of the UAS2 element by mutating either of its GCN4-responsive elements, GCRE2 or GCRE3. We furthermore

disturbed the natural configuration of UAS2 by inserting spacer DNA between GCRE2 and GCRE3. GCN4 is still able to confer transcriptional activation to an extent of 30–65% in such constructs. This GCN4-stimulated transcription, however, now starts at the basal initiator elements. It is therefore possible to activate the *TRP4* gene by GCN4 in a similar manner to that found for the *HIS3* and *HIS4* genes by introducing small alterations in the configuration of UAS2. Such small changes in the configuration of UAS2 possibly prevent the synergistic action of UAS1 and UAS2 that we found for the 'general control' promoter of the *TRP4* gene. No other 'general control' promoter has been found to be regulated by the synergistic action of different GCN4 responsive elements. Synergism in the regulation of the *TRP4* gene by the 'general control' system has the advantage that stimulation of transcription by GCN4 can be completely shut down by preventing the binding of GCN4 to either UAS1 or UAS2. This effect can be observed *in vivo* when yeast cells are starved simultaneously for amino acids and for inorganic phosphate. The binding of the transcriptional factor PHO2/BAS2 to UAS1 then represses any transcription of the *TRP4* gene driven by GCN4 (Braus *et al.*, 1989).

The main finding of this report is that a consensus TATA box can functionally replace the GCRE2 and GCRE3 elements. These two elements cannot be recognition sites for transcription factors of the basal control system as their deletion by point mutagenesis affects only the binding of GCN4 *in vitro* and the GCN4-driven transcription *in vivo*, but not the basal transcription of the *TRP4* gene. Basal transcription of the *TRP4* gene also remains unaffected when the deleted GCRE elements at UAS2 are further replaced by a consensus TATA box. Such a replacement, however, allows a promoter with a deleted UAS2 to be driven again by GCN4 and, moreover, allows the transcription machinery to use the initiation start sites at −31, −26 and −12 again when activated by GCN4. Our data suggest that GCN4 fulfills the TATA factor function for the 'general control' transcription in the *TRP4* gene. We cannot completely rule out the possibility that, *in vivo*, the three GCN4 sites serve as UASs that activate transcription in combination with a more downstream 'weak' TATA element which deviates somewhat from a TATAAA sequence. In addition other factors with binding properties similar to GCN4, e.g. the transcriptional factor yAP-1 (Harshman *et al.*, 1988) might also be involved in the function of UAS2. There is, however, additional evidence that the TATA factor function for the 'general control' transcription in the *TRP4* gene is fulfilled by GCN4: (i) GCN4 is able to interact specifically with RNA polymerase II *in vitro* (Brandl and Struhl, 1989). The region of GCN4 that contacts pol II resides within the DNA binding domain of the protein and not within the short acidic domain, which is required *in vivo* for transcriptional activation. (ii) GCN4 efficiently activates transcription in an artificial *GAL–HIS3* hybrid promoter in the absence of a TATA element when bound close to the mRNA initiation site (Chen and Struhl, 1989). These data demonstrate that there are other factors in yeast apart from the general transcription factor TFIID that recognize sequences unrelated to the consensus TATA box, but which are nevertheless able to perform the role of TFIID. Our data therefore suggest multiple functions of GCN4 *in vivo*, including the TATA factor function.

Materials and methods

Construction of yeast strains carrying TRP4 mutant alleles

All yeast strains carrying *TRP4* mutant alleles were constructed using the gene replacement technique (Rudolph *et al.*, 1985). The complete *TRP4* promoter was first evicted (from position −279 to +196, relative to the translational start site at position +1) and then substituted by the *URA3* gene. *TRP4* promoter mutant alleles obtained by site-directed point mutagenesis and/or subsequent standard cloning techniques were then re-introduced replacing the *URA3* gene. Linear fragment yeast transformations were performed using the lithium treatment method (Ito *et al.*, 1983). The integration of the mutant alleles at the original *TRP4* locus on the chromosome was confirmed using the Southern blot technique (Southern, 1975). *TRP4* gene replacements were performed in three isogenic derivatives of the *Saccharomyces cerevisiae* laboratory strain S288C: RH1385 (*MATaΔura3*), RH1378 (*MATaΔura3 gcd2-1*) and RH1408 (*MATa ura3-52 gcn4-103*). The *gcd2-1* and the *gcn4-103* mutations have been described earlier (Niederberger *et al.*, 1986; Hinnebusch, 1985). The *gcd2-1* mutation causes constitutively high amounts of GCN4 protein in the cell whereas the *gcn4-103* mutation contains a large deletion of the *GCN4* gene.

Site-directed mutagenesis

Point mutations were generated using the Muta-Gene *in vitro* mutagenesis kit from Bio-Rad (Richmond, CA), based on a method described by Kunkel (Kunkel, 1985). The promoter regions of all *TRP4* mutant alleles obtained by this procedure were completely sequenced using the dideoxy method (Sanger *et al.*, 1977) thereby ruling out possible second site mutations.

Gel retardation assays and DNase I footprint analysis

In vitro protein–DNA binding techniques using GCN4 protein, produced in *E.coli*, have been described earlier (Arndt and Fink, 1986; Braus *et al.*, 1989).

Northern analysis

Poly(A)$^+$ RNA was isolated as described earlier (Furter *et al.*, 1986). For Northern hybridization, poly(A)$^+$ RNA was separated on a formaldehyde agarose gel, electroblotted onto a nylon-membrane and hybridized with DNA fragments labeled according to the 'oligolabeling' technique described by Feinberg and Vogelstein (1984).

Primer extension analysis

Primer extension analysis was performed according to Kassavetis and Geiduschek (1982) using 100 μg of poly(A)$^+$ RNA and 5×10^6 c.p.m. of a 5′-end-labeled 50 bp primer (from position +29 to +79 relative to the translation start site of the *TRP4* gene).

Media and enzyme assays

Yeast strains were cultivated in YEPD complete medium or MV minimal medium supplemented with uracil (40 mg/l) and arginine (40 mg/l) as described earlier (Miozzari *et al.*, 1978). PR transferase activities were determined *in situ* as described (Furter *et al.*, 1986).

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