Mol Gen Genet (1991) 231:22–32

MGG

© Springer-Verlag 1991

A multi-component upstream activation sequence of the *Saccharomyces cerevisiae* glyceraldehyde-3-phosphate dehydrogenase gene promoter

Grant A. Bitter, Kenneth K.H. Chang, and Kevin M. Egan

Department of Molecular Genetics, Amgen Inc., 1840 Dehavilland Drive, Thousand Oaks, CA 91320, USA

Received May 6, 1991 / August 12, 1991

Summary. The majority of the activation potential of the *Saccharomyces cerevisiae TDH3* gene promoter is contained within nucleotides −676 to −381 (relative to the translation initiation codon). An upstream activation sequence (UAS) in this region has been characterized by in vitro and in vivo assays and demonstrated to be composed of two small, adjacent DNA sequence elements. The essential determinant of this upstream UAS is a general regulatory factor 1 (GRF1) binding site at nucleotides −513 to −501. A synthetic DNA element comprising this sequence, or an analogue in which two of the degenerate nucleotides of the GRF1 site consensus sequence were altered, activated 5′ deleted *TDH3* and *CYC1* promoters. The second DNA element of the UAS is a 7 bp sequence which is conserved in the promoters of several yeast genes encoding glycolytic enzymes and occurs at positions −486 to −480 of the *TDH3* promoter. This DNA sequence represents a novel promoter element: it contains no UAS activity itself, yet potentiates the activity of a GRF1 UAS. The potentiation of the GRF1 UAS by this element occurs when placed upstream from the TATA box of either the *TDH3* or *CYC1* promoters. The characteristics of this element (termed GPE for GRF1 site potentiator element) indicate that it represents a binding site for a different yeast protein which increases the promoter activation mediated by the GRF1 protein. Site-specific deletion and promoter reconstruction experiments suggest that the entire activation potential of the −676 to −381 region of the *TDH3* gene promoter may be accounted for by a combination of the GRF1 site and the GPE.

Key words: *TDH3* promoter – Upstream activation sequence – Potentiator element

Introduction

Promoters in *Saccharomyces cerevisiae* have several characteristic structural elements. The downstream region contains a TATA box the distance of which from the transcription start site varies in different promoters. The TATA box serves as the site of assembly of the components of the transcription initiation complex. Upstream activation sequences (UASs) both enhance the efficiency of initiation by these complexes and mediate their regulation. Yeast UAS elements function in either orientation and at variable distances from the TATA box, although they have not been observed to function when positioned 3′ to the transcription start site. Finally, there appears to be a downstream initiation region (IR) in yeast which fixes the transcription start site (Chen and Struhl 1985; Furter-Graves et al. 1991). This overall structural organization of yeast promoters, with the exception of the variable distance of TATA boxes from the transcription starts, is very similar to the organization of mammalian promoters.

Genes encoding glycolytic enzymes are expressed at very high levels in *S. cerevisiae*. The glycolytic enzymes constitute 25–60% of the cellular protein (Hess et al. 1986) and the specific mRNAs represent major components of the poly(A^+) RNA. The high level expression of glycolytic enzymes in yeast may be due, in large part, to efficient transcription initiation since promoters from these genes can be used for the efficient expression of heterologous genes (reviewed in Bitter et al. 1987). The yeast glycolytic enzyme genes appear to be constitutively expressed at high levels. It has been reported (Maitra and Lobo 1971) that steady-state levels of glycolytic enzymes are induced 3- to 100-fold by shifting from nonfermentable (e.g. glycerol, lactate) to fermentable carbon sources (such as glucose or galactose). This effect, however, is variable and in many strains enzyme synthesis appears to be constitutive (Clifton et al. 1978). A positive regulator of glycolytic enzyme synthesis has been genetically defined (Clifton and Fraenkel 1981); strains containing a *gcr1* mutation have glycolytic enzyme levels which are 5% or less relative to wild type. The gene encoding *GCR1* has been cloned (Baker 1986), and its absence (null mutants) demonstrated to result in a 50-fold decrease in mRNA levels for enolase and glyceraldehyde-3-phosphate dehydrogenase (Holland et al. 1987). These studies suggest that the *GCR1* gene product is,

Offprint requests to: G.A. Bitter

or affects the activity of, a *trans*-acting glycolytic gene transcription factor.

The yeast *RAP1* gene product (Shore et al. 1987) is a protein which binds to the silencer region of the *HML* and *HMR* genes, as well as to UAS regions of the *MATα*, *TEF2* and *RP51* genes. Biochemically characterized proteins, which are also probably encoded by the *RAP1* gene, include TUF which binds to the UAS of some ribosomal genes (Nishizawa et al. 1989; Vignais et al. 1987; Uemura et al. 1986), and GRF1 which binds to promoter UAS regions of the glycolytic enzyme genes *ENO1* and *ENO2* (Buchman et al. 1988a, b). Functionally characterized UAS regions of the *PYK* (Nishizawa et al. 1989) and *PGK* genes (Ogden et al. 1986; Stanway et al. 1987; Chambers et al. 1988, 1989) also include a GRF1 consensus DNA binding sequence (5′-RMACCCANNCAYY-3′; Buchman et al. 1988b), suggesting that GRF1 may be a common activator protein for yeast glycolytic enzyme gene promoters.

Previous studies (Bitter and Egan 1988) demonstrated that the −676 to −381 region of the yeast glyceraldehyde-3-phosphate dehydrogenase gene (*TDH3*) promoter contained sequences which are required for optimal activity. In this report, additional 5′ deletions of the *TDH3* promoter were constructed and their effect on promoter activity quantitated. These studies identify at least two activation regions within the *TDH3* gene promoter, with the majority of the activation potential contained within the −676 to −381 region. A bipartite UAS is characterized within nucleotides −516 to −477 which appears to be the major UAS of the *TDH3* promoter and which employs a novel mechanism for promoter activation.

Materials and methods

Strains, plasmids, culture conditions. Constructed plasmids were cloned in *Escherichia coli* HB101 (Bolivar et al. 1977). *S. cerevisiae* strains YSDP4 (*MATα pep4-3 trp1*; Zsebo et al. 1986), DFY67 (*MATα gcr1-1 leu2 lys1 trp1*; Clifton et al. 1978), DFY112 (*Matα ade6 gcr1-1 trp1*; Clifton and Fraenkel 1981), SE7-6 (*MATα pep4-3 trp1Δ*; constructed by S. Elliott of this laboratory) and CM-1 (*MATα pep4-3 trp1Δ ura3*; derived from SE7-6, S. Elliott, unpublished) were used for preparation of crude proteins and as recipients for plasmid transformations. Yeast strains were transformed by either electroporation (Delorme 1989) or by the LiCl method of Ito et al. (1983). *E. coli* transformants were grown in L broth containing 50 μg/ml ampicillin and selective medium for transformed yeast was SD, CAA (0.67% yeast nitrogen base without amino acids, 2% dextrose, 0.5% casamino acids; supplemented with uracil for strain CM-1). Plasmids YRp7ΔCEN3, pBR(UAS_G) and pGPD(s)-2 have been described (Bitter and Egan 1988). Plasmid pRBΔ20B was described previously (West et al. 1984). Vector constructions were performed using either plasmid pBR322 (Bolivar et al. 1977), coliphage M13mp19 (Norrander et al. 1983) or plasmid pUC119 (Vieira and Messing 1987) for subcloning procedures.

Vector constructions. Recombinant DNA manipulations utilized standard techniques (Maniatis et al. 1982). Oligodeoxyribonucleotides were synthesized on an Applied Biosystems Model 380B synthesizer. Restriction endonuclease recognition sequences for *Sal*I were engineered into the *TDH3* gene promoter as follows. The 1.6 kb *Sal*I-*Hin*dIII fragment extending from −381 in the *TDH3* promoter to the *Hin*dIII site in the *TRP1* gene was subcloned from pGPD(s)-2 into pUC119 to generate PUC119/HS-5. Site-directed mutagenesis was performed according to the method of Kunkel (1985). The oligonucleotide 5′-CAAGGCAATTGGTCGACGCATGTATC-3′ was utilized to introduce a *Sal*I site at position −264 of the *TDH3* promoter and generate pUC119/HS-5-1. Subsequently, the oligonucleotide 5′-TTTAAACCAGTCGACTGAAATTATT-3′ was used to introduce a *Sal*I site at position −171 of the *TDH3* promoter and generate pUC119/HS-5-1-1. Expression vector pGPD(s) was constructed by cloning the 1.95 kb *Hin*dIII fragment from pGPD(s)-2 containing the GPD (glyceraldehyde-3-phosphate dehydrogenase) promoter and PGK (phosphoglycerate kinase) terminator into the 6 kb *Hin*dIII fragment of YRp7ΔCEN3. The region of the GPD promoter from −676 to −381 was deleted to generate pGP381 by digesting pGPD(s) to completion with *Sal*I and partially with *Hin*dIII and religation with the synthetic linker

```
5'-AGCTTGGTACCGCCCGGG-3'
    3'-ACCATGGCGGGCCCAGCT-5'
```

The promoter region of pGP381 was replaced with the 240 bp *Sal*I-*Bam*HI fragment of pUC119/HS-5-1 to generate pGP264 or the 147 bp *Sal*I-*Bam*HI fragment of pUC119/HS-5-1-1 to generate pGP171. The nucleotide sequence of each promoter deletion was confirmed by dideoxy chain termination sequencing. The *E. coli lacZ* gene was isolated from pRBΔ20B as a *Bam*HI-*Eco*RI fragment. The 3′ end of the gene was reconstructed using a synthetic linker which rebuilt the codons of the *lacZ* gene from the *Eco*RI site to the termination codon followed by a *Bam*HI cohesive end and the 5′ end rebuilt using the synthetic linker

```
5'-GATCCAATAAACAAAATGGTTAGTCCAC-3'
    3'-GTTATTTGTTTTACCAATCAGGTGCTAG-5'.
```

After confirming the DNA sequence at the 5′ end, the *Lac*Z gene was cloned as a *Bam*HI fragment into each of the promoter vectors (above) to generate pGPD(s)/Z, pGP381/Z, pGP264/Z and pGP171/Z.

Assays of β-galactosidase in permeabilized yeast. Transformed yeast strains were grown in the appropriate selective medium and harvested at an OD_{600} of 0.5–1.5. Cells were collected by centrifugation and β-galactosidase activity quantitated on permeabilized cells as described previously (Guarente 1983). The increase in absorbance at 420 nm of the samples was monitored using the Kinetics II program on a Beckman DU-8 or DU-65 spectrophotometer. The *o*-nitrophenyl-β-D-galactoside hydrolysis rates were calculated by linear regression analysis of the absorbance versus time data. One unit is as de-

fined previously (Miller 1972). All data presented in Results represent the average of at least two independent experiments in which the correlation coefficient for the linear regression analysis was 0.9 or greater.

Preparation of yeast whole cell proteins. *S. cerevisiae* YSDP4, SE7-6, DFY67 or DFY112 were grown in 500 ml of YPD medium (1% yeast extract, 2% bactopeptone, 2% dextrose) to an OD_{600} of 1–2. Cells were harvested by centrifugation and washed once with TGE (100 mM TRIS-HCl pH 7.9, 10% glycerol, 1 mM EDTA) and subsequently maintained at 4° C. Cells were resuspended in 5 ml TGE containing 0.4 M $(NH_4)_2SO_4$, 5 mM dithiothreitol (DTT), 1 mM phenylmethylsulfonyl fluoride (PMSF), and 1 µg/ml each of aprotinin, pepstatin, and leupeptin. Cells were lysed by vortexing with glass beads and the whole cell extract (F1) centrifuged at $10000 \times g$ for 20 min. The supernatant (F2) was collected and solid $(NH_4)_2SO_4$ added to 40% saturation. The suspension was stirred for 30 min after all $(NH_4)_2SO_4$ was dissolved and then centrifuged at $18000 \times g$ for 30 min. The pellet was resuspended in 2 ml of TGE containing 0.1 M KCl, 1 mM DTT, 1 mM PMSF and 1 µg/ml each of aprotinin, pepstatin and leupeptin and dialyzed against 200–300 volumes of the same buffer for 15 h. The protein concentration in this fraction (F3) was determined by the method of Bradford (1976). The F3 was quick frozen in small aliquots and stored at −70° C.

Protein-DNA binding assays. The F3 proteins were incubated, unless otherwise indicated, at 30° C for 20 min with specific DNA probes (labeled by end-filling with α-[^{32}P] deoxynucleotide triphosphates using Klenow fragment) or synthetic oligonucleotides (end-labeled with T4 polynucleotide kinase) in 20 µl of 50 mM TRIS-HCl pH 7.9, 50 mM KCl, 5 mM $MgCl_2$, 0.5 mM EDTA. The specific probe utilized, the mass of F3 proteins and the non-specific carrier DNA used in each experiment are detailed in the figure legends. One-tenth volume of 0.5 mg/ml bromphenol blue was added at the end of the incubation and the samples electrophoresed in 5% (8% for the experiment in Fig. 5) polyacrylamide gels (acrylamide:bisacrylamide, 30:0.8 in 0.5× TBE buffer) at less than 25 mA. The gels were dried and exposed to Kodak XAR film at −70° C using intensifier screens. DNase I footprint conditions are detailed in the figure legend.

Results

Activity of TDH3 *promoter 3′ deletions*

The yeast *TDH3* promoter element utilized previously (Bitter and Egan 1984) spans nucleotides −676 to −24 upstream of the *TDH3* gene coding region. This segment includes the transcription initiation start site with the TATA box occupying nucleotides −141 to −134. A *SalI* restriction site was engineered at position −381 to generate the GPD(s) promoter (Bitter and Egan 1988). For the present studies, this promoter was incorporated into vectors to express the *E. coli lacZ* gene. These vectors utilize the yeast *PGK* gene 3′ region for transcription termination and the yeast *TRP1/ARS1* 1.4 kb fragment for selection and replication in yeast. The vector pGPD(s)/Z (Fig. 1 A) also includes the yeast *CEN3* sequence which maintains the plasmid at a constant 1–2 copies per cell (Tschumper and Carbon 1983). These studies utilized exclusively centromere-containing plasmids so that promoter activities measured are not complicated by the wide fluctuations in plasmid copy number observed with 2 µm based plasmids (Bitter et al. 1987). Deletions of the *TDH3* promoter were con-

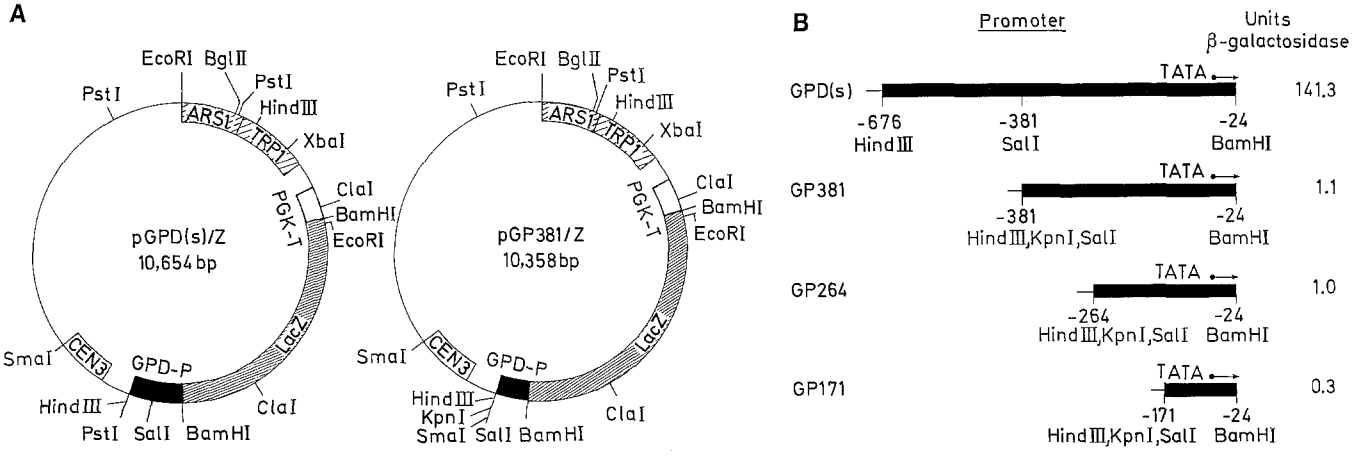

Fig. 1. A Restriction endonuclease map of plasmids pGPD(s)/Z and pGP381/Z. The structure of plasmids pGP264/Z and pGP171/Z are identical to pGP381/Z with the exception of the DNA sequences included within the promoter segment. **B** Structure of native GPD(s) and 5′ deleted GPD (glyceraldehyde-3-phosphate dehydrogenase) promoters. Nucleotide positions from the native GPD promoter (Bitter and Egan 1984) are indicated. The GPD(s) promoter has a 21 bp linker containing a *SalI* site inserted at position −381 (Bitter and Egan 1988); nucleotide positions represent those of the native promoter (lacking the *SalI* site) relative to the ATG translation initiation codon. The most 5′ nucleotide of each of the promoter deletions is indicated. The units of β-galactosidase produced in yeast strain SE7-6 from each promoter when incorporated into the vectors depicted in **A** was quantitated. The TATA box occupies nucleotides −141 to −134 of the promoter

structed (Materials and methods) with constant 3′ end points at −24 but variable 5′ deletion end points (Fig. 1B). The promoter of pGPD(s)/Z was replaced with these 5′ deletion promoters to generate vectors pGP381/Z (Fig. 1A), pGP264/Z and pGP171/Z. These vectors have identical structures with the exception of the DNA sequences included in the promoter segment.

The activity of the native and 5′ deleted *TDH3* promoters, as measured by β-galactosidase activity, is presented in Fig. 1B. Deletion of sequences between −676 and −381 (GP381 promoter) decreased the activity over 100-fold. Deletion of DNA sequences from −381 to −264 (GP264 promoter) did not further reduce promoter activity, while subsequent deletion of sequences from −264 to −171 (GP171 promoter) resulted in a further 3-fold decrease in promoter activity. By this sequential deletion analysis, therefore, it appears that the *TDH3* gene promoter contains at least two activation domains, with the majority of the activity contained within the upstream 295 bp.

Identification of a protein which binds to the TDH3 *upstream activation region*

Crude, whole cell extracts (F3) were prepared and tested for specific binding to the GPD(s) promoter fragment by gel mobility shift assays. When the entire promoter (−676 to −24) was incubated with the F3, one major protein:DNA complex was observed (Fig. 2A). This complex represents a specific interaction between a protein(s) in the F3 and the promoter since formation of the complex was inhibited by SDS/heat treatment of the extract (Fig. 2A, lane 5) or inclusion of excess unlabeled promoter fragment in the binding reaction (Fig. 2A, lanes 7–9). The −381 to −24 promoter fragment did not function as a competitor for formation of this complex (Fig. 2B). Similarly, the promoter fragments −264 to −24 and −171 to −24 did not compete for complex formation when included in equivalent molar excess (data not shown). These results indicate that, under these conditions, the major protein:DNA complex formed on the promoter represents binding of a protein to the region between −676 and −381. This was corroborated by a gel mobility shift assay using the −676 to −381 region as a probe (Fig. 2C). A single complex of decreased mobility was observed whose formation was completely inhibited by the presence of unlabeled GPD(s) promoter DNA.

The glyceraldehyde-3-phosphate dehydrogenase mRNA levels in yeast strains containing the *gcr1-1* mutation are reduced more than 50-fold from wild type (Holland et al. 1987). This reduction in mRNA abun-

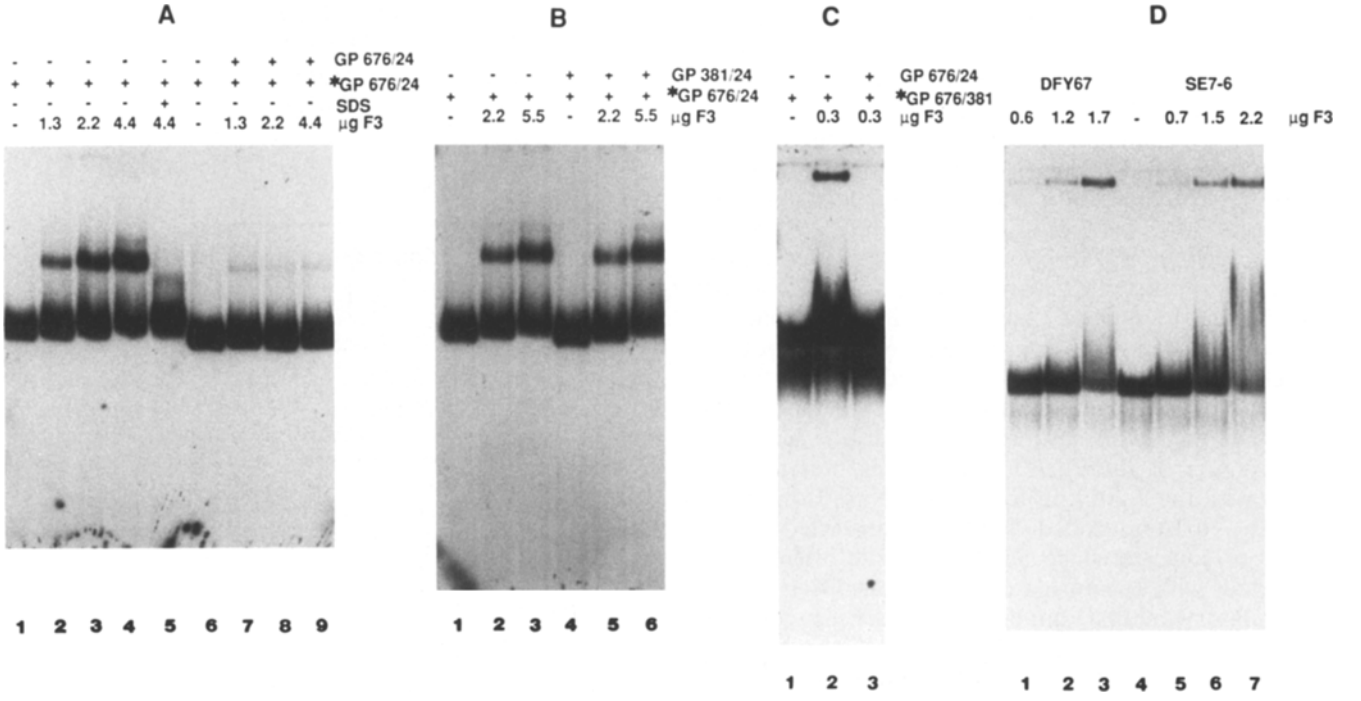

Fig. 2A–D. Gel mobility shift assays of GPD(s) promoter fragments. **A** All reactions contained 2 µg sonicated salmon sperm DNA and 0.3 ng labeled GPD(s) promoter fragment (nucleotides −676 to −24) and the indicated mass of F3 proteins from strain YSDP4. In addition, reaction 7 contained 100 ng and reactions 8 and 9 contained 300 ng of unlabeled GPD(s) promoter fragment. Reaction 5 was made 0.5% with SDS and heated to 58° C for 5 min after the binding reaction. **B** All reactions contained 4 µg sonicated salmon sperm DNA and 0.2 ng labeled GPD(s) promoter fragment and the indicated mass of F3 proteins from strain YSDP4. Reactions 5,6 also contained 25 ng of unlabeled GP381 promoter fragment (nucleotides −381 to −24). **C** The *Hin*dIII-*Sal*I fragment of the GPD(s) promoter (encompassing nucleotides −676 to −381) was used as probe (5 ng) and all reactions contained 2 µg of salmon sperm DNA. Reactions 2,3 contained 0.3 µg of F3 proteins from strain SE7-6. In addition, reaction 3 contained 450 ng of unlabeled GPD(s) promoter (nucleotides −676 to −24). **D** Binding reactions utilized 5 ng of the *Hin*dIII-*Sal*I (−676 to −381) probe, 2 µg salmon sperm DNA and contained the indicated mass of F3 proteins from either strain DFY67 (lanes 1–3) or strain SE7-6 (lanes 5–7)

dance is comparable to the reduction in promoter activity observed when nucleotides −676 to −381 were removed from the *TDH3* promoter (Fig. 1B). Accordingly, we determined whether the protein identified above, which binds to this region of the promoter, is present in strains carrying the *gcr1-1* mutant allele (Fig. 2D). Binding reactions were performed on the −676 to −381 probe using a titration of F3 proteins from wild type (SE7-6) and *gcr1-1* mutant (DFY67) cells. The amount of probe converted to complex at any given concentration of proteins is approximately the same for the F3 prepared from each strain. Similar results were observed for F3 proteins from another *gcr1-1* mutant strain (DFY112; data not shown). It should be noted, furthermore, that the migration of the protein:DNA complex is indistinguishable in the three strains. The *gcr1-1* mutant allele contains an in-frame termination codon which reduces the size of the gene product from 844 amino acids to 315 amino acids (Holland et al. 1987). It appears, therefore, that the protein detected in these binding assays is not the *GCR1* gene product, nor a protein whose binding activity is controlled by the *GCR1* gene product.

The protein which binds to the upstream activation region is GRF1

The −676 to −381 promoter fragment was uniquely labeled at the *Hin*dIII site (Fig. 1A) and incubated with F3 proteins under various conditions, followed by digestion with DNase I (Fig. 3A). Compared to the digestion pattern of free DNA (Fig. 3A, lane 1), all reactions in which the DNA was complexed with F3 proteins showed a region of reduced DNase I cleavage efficiency. Essentially complete protection was observed after binding reactions at 30° C for 3 min (Fig. 3A, lane 3) or 25 min (Fig. 3A, lane 4) prior to DNase I digestion. Partial protection was observed after only a 15 s binding reaction at 4° C prior to DNase I digestion (Fig. 3A, lane 2). While the borders of the protected region cannot be precisely determined with probe labeled on this strand, the binding site is clearly within 175–205 nucleotides from the 5′ end of the probe. The nucleotide sequence of this region of the *TDH3* promoter is presented in Fig. 3B with the region protected by DNase I digestion (−520 to −489) bracketed. Within the protected region is a 13 bp sequence which, in the opposite orientation, corresponds with one mismatch to the yeast GRF1 (general regulatory factor 1) binding site consensus sequence presented by Buchman et al. (1988b).

The GRF1 consensus sequence includes the sequence previously identified as the binding site of the RAP1 protein (Shore et al. 1987; Shore and Nasmyth 1987). Gel mobility shift assays utilizing the *TDH3* GRF1 site were therefore performed using oligonucleotide competitors in order to determine whether the protein we have detected is RAP1 (Fig. 4). Inclusion of unlabeled *TDH3* GRFI oligonucleotide prevented complex formation (lane 4) whereas two unrelated oligonucleotides did not compete at equivalent molar excess (lanes 8, 10). An oligonucleotide containing the RAP1 protein binding

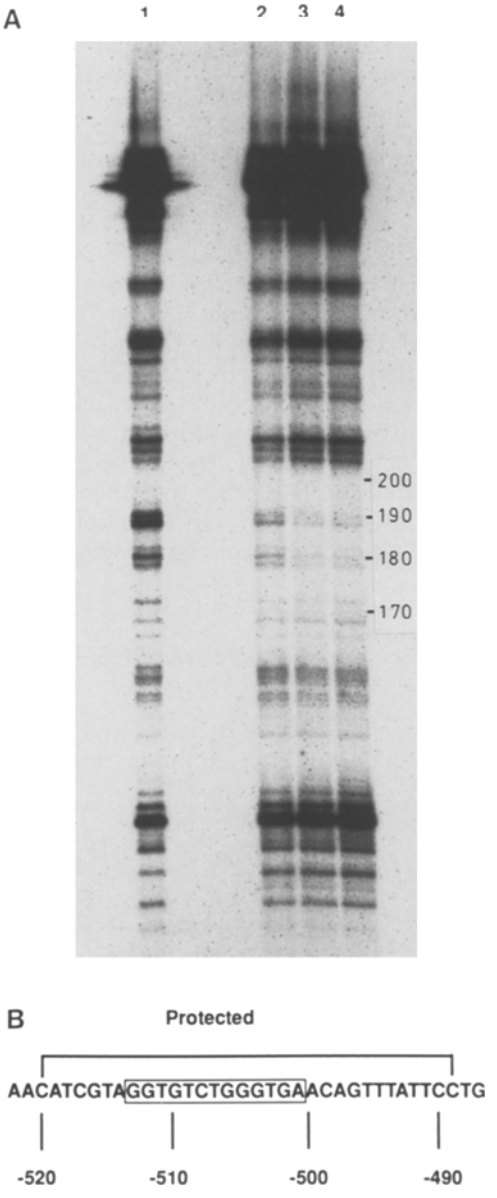

Fig. 3A, B. DNase I footprint of GP−676/−381 protein binding site. **A** The *Hin*dIII-*Sal*I fragment (−676 to −381) of the GPD(s) promoter was labeled at the *Hin*dIII site, incubated with F3 proteins from strain SE7-6, and digested with DNase I. Lane 1 contains protein-free DNA probe which was purified by polyacrylamide gel electrophoresis. Protein binding reactions for lanes 2, 3 and 4 included 2 μg of salmon sperm DNA, 2 ng of probe DNA and 79 μg F3 proteins. Reactions were allowed to proceed for 10 s at 4° C (lane 2), 3 min at 30° C (lane 3) or 25 min at 30° C (lane 4), followed by a 5 min incubation at 4° C with 2 μg of DNase I. Samples were phenol extracted, denatured and electrophoresed in a 6% polyacrylamide gel in TBE buffer containing 8 M urea. An unrelated DNA sequence ladder was run in adjacent lanes for size markers. **B** The DNA sequence of the GPD promoter protected from DNase I digestion in Fig. 4 is depicted with the homology to the GRF1 consensus sequence enclosed in a *box*. Recent DNA sequence analysis of the *TDH3* gene promoter revealed several sequence differences in this region of the promoter from that previously published (5′-AACACTGATGGTGCTTGGGTGAACAGGTTTATTCCTG-3′; Bitter and Egan 1984). The DNA sequence depicted is that recently determined on the promoter used in these studies

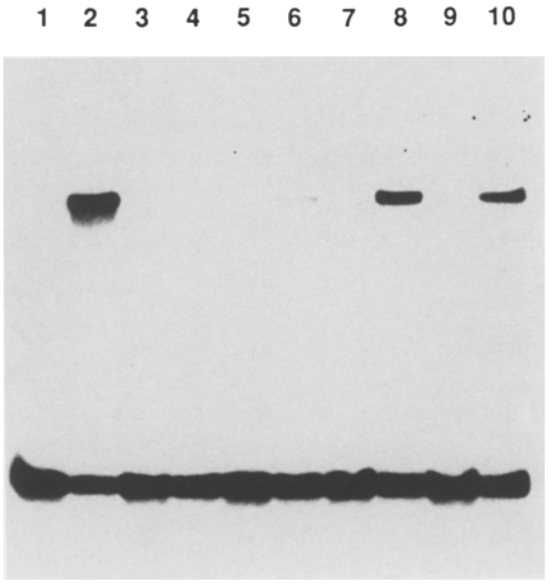

Fig. 4. Gel mobility shift assays in the presence of competitor oligonucleotides. The oligonucleotide GRF1 (5′AGAATATATAACATCGTAGGTGTCTGGGTGAACAGTTTATTCCTGGCA-3′) was end-labeled with T4 polynucleotide kinase. Approximately 1 fmole of probe and 4 μg salmon sperm DNA were incubated in the absence (lanes 1, 3, 5, 7, 9) or presence (lanes 2, 4, 6, 8, 10) of 14 μg F3 proteins from strain SE7-6. In addition, 1000 fmole of the following unlabeled duplex oligonucleotides were included in certain reactions: GRF1 oligonucleotide, lanes 3, 4; HMR oligonucleotide (5′-CTTGCAAAAACCCATCAACCTTGAAAAAAAGTAGAC-3′), lanes 5, 6; CK-1 oligonucleotide (5′-CGATCAGAGATTCCACAATTTCTCGAGGATCAGAGATTCCACAATTTG-3′), lanes 7, 8; SRE oligonucleotide (5′-CGATGTCCATATTAGGACAG-3′), lanes 9, 10

site from the *HMR* silencer region also effectively competed for protein binding to the *TDH3* GRF1 site (lane 6). This result suggests that the protein factor which binds to the *TDH3* GRF1 site is encoded by the *RAP1* gene. At equivalent molar excess, the *TDH3* oligonucleotide competes more effectively (lane 4) than the HMR oligonucleotide (lane 6), suggesting that the protein has a higher affinity for the binding site in the *TDH3* gene promoter.

UAS activity of GRF1 binding site DNA sequences

It has been demonstrated previously that DNA sequences present in the 5′ flanking region of a number of yeast genes which match the GRF1 consensus provide UAS function to heterologous yeast test promoters (Buchman et al. 1988b). The −676 to −381 region of the *TDH3* promoter activated the region downstream of −381 approximately 100-fold (Fig. 1B). Since this region contains a GRF1 site consensus sequence homology to which specific protein binding could be demonstrated, it seemed likely that this element provided a UAS function to the *TDH3* promoter. Several oligonucleotides representing DNA sequences within the region protected from DNase I digestion were chemically synthesized and cloned into pGP381/Z (Fig. 5). An element (GRF1-n), which duplicates nucleotides −516 to −497 of the native promoter and includes the entire sequence which differs by only one mismatch from the GRF1 consensus, activated the GP381 promoter 8.4-fold. An analogue of this sequence (GRF1) in which two of the degenerate nucleotides of the GRF1 consensus were altered, also activated the GP381 promoter (5.4-fold). In contrast to the first two elements, an element which included only 8 of the 13 nucleotides of the GRF1 consensus (GRF1/2) did not activate the GP381 promoter deletion. These results indicate that the sequence responsible for protein binding and promoter activation of this region of the *TDH3* promoter is the element that differs by one mismatch from the GRF1 consensus binding site.

Each of these three DNA elements was also tested for UAS activity on a heterologous yeast promoter. A 5′ deleted *CYC1* gene promoter was chemically synthesized and cloned as a *Sal*I-*Bam*HI fragment to construct pCY247/Z. This vector has a structure identical to pGP381/Z (Fig. 1A) with the exception that the promoter region between the *Sal*I and *Bam*HI sites consists of nucleotides −247 to −4 of the yeast *CYC1* gene promoter (McNeil and Smith 1986). The response of this promoter deletion (CY247) to each of the three sequence elements was qualitatively similar to the GP381 promoter deletion (Fig. 5). The magnitude of activation by the GRF1-n and GRF1 elements was two- to threefold greater on the CY247 promoter than observed with the GP381 promoter.

Potentiation of the GRF1 UAS by a flanking DNA sequence element

Computer-assisted analysis of the 5′ flanking regions of yeast glycolytic enzyme gene promoters (Table 1; Discussion) revealed a conserved DNA sequence with the consensus 5′-GMWTCCW-3′. This consensus sequence appears in the *TDH3* gene promoter at −486 to −480. A GRF1 site alone activates the GP381 promoter only 5- to 8-fold (Fig. 5), while the native promoter is approximately 100-fold more active than the GP381 promoter deletion (Fig. 1B). Therefore, the role of the conserved sequence at −486 to −480 of the *TDH3* promoter was further investigated.

A synthetic DNA element which duplicates nucleotides −489 to −477 did not activate when installed in either orientation upstream of the GP381 promoter (Fig. 5). This DNA element was similarly inert when positioned upstream of the CY247 promoter. Therefore, this conserved DNA sequence element is not a UAS. However, when the element was placed between a GRF1 site and the GP381 promoter, the UAS activity of the combined element was 3.5-fold greater than the GRF1 site alone. This potentiation of the GRF1 UAS also occurred with the conserved region (termed GPE for *G*RF1 site *p*otentiator *e*lement) in the opposite orientation relative to the GRF1 site. The effect of the GPE was also tested when positioned upstream of the GRF1 UAS. Potentiation of the GRF1 UAS was observed with the GPE in either orientation. The GRF1 and GPE sites

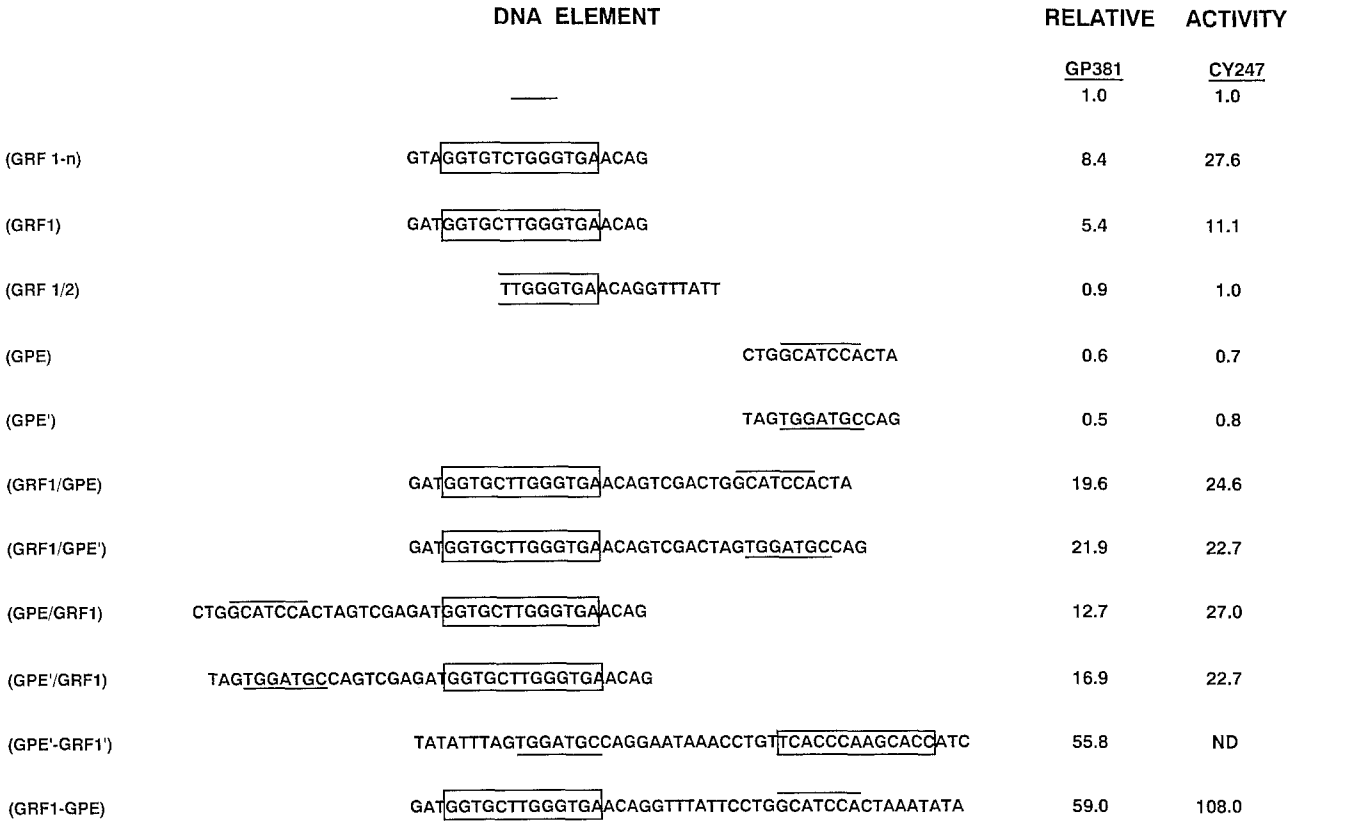

Fig. 5. Effects of cloned synthetic DNA sequence elements on 5′ promoter deletions. The indicated DNA sequence elements were synthesized and assembled between the *Kpn*I and *Sal*I sites of pGP381/Z (Fig. 1A) or pCY247/Z; nucleotides constituting the restriction site cohesive ends which were included in the oligonucleotide are not depicted. Promoter activity in strain SE7-6 (GP381 promoter and derivatives) or strain CM-1 (CY247 promoter and derivatives) was quantitated by β-galactosidase assays. Promoter activity is expressed as activity relative to that obtained with the parent pGP381/Z (1.9 units) or pCY247/Z (1.2 units) vectors. Nucleotides of each element corresponding to the GRF1 binding site homology are enclosed in a *box*. The homology to the GRF1 consensus binding site is enclosed in a *box* and the GPE is *overlined* in the native orientation and *underlined* in the opposite orientation

Table 1. Association of GPE homologies with GRF1 binding sites in yeast glycolytic enzyme gene promoters

Gene			
GRF1 consensus		5'-MACCCANNCAY-3' 3'-KTGGGTNNGTR-5'	
GPE consensus			5'-GMWTCCW-3' 3'-CKWAGGW-5'
ADH1	5'-AACC	AACCCATACAT	GGGATTCCTATAA-3'
ENO1	5'-CCGAGCTTCCACTAGGATA	CACCCAAACAC	CTGCA-3'
PGK	5'-GAAAA	AACCCAGACGC	TCGACTTCCTGTCTTCCTATTGATTGCAGCTTCCAATTT-3'
PYK	5'-ATGT	CACCCAGACAT	GGGCTTCCATAAT-3'
TDH3	5'-CGTAG	GTGTCTGGGTG	AACAGTTTATTCCTGGCATCCACTAA-3'

DNA sequences from the *ADH1* (−668 to −639; Santangelo and Tornow 1990), *ENO1* (−447 to −412; Buchman et al. 1988a), *PGK* (−479 to −423; Chambers et al. 1989), *PYK* (−659 to −630; Nishizawa et al. 1989) and *TDH3* (−516 to −475; this work) gene promoters are depicted. The GRF1 consensus sequence derived by Buchman et al. (1988b) was modified to be consistent with the data in this paper. The GPE consensus was derived by computer-assisted homology searches of yeast glycolytic enzyme gene promoters. Homologies to the GRF1 and GPE consensus sequences are indicated by overlining. IUB codes are K=G or T; M=A or C; N=A or G or C or T; R=A or G; W=A or T; Y=C or T

are separated by 14 bp in the native promoter whereas they are separated by 10, 11 or 12 bp, and the sequence between the two elements is altered, in these constructions. When the GPE was present in the native orientation 15 bp from a GRF1 site, the element was over 10-fold more active as a UAS than the GRF1 site alone (56-fold activation of the GP381 promoter). This combination of the elements was equally effective as a UAS in the opposite orientation relative to the GP381 promoter.

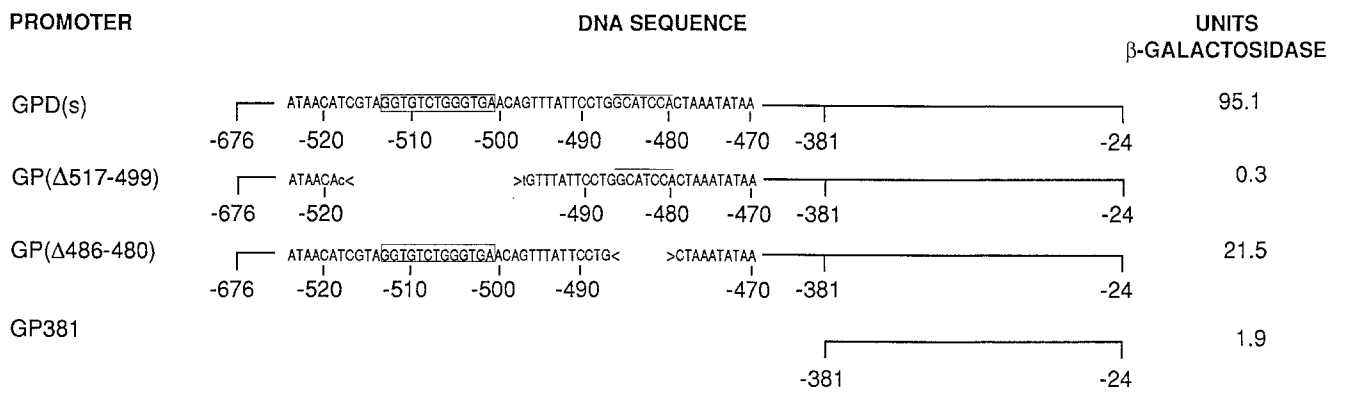

Fig. 6. Effect of specific deletions within the native GPD(s) promoter. The indicated deletions were introduced into the native promoter to generate pGP(Δ517-499) or pGP(Δ486-480). The units of β-galactosidase produced in strain SE7-6 by pGPD(s)/Z, pGP381/Z or the two deletion mutants was quantitated. The homology to the GRF1 consensus binding site is enclosed in a *box* and the GPE sequence is *overlined*. In four separate experiments, the β-galactosidase levels produced by pGP(Δ517-499) ranged from 3- to 10-fold lower than that produced by pGP381/Z

Each of the combinations of the GRF1 and GPE was also tested on the 5′ deleted *CYC1* gene promoter (Fig. 5). The results demonstrate that the GPE potentiates the GRF1 UAS approximately twofold when placed upstream of the CY247 TATA box. Furthermore, this potentiation occurred on either side of, and in either orientation relative to, the GRF1 UAS.

It was also determined whether the GPE would potentiate a different yeast UAS. The α-specific UAS from the yeast *STE3* gene (α1 UAS; Jarvis et al. 1988) activated the GP381 promoter 23.7-fold and the CY247 promoter 4.3-fold. When the GPE was cloned between the α1 UAS and the TATA box of either promoter, no potentiation was observed (data not shown). These results suggest that potentiation by the GPE is specific for UAS elements which bind the GRF1 protein.

Contribution of GRF1 and GPE elements to total activation potential of the upstream region

Deletion of the −676 to −381 region of the *TDH3* promoter decreases promoter activity approximately 100-fold. A synthetic DNA element containing both a GRF1 site and the GPE activates the GP381 promoter over 50-fold indicating that a combination of these two elements may constitute the major, if not sole, determinant of activation in this region. To test for the presence of additional activation domains within the upstream region, the GRF1 and GPE sites were individually deleted from the native promoter, and the activity of these promoter derivatives quantitated relative to the native and GP381 promoters.

Deletion of the GPE (nucleotides −486 to −480) decreased the promoter activity fourfold (Fig. 6). The resultant promoter deletion was tenfold more active than the GP381 promoter deletion, demonstrating that the activation potential of the upstream 295 bp was only partially disabled by deletion of the GPE. When the GRF1 site was deleted from the native promoter, the resultant promoter had an activity which was lower than that of the GP381 promoter deletion (Fig. 6). These results are consistent with the potentiation of the GRF1 UAS by the GPE determined with synthetic DNA elements (Fig. 5). The results further demonstrate that the UAS activity of the −676 to −381 region is dependent upon GRF1 protein binding.

Discussion

In the present study, a UAS within the −676 to −381 region of the *TDH3* gene promoter was characterized. Specific protein binding was demonstrated to occur within nucleotides −510 to −489, a region which includes a homology to the GRF1 protein binding site consensus sequence. Oligonucleotide competitor experiments suggest that the protein which binds to this site is RAP1. This GRF1 homology activated 5′ deleted *TDH3* and *CYC1* gene promoters.

The essential component of the UAS described in this study is the GRF1 protein binding site. We also characterized a second DNA element of the *TDH3* gene promoter which we have termed GPE (for *G*RF1 site *p*otentiator *e*lement). Site-specific deletion and promoter reconstruction experiments demonstrate that the GPE contributes to promoter efficiency. This element functioned in either orientation with respect to, and on either side of, the GRF1 site. Thus, it does not appear merely to make the GRF1 sequence a more efficient protein binding site. The position and orientation independence of the GPE indicate that it is a binding site for another protein. Our results further demonstrate that this element does not directly activate the promoter (it is not a UAS), but potentiates the activation mediated by the GRF1 protein. The GPE appears to potentiate the GRF1 UAS independently of the TATA element (e.g. either *TDH3* or *CYC1*) but does not appear to potentiate other yeast UASs (e.g. *STE3* α1 UAS).

DNA sequence elements similar to the GPE (Table 1) have been noted previously in other yeast glycolytic enzyme gene promoters. The sequence 5′-CTTCC-3′ exists

at three positions downstream from the *PGK* promoter GRF1 site, and deletion of these sites decreases the promoter activity (Chambers et al. 1988). One of the sites matches the GPE consensus we developed (Table 1). A similar sequence near the GRF1 binding sites of the *ENO1* and *PYK* gene promoters was noted by Buchman et al. (1988b). Mutation of this sequence decreased the in vivo UAS activity of the DNA without affecting the in vitro binding affinity of GRF1. Thus, the *PKG*, *ENO1* and *PYK* gene promoters all have sequences which match the GPE consensus and contribute to promoter activity. For these promoters, however, it has not been determined whether the noted sequence elements are themselves UASs or contribute to promoter activity via other mechanisms. The proximity of these elements to known GRF1 UASs suggest that these elements are GPEs within each respective promoter. A sequence which matches the GPE consensus exists near a GRF1 consensus binding site sequence in the *ADH1* promoter (Table 1), but the functional significance of this sequence has not been determined.

It is becoming increasingly apparent that eukaryotic promoter enhancers can act synergistically. This is also true for yeast UASs. Thus, DNA sequences recognized by the *ARS*-binding protein I and the *DED1* UAS are each weak activators of a 5′ deleted promoter. When both sequence elements are present, however, they act synergistically to activate the promoter to a level greater than expected for the sum of each individual activator sequence (Buchman and Kornberg 1990). Similarly, the GRF2 binding site is a weak UAS but also acts synergistically with the *DED1* UAS (Chasman et al. 1990). The *TDH3* UAS described in this work is mechanistically distinct from two weak UASs acting synergistically. The GRF1 binding site is a UAS (5- to 8-fold activation), while the GPE is not itself a UAS. However, the two elements function synergistically to yield an activation greater than that observed with the GRF1 site alone. Recent studies (Lin et al. 1990; Carey et al. 1990) indicate that eukaryotic transcriptional activators act synergistically not by directly interacting, but by individually contacting some component(s) of the transcription initiation apparatus. The GPE is a transcription potentiator rather than a transcription activator. Although it is capable of potentiating the GRF1 UAS, it remains to be determined whether this is mediated through direct interactions with the GRF1 protein or with other components of the transcription apparatus.

There are examples of eukaryotic enhancers which require two different proteins for activation. The mammalian transcription factor AP-1 is a heterotypic dimer of c-jun and c-fos (reviewed in Karin 1990). While the c-jun protein alone is capable of activating promoters, it does so as a homotypic dimer and recognizes the same DNA sequence element as the heterotypic dimer. This is mechanistically different from the *TDH3* UAS which is composed of two distinct and separable DNA elements. The α-specific genes in yeast (reviewed in Herskowitz 1989) contain a UAS which requires two proteins, MCM1 and α1, for activation. However, for these promoters, both proteins are required for activation. In contrast, the GRF1 binding site alone functions as a UAS. To our knowledge, the GPE described in this study represents the first example of a eukaryotic promoter element which is not itself a transcription activator, but a potentiator of the activation mediated by a separate enhancer element.

The present study demonstrates that the GRF1 site, in combination with the GPE, can account for the majority, if not all, of the activation potential of the −676 to −381 region of the *TDH3* gene promoter. Several previous studies (Baker 1986; Holland et al. 1987) demonstrated that mutation of the *GCR1* gene drastically decreases the activity of yeast glycolytic gene promoters, including *TDH3*, suggesting that the *GCR1* gene product is, or controls the activity of, a positive activator of glycolytic gene promoters. The abundance of the GRF1 protein which binds to the *TDH3* promoter was not altered in *gcr1-1* mutants. Thus, it is unlikely that the target of *GCR1* regulation is the GRF1 protein(s). It is possible that the *GCR1* gene product exerts its positive regulation of the *TDH3* promoter through the GPE, although site-specific deletion of this element resulted in only a fourfold decrease in promoter activity. The *GCR1* gene product may be a positive activator of another, as yet uncharacterized, *TDH3* promoter UAS. Alternatively, the positive regulation of yeast glycolytic enzyme genes by the *GCR1* gene product may be effected by antagonizing a silencer function (Holland et al. 1990; Brindle et al. 1990). Consistent with this second possibility is the observation (Pavlovic and Hörz 1988) that the *GCR1* gene product maintains the region of the *TDH3* promoter downstream from −370 in an open chromatin conformation, whereas two nucleosome core particles are positioned in this region in *gcr1* deletion strains. It will be of interest to determine the molecular mechanism of coordinate regulation of yeast glycolytic enzyme genes by the *GCR1* gene product.

Acknowledgements. We are grateful to Dr. K. Dane Wittrup for helpful discussions and for pointing out the GPE homology within glycolytic gene promoters, Dr. Norman Davidson for discussions on protein:DNA interactions and to Dr. Arnold J. Berk for comments on the manuscript. We also thank Cathleen Sugarman for technical assistance during the early stages of this work, Drs. D. Fraenkel and S. Elliott for yeast strains, Charles Herrera and Holly Erjavec for DNA synthesis; Jessica Katzowitz and Arlette Harcourt for DNA sequencing, and Joan Bennett for preparation of the manuscript.

References

Baker HV (1986) Glycolytic gene expression in *Saccharomyces cerevisiae*: nucleotide sequencing of GCR1, null mutants and evidence for expression. Mol Cell Biol 6:3774–3784

Bitter GA, Egan KM (1984) Expression of heterologous genes in *Saccharomyces cerevisiae* from vectors utilizing the glyceraldehyde-3-phosphate dehydrogenase gene promoter. Gene 32:263–274

Bitter GA, Egan KM (1988) Expression of heterologous genes in *S. cerevisiae* from vectors utilizing the glyceraldehyde-3-phosphate dehydrogenase gene promoter. Gene 69:193–207

Bitter GA, Egan KM, Koski RA, Jones MO, Elliott SG, Giffin JC (1987) Expression and secretion vectors for yeast. Methods Enzymol 153:516–544

Bolivar F, Rodriguez RL, Greene PJ, Betlach MC, Heyneker HC, Boyer HW, Crosa JH, Falkow S (1977) Construction and characterization of new cloning vehicles II. A multipurpose cloning system. Gene 2:95–113

Boyer HW, Crosa JH, Falkow S (1977) Construction and characterization of new cloning vehicles II. A multipurpose cloning system. Gene 2:95–113

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of dye-ligand binding. Anal Biochem 72:248–254

Brindle PK, Holland JP, Willett CE, Innis MA, Holland MJ (1990) Multiple factors bind the upstream activation sites of the yeast enolase genes *ENO1* and *ENO2*: ABFI protein, like repressor activator protein *RAP1*, binds *cis*-acting sequences which modulate repression or activation of transcription. Mol Cell Biol 10:4872–4885

Buchman AR, Kornberg RD (1990) A yeast ARS-binding protein activates transcription synergistically in combination with other weak activating factors. Mol Cell Biol 10:887–897

Buchman AR, Kinnerly WJ, Rine J, Kornberg RD (1988a) Two DNA-binding factors recognize specific sequences at silencers, upstream activating sequences, autonomously replicating sequences, and telomeres in *Saccharomyces cerevisiae*. Mol Cell Biol 8:210–225

Buchman AR, Lue NF, Kornberg RD (1988b) Connections between transcriptional activators, silencers and telomeres as revealed by functional analysis of a yeast DNA-binding protein. Mol Cell Biol 8:5086–5079

Carey M, Lin Y-S, Green MR, Ptashne M (1990) A mechanism for synergistic activation of a mammalian gene by *GAL4* derivatives. Nature 345:361–364

Chambers A, Stanway C, Kingsman AJ, Kingsman SM (1988) The UAS of the yeast *PGK* gene is composed of multiple functional elements. Nucleic Acids Res 16:8245–8260

Chambers A, Tsang JSH, Stanway C, Kingsman AJ, Kingsman SM (1989) Transcriptional control of the *Saccharomyces cerevisiae PGK* gene by RAP1. Mol Cell Biol 9:5516–5524

Chasman DI, Lue NF, Buchman AR, LaPointe JW, Lorch Y, Kornberg RD (1990) A yeast protein that influences the chromatin structure of UAS_G and functions as a powerful auxiliary gene activator. Genes Dev 4:503–514

Chen W, Struhl K (1985) Yeast mRNA initiation sites are determined by specific sequences, not by the distance from the TATA element. EMBO J 4:3273–3280

Clifton D, Fraenkel DG (1981) The *gcr* (glycolysis regulation) mutation of *Saccharomyces cerevisiae*. J Biol Chem 256:13074–13078

Clifton D, Weinstock SB, Fraenkel DG (1978) Glycolysis mutants in *Saccharomyces cerevisiae*. Genetics 88:1–11

Cohen R, Yokoi T, Holland JP, Petter AE, Holland MJ (1987) Transcription of the constitutively expressed yeast enolase gene *ENO1* is mediated by positive and negative cis-acting regulating sequences. Mol Cell Biol 7:2753–2761

Delorme E (1989) Transformation of *Saccharomyces cerevisiae* by electroporation. Appl Environ Microbiol 55:2242–2246

Furter-Graves EM, Furter R, Hall BD (1991) *SHI*, a new yeast gene affecting the spacing between TATA and transcription initiation sites. Mol Cell Biol 11:4121–4127

Guarente L (1983) Yeast promoters and *lacZ* fusions designed to study expression of cloned genes in yeast. Methods Enzymol 101:181–191

Herskowitz I (1989) A regulatory hierarchy for cell specialization in yeast. Nature 342:749–757

Hess B, Boiteux A, Kruger J (1986) Coordinate control of glycolytic enzymes. Adv Enzyme Regul 7:149–167

Holland MJ, Yokoi T, Holland JP, Myambo K, Innis MA (1987) The GCR1 gene encodes a positive transcriptional regulator of the enolase and glyceraldehyde-3-phosphate dehydrogenase gene families in *Saccharomyces cerevisiae*. Mol Cell Biol 7:813–820

Holland JP, Brindle PK, Holland MJ (1990) Sequences within an upstream activation site in the yeast enolase gene *ENO2* modulate repression of *ENO2* expression in strains carrying a null mutation in the positive regulatory gene *GCR1*. Mol Cell Biol 10:4863–4871

Huet J, Coutrelle P, Cool M, Vignais ML, Thiele D, Marck C, Buhler JM, Sentenac A, Fromageot P (1985) A general upstream binding factor for genes of the yeast transcriptional apparatus. EMBO J 4:3539–3547

Ito H, Fukuda K, Kimura A (1983) Transformation of intact yeast cells with alkali ions. J Bacteriol 153:163–168

Jarvis EE, Hagen DC, Sprague GF (1988) Identification of a DNA sequence that is necessary and sufficient for α-specific gene control in *Saccharomyces cerevisiae*: implications for regulation of α-specific and a-specific genes. Mol Cell Biol 8:309–320

Karin M (1990) The AP-1 complex and its role in transcriptional control by protein kinase C. In: Cohen P, Foulkes G (eds) Molecular Aspects of Cellular Regulation, vol 6. Elsevier/North Holland Biomedical Press, Amsterdam, pp 143–161

Kunkel TA (1985) Rapid and efficient site-specific mutagenesis without phenotypic selection. Proc Natl Acad Sci USA 82:488–492

Lin S-Y, Carey M, Ptashne M, Green MR (1990) How different eukaryotic transcriptional activators can cooperate promiscuously. Nature 345:359–361

Machida M, Uemura H, Jigami Y, Tanako H (1988) The protein factor which binds to the upstream activating sequence of *Saccharomyces cerevisiae ENO1* gene. Nucleic Acids Res 16:1407–1422

Maitra PK, Lobo Z (1971) A kinetic study of glycolytic enzyme synthesis in yeast. J Biol Chem 246:475–481

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York

McNeil JB, Smith M (1986) Transcription initiation of the *Saccharomyces cerevisiae* iso-1-cytochrome C gene. J Mol Biol 187:363–378

Miller JH (1972) Experiments in molecular genetics. Cold Spring Harborg Laboratory Press, Cold Spring Harbor, New York, pp 352–353

Nishizawa M, Arakai R, Teranishi Y (1989) Identification of an upstream activating sequence and an upstream repressible sequence of the pyruvate kinase gene of the *Saccharomyces cerevisiae*. Mol Cell Biol 9:442–451

Norrander J, Kempe T, Messing J (1983) Construction of improved vectors using oligonucleotide directed mutagenesis. Gene 26:101–106

Ogden JE, Stanway C, Kim S, Mellor J, Kingsman AJ, Kingsman SM (1986) Efficient expression of the *Saccharomyces cerevisiae PGK* gene depends on an upstream activation sequence but does not require TATA sequences. Mol Cell Biol 6:4335–4343

Pavlovic B, Hörz W (1988) The chromatin structure at the promoter of a glyceraldehyde phosphate dehydrogenase gene from *Saccharomyces cerevisiae* reflects its functional state. Mol Cell Biol 8:5513–5520

Santangelo GM, Tornow J (1990) Efficient transcription of the glycolytic gene *ADH1* and three translational component genes requires the *GCR1* product, which can act through the TUF/GRF/RAP binding sites. Mol Cell Biol 10:859–862

Shore D, Nasmyth K (1987) Purification and cloning of a DNA binding protein from yeast that binds to both silencer and activator elements. Cell 51:721–732

Shore D, Stillmann DJ, Brand AH, Nasmyth KA (1987) Identification of silencer binding proteins from yeast: possible roles in *SIR* control and DNA replication. EMBO J 6:461–467

Stanway C, Mellor J, Ogden JE, Kingsman AJ, Kingsman SM (1987) The UAS of the yeast *PGK* gene contains functionally distinct domains. Nucleic Acids Res 15:6855–6873

Tschumper G, Carbon J (1983) Copy number control by a yeast centromere. Gene 23:221–232

Uemura H, Shiba T, Paterson M, Jigami Y, Taneka H (1986)

Identification of a sequence containing the positive regulatory region of *Saccharomyces cerevisiae* gene *ENO1*. Gene 45:67–75
Vieira J, Messing J (1987) Production of single-strand plasmid DNA. Methods Enzymol 153:3–11
Vignais ML, Woudt LP, Wassenaar GM, Mager WH, Sentenac A, Planta RJ (1987) Specific binding of TuF factor to upstream activation sites of yeast ribosomal protein genes. EMBO J 6:1451–1457
West RW, Yocum RR, Ptashne M (1984) *Saccharomyces cerevisiae* GAL1–10 divergent promoter region: location and function of the upstream activating sequencing UAS_G. Mol Cell Biol 4:2467–2478
Woudt LP, Smith AB, Mager WH, Planta RJ (1986) Conserved sequence elements upstream of the gene encoding yeast ribosomal protein 125 are involved in transcription activation. EMBO J 5:1037–1040
Zsebo KM, Lu HS, Fieschko JC, Goldstein L, Davis J, Duker K, Suggs SV, Lai PH, Bitter GA (1986) Protein secretion from *Saccharomyces cerevisiae* directed by the prepro-α-factor leader region. J Biol Chem 261:5858–5865

Communicated by C.P. Hollenberg