
The UAS of the yeast *PGK* gene contains functionally distinct domains

Clive Stanway*, Jane Mellor, Jill E.Ogden, Alan J.Kingsman and Susan M.Kingsman

Department of Biochemistry, University of Oxford, Oxford OX1 3QU, UK

Received June 10, 1987; Revised and Accepted August 10, 1987

ABSTRACT

The upstream activation site (UAS) of the yeast phosphoglycerate kinase gene (*PGK*) has been localised by deletion analysis (1). Here we show that the UAS_{*PGK*} contains two functionally distinct domains. These two domains, designated activator (A) and modulator (M), appear to be located within bases -460 to -402 and -531 to -461, respectively, relative to the initiating ATG; although it is possible that part of the M domain resides within the A domain. They have been shown, using a heterologous assay promoter, to have distinct transcriptional functions. Domain A is responsible for activation of transcription whilst domain M is required for carbon source dependent regulation of transcription. Protein-DNA binding studies have demonstrated that the DNA fragment containing domain M has high affinity for at least one specific DNA-binding protein, whilst domain A does not appear to interact strongly in protein-binding assays under the same conditions. The domain M binding activity is dependent on the carbon source in the growth medium and may be functional in the carbon source control of *PGK* expression.

INTRODUCTION

The phosphoglycerate kinase gene (*PGK*) of *Saccharomyces cerevisiae* is highly expressed *in vivo* as it possesses a number of efficient transcriptional signals located both in the promoter and in the coding region, (1, 2, 3, J. Mellor, M.J. Dobson, A.J. Kingsman & S.M. Kingsman, manuscript submitted, Nucleic Acids Res.). The efficiency of the *PGK* promoter has been demonstrated by its use in directing high level expression of various foreign genes in yeast, including interferon and chymosin, (4, 5, 6). Recently a linker-scanning deletion series was created to examine the *PGK* promoter, and this identified the position of the upstream activation site (UAS); deletion of this element causes a significant drop in the level of transcription, (1). Various yeast genes have been shown to possess a UAS (for a review, see

7) and in certain cases it is now known that these sites are involved in specific protein-DNA interactions, e.g. GAL 1 -10, HIS 3, CYC 1, (8, 9, 10, 11).

Glycolytic enzyme levels and their respective steady state mRNA levels are "induced" by glucose, although the degree of "induction" for certain enzymes, e.g. PGK, is not clear from a review of the literature (12, 13, 14, 15). Heterologous gene expression directed by the PGK promoter is also subject to glucose induction (4). Hence it is reasonable to conclude that the PGK promoter is subject to carbon source regulation, however the degree of induction has not yet been accurately determined. The overall control of glycolytic gene expression in yeast has not yet been elucidated, however Clifton and Fraenkel (15) have reported the identification of one gene believed to be involved in this process, namely GCR ('glycolysis regulation'). Mutations in this gene result in 50-80% and 90-95% reductions in the levels of glycolytic enzymes on fermentable and non-fermentable substrates, respectively. This suggests that the product of GCR is involved in the mechanism that controls glycolytic gene expression.

Here we report a further analysis of the UAS_{PGK} which extends our understanding of this element with regard to the control of PGK transcription. Using a heterologous promoter assay and protein-DNA binding studies we have been able to dissect the UAS into two functionally distinct domains; one being an activator, the other being a modulator. The activator domain corresponds to the core UAS region identified by deletion analysis of the promoter (1); whilst the modulator domain resides, at least in part, just upstream of the activator. The modulator is shown here to be involved in regulation of transcription in response to a carbon source shift, which is accompanied by alterations in the protein interactions at this location.

MATERIALS AND METHODS

Strains, media and growth conditions.

Strains used were Escherichia coli AKEC = C6000 (thrC leuB6 thyA trpC117 hsdR hsdM) and Saccharomyces cerevisiae MD40/4c (ura2 trp1 leu2.3 leu2.112 his3.11 his3.15). E.coli cultures were grown on Luria broth (16) with ampicillin (Sigma) at 25 µg/ml when necessary. Yeast cultures were inoculated at a cell

density of ca. 10^4 cells/ml and were grown at 30°C in YEPD (1% yeast extract, 2% peptone and 2% glucose) or in defined minimal medium (17) with a carbon source of glucose (1%) or sodium acetate (60 mM; medium adjusted to pH 6). All cultures were harvested at 6 to 8×10^6 cells/ml. For the acetate/glucose or acetate/acetate (control) experiments, the cultures were grown in acetate minimal medium to 2×10^6 cells/ml, harvested, resuspended in fresh medium (either acetate or glucose) and grown to 6 to 8×10^6 cells/ml.

DNA manipulations and plasmid constructions

Restriction enzymes, T4 DNA ligase and Klenow fragment were purchased from BRL, Inc.. Radioactively labelled isotopes, [α - ^{32}P] dTTP (400Ci/mmol) and [α - ^{32}P] dGTP (3000Ci/mmol) were purchased from Amersham International. DNA manipulations and plasmid constructions followed standard procedures (18). All reagents used were analytical grade.

The UAS_{PGK} was previously isolated on a BamHI-HaeIII fragment (-538 to -402), here designated fragment x, from a deletion derivative of the PGK promoter (1; see Fig. 1a), and inserted into pSP46(1). The x fragment was divided at the TagI site at base -460. Both subfragments were subcloned into pSP46; the upstream region as a TagI fragment (-531 to -461), here designated y; whilst the downstream region was subcloned as a TagI-BamHI fragment (-460 to -402), here designated z. These fragments were assayed for transcriptional function by insertion into an assay vector pMA1557-14, which is a high copy number yeast-E.coli shuttle vector, consisting of pBR322 and a $2\mu\text{m}$ -LEU2 cassette carrying the human interferon $\alpha 2$ coding sequence (hu-IFN $\alpha 2$) fused to a PGK terminator sequence (19; Fig. 1b). Transcription of hu-IFN $\alpha 2$ from pMA1557-14 is directed by a deletion derivative of the yeast TRP1 promoter which does not possess the UAS for transcript I and therefore only transcript II is produced (19, Fig. 1b). The x fragment and subfragments, y and z, were individually cloned into pMA1557-14 so that they replaced the deleted transcript I UAS. The three plasmids thus created have been designated pMA1557-14-x, pMA1557-14-y and pMA1557-14-z for the insertion of the UAS-bearing fragment (x), the upstream subfragment (y) and the downstream subfragment (z), respectively, (Fig. 1b).

Yeast Transformation

Yeast transformations were performed following the procedure of

Hinnen *et al.* (20). Transformed strains were designated T, e.g. T1557-14 is a strain transformed with pMA1557-14.

Analysis of transcription

RNA was isolated from yeast cultures grown as described above, according to the method of Dobson *et al.* (21). Total DNA was prepared by the method of Cryer *et al.* (22) from the same culture as was used for the RNA isolation. Northern and Southern gels were electrophoresed and blotted following standard procedures (3, 23, 24). Filters were hybridized according to Thomas (23) using nick-translated probes (25) from a human interferon $\alpha 2$ fragment (hu-IFN $\alpha 2$; 3) and a yeast ribosomal DNA fragment (26). These probes were labelled to a specific activity of $> 5 \times 10^7$ cpm/ug.

Preparation of nuclear protein extracts

Yeast cultures were grown in either acetate or glucose containing media as described above and nuclei were prepared using the method of Nelson and Fangman (27), except that in addition to PMSF (1mM), the protease inhibitors antipain, chymostatin, leupeptin and pepstatin A were added at a final concentration of 0.5 μ g/ml. Typically from a 500ml culture the nuclei were resuspended in 1ml of 1M sorbitol, 20mM PIPES (pH 6.4), 0.1mM CaCl₂.

For nuclear protein extraction the nuclei were pelleted at 12,000g for 10' at 4°C and resuspended in 0.6M NaCl, 5mM EDTA, 10mM B-mercaptoethanol, 10mM Tris (pH 7.5), 0.5mM PMSF and antipain, chymostatin, leupeptin and pepstatin A, all at 0.5 μ g/ml. The nuclear suspension was incubated on ice for 30 minutes with occasional vortexing, following which the nuclei were re-pelleted (12,000g, 10 minutes, 4°C). The supernatant was dialysed against 10mM Tris (pH 7.5), 10mM B-mercaptoethanol, 1mM EDTA, 0.5mM PMSF at 4°C for 3 - 12h with two changes. The protein concentration of the extract was estimated by the method of Bradford (28). The extract was adjusted to 15% glycerol and stored at -70°C.

Protein-DNA binding reactions and gel electrophoresis

Protein-DNA binding reactions were performed essentially according to Toliaas and Du Bow (29). End-labelled DNA fragment (1 to 5ng; *ca* 2,500 cpm) was incubated in buffer (5% glycerol, 1mM EDTA, 10mM B-mercaptoethanol, 25mM Tris (pH 7.5), 25mM NaCl) with varying amounts of competitor DNAs (see figure legends for details) and 1 - 5 μ g of nuclear protein (which was added last).

Reactions were allowed to proceed for 15 minutes at 22°C before loading directly onto a native, low ionic strength, 4% polyacrylamide gel (30). These gels were electrophoresed for 1 - 2h at 15 V/cm, dried and autoradiographed.

DNase I protection studies

This method was developed by Galas and Schmitz (31) and has since been modified. The procedure followed here was based largely on that of Parker and Topol (32). Initially a typical binding reaction was set up (see above), to this was added 5 μ l (ca 10 units) of DNase I (BRL); freshly diluted in binding buffer plus 10mM MgCl₂. After a 2.5 minute incubation the reaction was stopped by the addition of 100 μ l of 1% Sarkosyl, 100mM NaCl, 100mM Tris (pH 8.0), 10mM EDTA, 0.5mg/ml proteinase K, 50 μ g/ml tRNA, followed by incubation at 37°C for 15 minutes, then 5 minutes at 90°C. Subsequently the reaction was phenol extracted and ethanol precipitated. The DNA pellet was resuspended in 4 μ l 80% formamide gel loading buffer, heated to 100°C for 1 minute and then electrophoresed on an 8% denaturing polyacrylamide gel. The end-labelled fragment was also sequenced using the chemical cleavage method (33) to provide markers for the DNase I cleavage/protection pattern.

RESULTS

The UAS_{PGK} directs activation and regulation of transcription when inserted into a heterologous promoter.

The organization of the PGK promoter and the fragments of it used in this study are outlined in Fig. 1a. The UAS_{PGK}, borne on fragment x, was shown by Ogden *et al.* (1) to be capable of activating transcription when it is positioned 5' to a TRP1 promoter deletion in a plasmid similar to pMA1557-14 (Fig. 1b). The assay promoter plasmid (pMA1557-14) used in this study is a high copy number yeast-E.coli shuttle vector bearing a derivative of the TRP1 promoter, that does not possess the UAS for transcript I, fused to the hu-IFN α 2 coding sequence(19). Here we have investigated the ability of the UAS_{PGK} not only to activate, but also to regulate transcription. In order to do this the x fragment was inserted, in its normal promoter orientation, into the TRP1 promoter in pMA1557-14, thus creating pMA1557-14-x. The x fragment is therefore in the position previously occupied by the UAS of TRP1 transcript I (Fig. 1b).

Figure 2a shows an autoradiograph of a Northern blot

analysis of strain MD40/4c transformed with pMA1557-10, which bears the undeleted TRP1 promoter (Fig. 1b), pMA1557-14 and pMA1557-14-x. These transformants, designated T1557-10, T1557-14 and T1557-14-x respectively, were grown in acetate changed to fresh acetate, or acetate shifted to glucose media. On either carbon source two transcripts (I and II) are produced by the undeleted promoter, pMA1557-10, (data only shown for acetate shifted to glucose grown cells; lane 1), however T1557-14 only produces transcript II, as it no longer possesses the transcript I UAS, (lanes 2 and 3). T1557-14-x synthesizes transcript I when glucose is the carbon source, (lane 5), which is in keeping with the previous observation (1), however no transcript I is detected from acetate-grown cells, (lane 4). Figure 2b is an autoradiograph of the same filter re-probed for ribosomal RNA and shows that there are no loading discrepancies which might account for this carbon source effect on T1557-14-x. Furthermore the copy number of the plasmids studied were equivalent and did not vary on nutritional shift (data not shown). Therefore the synthesis of transcript I by T1557-14-x in glucose is directed by the UAS_{PGK} and moreover this activation is carbon source dependent.

Distinct regions of the UAS_{PGK} have different transcriptional capacities.

In order to study the UAS_{PGK} fragment further we divided it at the TagI site at base -460, to give a downstream fragment, designated z, largely corresponding to the activation region identified by deletion analysis (1) and an upstream fragment, designated, y (Fig. 1a). These fragments were subcloned via pSP46 into the assay promoter plasmid pMA1557-14 in the same orientation and position as described for the x fragment (see above; Fig. 1b). These plasmids were designated pMA1557-14-y and pMA1557-14-z; the former contains the upstream region of the UAS_{PGK}, i.e. bases -531 to -461, and the latter contains the downstream region of UAS_{PGK}, i.e. bases -460 to -402. These constructions were analyzed in strain MD40/4c as transformants designated T1557-14-y and 1557-14-z, respectively, by Northern blotting, (see Fig. 2), after growth in acetate changed to fresh acetate, or acetate shifted to glucose media. In glucose T1557-14-z produces as much transcript I as T1557-14-x, whereas none is produced by T1557-14-y, (lanes 9, 5 and 7, respectively). This shows that the activating element of fragment x lies within the boundaries of fragment z and that fragment y has no activating

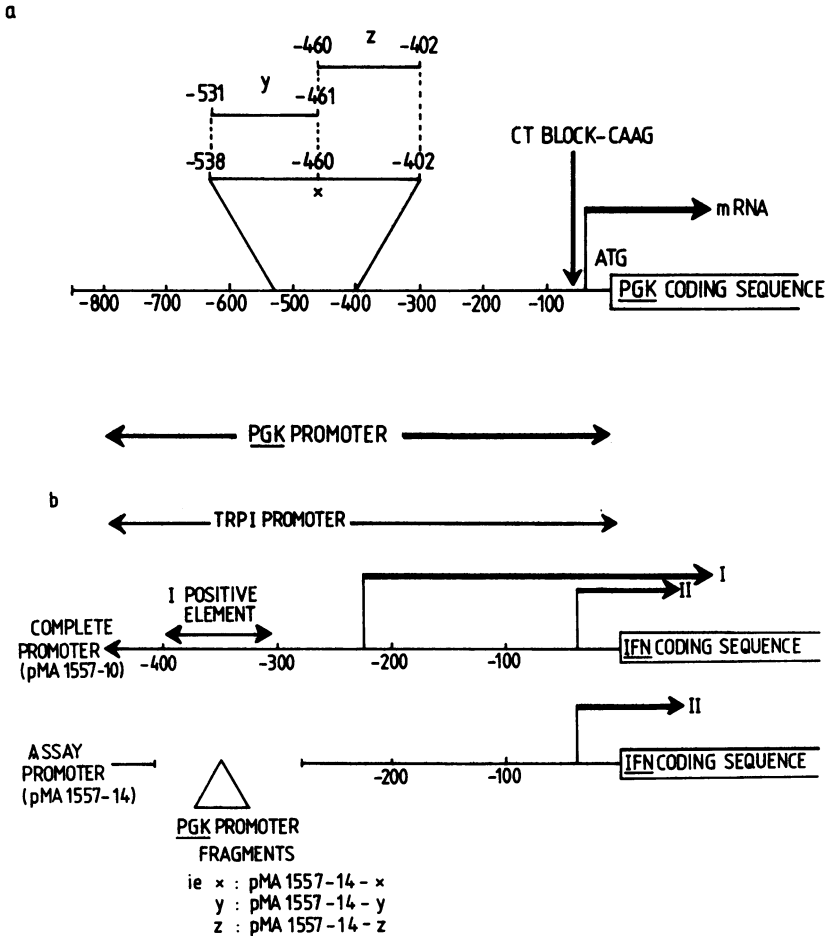


FIG. 1

a. Diagram of the structure of the PGK promoter. The fragment, designated x, bearing the UAS_{PGK} (-538 to -402; 1) is shown as an enlargement and the two subfragments, y and z, are indicated. All co-ordinates are relative to the A of the ATG being +1.

b. Diagram of the TRP1 promoter structure and the derivative of it used in this study. The wild type promoter (pMA1557-10) directs both transcript I & II, whilst the assay promoter (pMA1557-14) does not possess the transcript I UAS and therefore does not direct any transcript I synthesis; however transcript II is unaffected (19). The three PGK fragments x, y and z (see Fig.1a) were inserted into the assay promoter at the position indicated, yielding pMA1557-14-x, pMA1557-14-y and pMA1557-14-z, respectively.

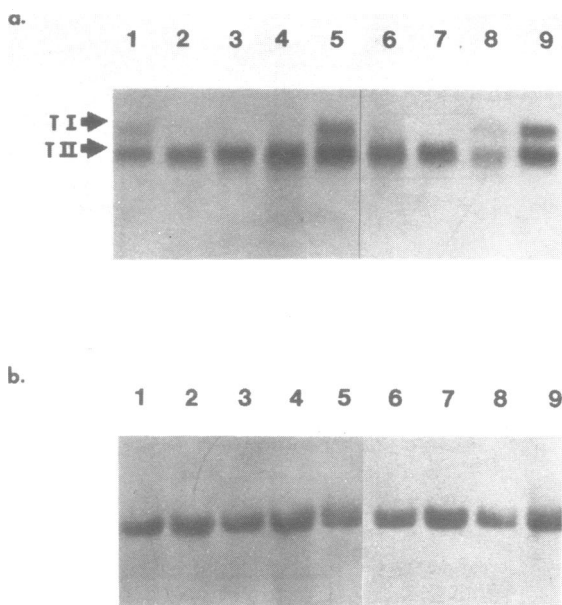


FIG. 2

a. Northern blot analysis of the mRNA levels directed by the TRP1 promoter constructions described in Fig. 1b, grown either in acetate/acetate (A/A) or acetate/glucose (A/G). The filter was probed with a nick-translated hu-IFN $\alpha 2$ fragment (3) which detects both transcript I & II. Lane 1, T1557-10 (wild type, A/G); lane 2, T1557-14 (A/A); lane 3, 1557-14 (A/G); lane 4, 1557-14-x (A/A); lane 5, 1557-14-x (A/G); lane 6, 1557-14-y (A/A); lane 7, 1557-14-y (A/G); lane 8, 1557-14-z (A/A); lane 9, 1557-14-z (A/G).

b. Internal loading control for the Northern blot in Fig. 2a. The filter was re-probed with a nick-translated rDNA probe (26). Lanes are the same as the same as described in 2a.

function. However in contrast to T1557-14-x, T1557-14-z produces the same levels of transcript I in both acetate and glucose, (lanes 8 and 9; lane 8 being slightly underloaded relative to lane 9), suggesting that all or part of a carbon source regulatory component is not present in fragment z, but by deduction is located, at least in part, in fragment y. Clearly then the UAS_{PGK} contains two domains that may or may not overlap. The first is the activator (A) domain, present in fragment z, and the second is the modulator (M) domain, which appears to be responsible for carbon source regulation, and which is located, at least in part, in the y fragment. It is also possible that the

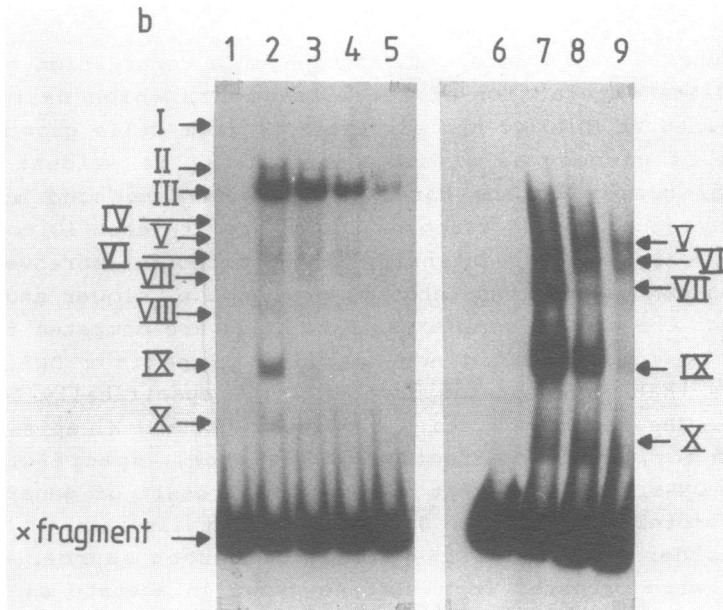
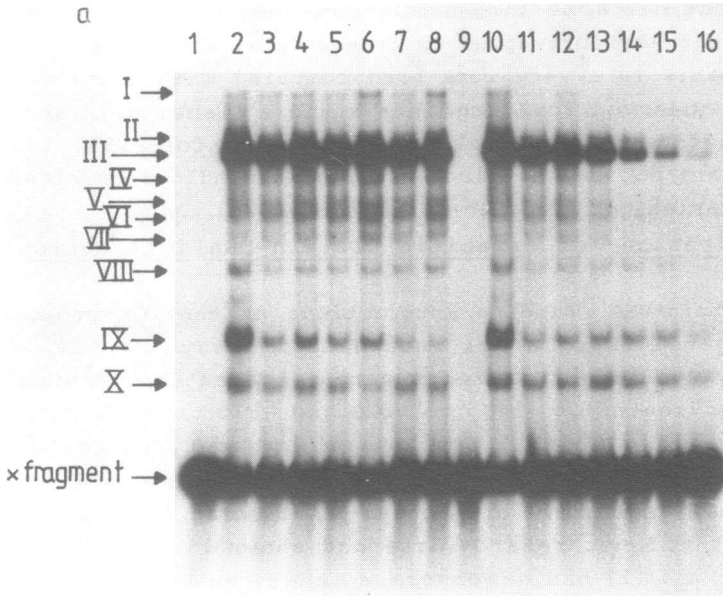
y fragment has some limited activation function, as a diffuse signal that may correspond to transcript I can be seen in lane 6. However this is a very weak band compared with the transcript I signals observed for fragments x and z (lanes 5, 8 and 9), and its significance is not clear. As observed for T1557-14-x these results are not due to discrepancies in loadings or plasmid copy number variations (Fig. 2b; data not shown).

Identification of specific protein-DNA interaction at the UAS_{PGK}.

Protein-DNA binding interactions at the PGK promoter were investigated using a gel retardation assay (28, 29, 34, 35). Initially two large fragments were selected for testing; these had co-ordinates of -1405 to -820 and -820 to -315, relative to the initiating ATG (2). The latter fragment, incubated with a nuclear extract from strain MD40/4c, showed discrete retardation complexes, indicative of protein-DNA binding, whilst the former fragment did not retard (data not shown). As the analysis of Ogden *et al.* (1) had demonstrated that the UAS_{PGK} resided on a fragment with co-ordinates -538 to -402, it seemed highly likely that the UAS was responsible for the retardation complex observed.

Figure 3a shows an autoradiograph of a retardation gel used to assay the interaction of the x fragment, which carries the UAS_{PGK}, with an MD40/4c nuclear extract, from cells grown in the presence of glucose as a carbon source. It is evident that a number of complexes are formed, which have reduced mobility relative to the unbound fragment. There are at least 10 complexes apparent, which vary in intensity and specificity; moreover these complexes can be resolved into two groups, i.e. slower and faster migrating. The slower complexes (I to VIII) are competed for more readily by specific than non-specific competitor DNA, which indicates that the proteins involved bind specifically to the x fragment. The faster migrating complexes (IX and X) appear to be competed for, equally effectively by either, specific or non-specific DNAs, implying that they are the result of general DNA-binding proteins, (see Fig. 3 legend for details).

In order to compare the effect of carbon source, nuclear extracts were prepared from MD40/4c grown in acetate as well as glucose minimal media. These were assayed by retardation gel analysis using labelled x fragment (Fig. 3b). The "glucose" extract shows the same profile as observed in Fig. 3a, although



it is slightly underexposed here, (Fig. 3b, left panel), which emphasizes the contrast with the profile produced by the "acetate" extract. The most significant difference is that complex III is missing in the "acetate" extract profile. There also appear to be differences in some of the other complexes formed by the "glucose" extracts (Fig. 3a, lane 2; Fig. 3b, lane 2) compared with those formed by the "acetate" extracts (Fig. 3b, lane 7). Using the "acetate" extract complexes V, VI, VII, IX and X are clearly present, but even on this relatively overexposed autoradiograph there is no suggestion of complexes I, II, IV and VIII, which are produced with "glucose" extracts. These data show that the protein-nucleic interactions at the UAS_{PGK}, particularly those leading to the formation of complex III are altered when cells are grown on different carbon sources.

In order to correlate protein binding activity with the transcriptional domains identified, two approaches were taken. Firstly the two fragments, y and z, subcloned into pSP46, were used as competitor DNAs against labelled x fragment and compared with the competitive effect of the x fragment subcloned into pSP46 (see Fig. 4 and legend for details). The effect of y fragment competition is essentially the same as that produced by the x fragment, i.e. the slower migrating complexes are more

Fig. 3

a. The x fragment which bears the UAS_{PGK} forms multiple protein complexes. This figure shows a retardation gel assay (29, 30) employing ³²P-labelled x fragment and a nuclear extract from yeast strain MD40/4c grown in YEPD. Binding reactions for each lane contained 2ng labelled fragment (ca. 2500 cpm), the reactions for lanes 2 to 8 and 10 to 16 contained 5µg nuclear protein. Competitor DNAs were added to the reactions as follows; reactions 3 to 8 and 11 to 16 contained 100ng of sonicated calf thymus DNA; reactions 4 to 8 respectively contained 10ng, 100ng, 500ng, 1µg and 2µg pSP46 DNA cut with Sau3A; whilst reactions 12 to 16 respectively contained 10ng, 100ng, 500ng, 1µg and 2µg pSP46-x DNA cut with Sau3A. Complexes formed are indicated and so is the free fragment.

b. Effect of carbon source on the protein complexes formed by the x fragment. The effect of carbon source was investigated in a retardation gel assay using two nuclear extracts from MD 40/4c, both grown in defined minimal medium, one using glucose, the other acetate as a carbon source. Lane 1: Labelled x fragment, lanes 2 to 5: x fragment plus glucose nuclear extract with competitor DNA (sonicated calf thymus) at 10ng, 100ng, 1µg and 10µg, respectively. Lane 6: labelled x fragment; lanes 7 to 9; x fragment, plus acetate nuclear extract with competitor DNA (sonicated calf thymus) at 100ng, 1µg and 10µg, respectively.

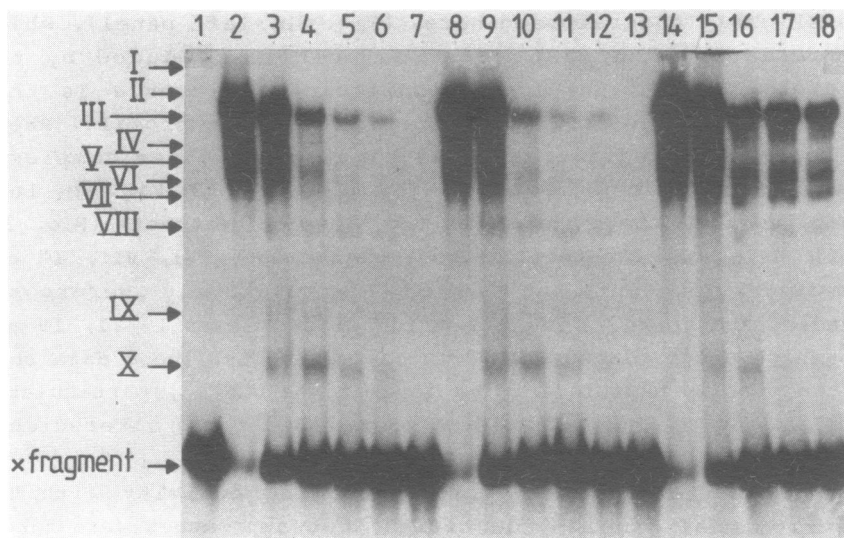


FIG. 4

Sub-fragment competition against the x fragment (UAS_{PGK}) protein binding. The effect of the y and z fragments as competitor DNAs on the x fragment protein-binding profile was investigated using a gel retardation assay as follows. All lanes contained labelled x fragment, lanes 2 to 6, 8 to 12 and 14 to 18 contained MD 40/4c nuclear extract (from YEPD grown cells) and 100ng sonicated calf thymus DNA. Lanes 3 to 6 respectively contained 100ng, 500ng, 1µg and 2µg of pSP46-y DNA, cut with Sau3A; lanes 9 to 12 respectively contained 100ng, 500ng, 1µg and 2µg of pSP46-x DNA cut with Sau3A; lanes 15 to 18 respectively contained 100ng, 500ng, 1µg and 2µg of pSP46-z DNA cut with Sau3A.

readily competed for than the faster migrating group. The z fragment does not compete for the slower migrating complexes and therefore does not interact with the proteins responsible. These results imply that the DNA fragment that bears all, or part of, domain M is the subject of specific protein binding, involving a number of proteins, (some possibly as protein-protein contacts), whilst domain A, although it is a transcriptional activator, at the most has weak protein binding affinity under these conditions.

The second approach employed to investigate the protein - binding affinities of domains M and A was to use each fragment, y and z, labelled with ^{32}P , incubated with a nuclear extract and specific and non-specific DNAs in an experimental design

identical to that described for the x fragment (Fig. 3a). Under these conditions fragment z did not yield a specific protein - DNA complex. On the other hand fragment y was observed to form a series of slow-migrating protein-DNA complexes, very similar to those observed for the fragment x (see Fig. 5a). Furthermore the two competition series show that these complexes are specific to the y fragment. The striking similarity between the number, pattern and relative intensity of the specific y complexes and those observed for the x fragment (Fig. 3a), and also the competitive effect of y DNA on the x-specific complexes (Fig. 4), strongly suggest that the respective complexes are essentially the same in protein composition. Therefore the same numbering system has been applied to the y-specific complexes as was applied to the x-specific complexes. It is interesting to note the appearance of two faster migrating complexes at high levels of specific DNA competition, i.e. lanes 14 and 15, Fig. 5a. Presumably these are the result of non-specific DNA-binding proteins that have a lower affinity for y DNA than the specific complex proteins, as they are unable to bind y DNA when the specific complexes are formed.

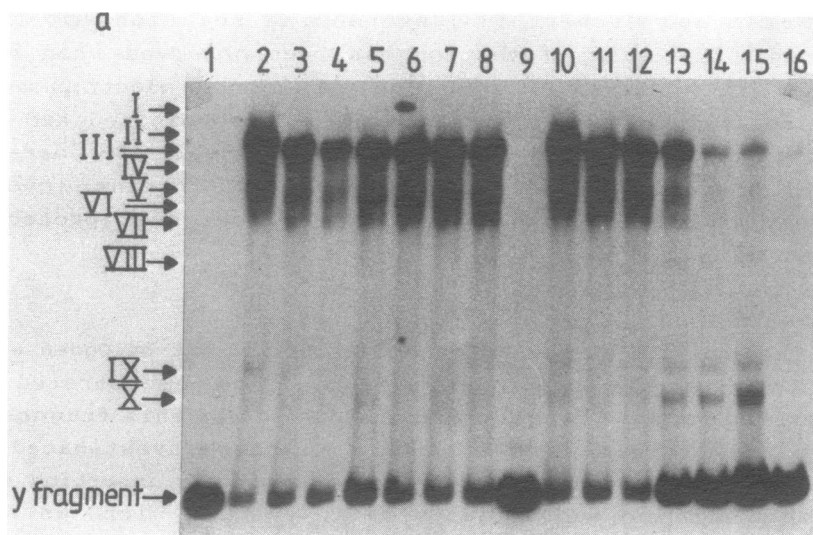
In order to discover the area(s) of specific contact with the y fragment DNA, footprinting or DNase I protection experiments were conducted with bound and unbound y DNA (Fig. 5b). A clear footprint is formed over bases -523 to -496, the region of protection being identified by co-electrophoresis of the Maxam and Gilbert (33) sequencing reactions of the y fragment. The same protection has been observed when bound complex III, which was DNase I digested prior to electrophoresis, was isolated from a retardation gel, deproteinized and then resolved on a denaturing 8% polyacrylamide gel, (36; data not shown). Therefore the protein, or one of the proteins, involved in complex III is responsible for the observed protection pattern.

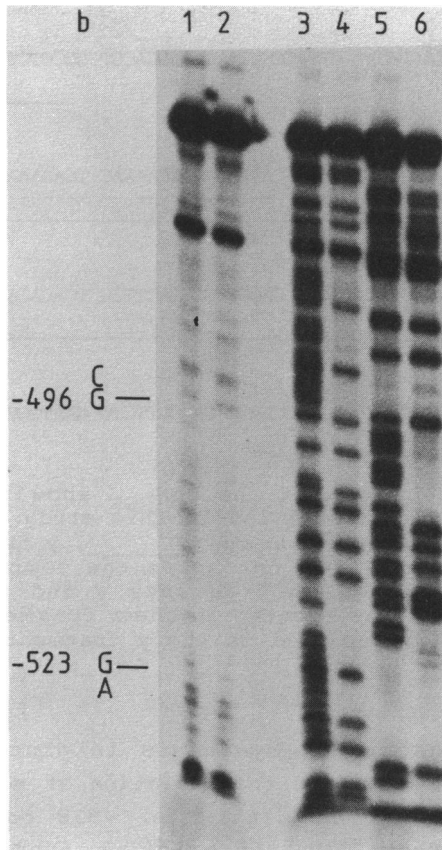
DISCUSSION

The analysis of the PGK promoter carried out by Ogden et al. (1) identified the location of the UAS_{PGK} and demonstrated that it resided on the x fragment; therefore it was this fragment on which the present work focussed. We have investigated the transcription functions located in this region by inserting the x fragment and two subfragments of x, y and z, into an assay

promoter. The activation potential of the UAS_{PGK} was confirmed and furthermore we have observed that the control of transcription exerted by the UAS_{PGK} is subject to carbon source regulation. In addition we have shown that the two subfragments, z and y, define two functionally distinct domains within the UAS, an activator domain (A) and a modulator domain (M), respectively; however, it should be noted that part of the M domain may overlap with the A domain. The demonstration of activation of transcription by the A domain confirms the deduction of the previous deletion study (1), i.e. that the core of the activation region must lie within bases -479 to -402. On the other hand the M domain, or that part of it that resides on the y fragment, evidently has no activation activity, however we have shown, by implication, that the M domain is able to modulate control of transcription when the carbon source is switched from non-fermentable to fermentable, because under these conditions the UAS_{PGK} exerts regulation on transcription whereas the A domain functions as a constitutive activator. The fragments used in this study, and the domains identified are summarised in Fig. 6.

It appears likely that the function of the M domain in acetate medium is that it mediates a repression of transcription, as pMA1557-14-z directs approximately equal transcript I synthesis in both acetate and glucose media, whereas pMA1557-14-x



**FIG. 5**

a. The γ fragment which bears all, or part of the M domain forms multiple protein complexes. Labelled γ fragment was assayed for protein binding affinity using gel retardation as follows. All lanes contained labelled γ fragment, lanes 2 to 8 and 10 to 16 contained MD40/4c nuclear protein extract; lanes 3 to 8 and 11 to 16 contained 100ng sonicated calf thymus DNA. Lanes 4 to 8 respectively contained 10ng, 100ng, 500ng, 1 μ g and 2 μ g pSP46 DNA cut with Sau3A. Lanes 12 to 16 contained 10ng, 100ng, 500ng, 1 μ g and 2 μ g of pSP46- γ DNA cut with Sau3A.

b. DNase I protection of the γ fragment. Labelled γ fragment was partially digested with DNase I with or without MD40/4c nuclear extract present. The resulting DNA fragments were resolved on an 8% denaturing polyacrylamide gel. The chemical cleavage products (33) of the γ fragment were electrophoresed simultaneously as markers. Lane 1: unbound γ fragment (DNased), lane 2: bound γ fragment (DNased), lanes 3 to 6: γ fragment sequence, 3: AG, 4: G, 5: CT, 6: C.

```

-580
                                     ***
      TOCCTCCTTC TTGAATTGAT GTTACCTCA TAAAGCACGT GGCCTCTTAT CGAGAAAGAA
                                     -- -----
-520
      ***** ***** *****
      ATTACCGTCG CTCGIGATTT GTTTGCAAAA AGAACAAAAC TGAAAAAACC CAGACACGCT
      -----
-460
      CGACTTCCTG TCTTCTATT GATTGCAGCT TCCAATTCG TCACACAACA AGGTCCTAGC
      .....
-400
      GACGGCTCAC AGGTTTGTGTA ACAAGCAATC GAAGGTTCTG GAATGGCGGG AAAGGGTTTA
  
```

FIG. 6 Nucleotide sequence of the UAS_{PGK} showing the fragments used, and the domains identified in this study. Each fragment is underlined as follows: x fragment _____, y fragment -----, z fragment The x fragment bears the complete UAS, whilst domains M and A reside on fragments y and z, respectively; however part of M may be located in the z fragment. The region of DNase I protection identified in the y fragment (see Fig. 5b) is indicated by *****.

only directs transcript I synthesis in glucose. This is in keeping with the observation that deletion of most of the region encompassed by the y fragment, i.e. -538 to -479, does not detrimentally affect PGK transcription from cells grown in glucose (1). It is quite possible however, that the regulation of PGK transcription involves more than just the M domain.

The protein-DNA binding profile of the x fragment in Figure 3a shows that there are a group of slow-migrating specific complexes, whilst the fast migrating complexes are the result of general DNA-binding proteins. The y fragment also forms a number of protein-DNA complexes which appear remarkably similar in distribution and intensity to those of the x fragment (Fig. 5a); and the cross competition experiment (Fig. 4) demonstrates that all the specific complexes that form with the x fragment also form with the y fragment. Consequently we propose that the protein composition of the respective x- and y-specific complexes are identical and therefore have been ascribed the same numbering. The DNase I protection pattern observed in the y fragment (Fig. 5b), which is the result of complex III, covers 28

base pairs in which there are no obvious repeats or unusual features. The retardation gel assays also show that the z fragment, although it is a transcriptional activator, does not appear to form any specific protein contacts. This fragment, however does contain 3 repeats of the sequence CTTCC, which may be of functional significance.

The effect on the DNA-binding profile of the x fragment resulting from growth in acetate is that the predominant complex (III) is lost, along with complexes I, II, IV and VIII, however the other specific protein interactions remain (Fig. 3b). This is interesting in two respects; firstly as the major complex that disappears, i.e. III, is known, from the footprint (Fig. 5b) to map to the y fragment, which contains part or all of the M domain, it appears likely that this change is linked to the transcriptional modulation effected by this region of DNA. However, if, as proposed above, the M domain is indeed the site of action of a repressor of transcription it is unclear how the loss of this protein-binding event serves this purpose. Secondly the less intense but specific complexes (V, VI and VII) remain in the "acetate extract" profile, even though no footprint evidence has as yet been observed for them. Perhaps in growth on non-fermentable carbon sources these proteins (V, VI and VII) bind in vivo and cause transcription repression, whilst the DNA-protein complex III may block this repression in vivo on fermentable carbon sources. It is therefore apparent that there are at least 8 specific protein-DNA interactions at the UAS_{PGK} and that these are restricted to the y subfragment. Furthermore it seems probable that these interactions are involved in M domain function.

At present little is known regarding the mechanism whereby proteins regulate and activate transcription via UASs. Recently Ptashne (37) has proposed that transcriptional regulation and activation is achieved by specific protein-DNA interactions and subsequent protein-protein interactions. The evidence from the studies on GAL1-10 and HIS3 regulation by the gene products of GAL4 and GCN4, respectively, concur with these proposals. In both cases the regulatory protein binds to a specific DNA site via one domain, whilst a second domain is believed to be responsible for transcriptional activation by protein-protein interactions which are as yet undefined (9, 38, 39). It appears likely that the UAS_{PGK} is different in its structure and mode of action when

compared with the regulation and activation conferred by the GAL 1-10 or the HIS 3 UAS. Firstly the DNA domain through which activation of PGK is mediated appears not to be a site of strong primary protein recognition. It is possible, however that an activator protein binds first to another sequence before contacting the A domain. Secondly the M domain, although it is not a transcriptional activator is, or is directly adjacent to, a site of primary protein-DNA contact. Presumably this interaction is crucial to the function of the M domain; however the manner in which the proteins interacting at this site may achieve modulation of transcription has not yet been resolved.

ACKNOWLEDGEMENTS

We would like to thank SERC/Celltech for financial support and Frank Caddick for photography.

*To whom correspondence should be addressed

REFERENCES

1. Ogden, J.E., Stanway, C., Kim, S., Mellor, J., Kingsman, A.J. and Kingsman, S.M. (1986) *Mol. Cell. Biol.* **6**, 4335-4343.
2. Dobson, M.J., Tuite, M.F., Roberts, N.A., Kingsman, A.J., Kingsman, S.M., Perkins, R.E., Conroy, S.C., Dunbar, B. and Fothergill, L.E. (1982) *Nucleic Acids Res.* **10**, 2625-2637.
3. Mellor, J., Dobson, M.J., Roberts, N.A., Kingsman, A.J. and Kingsman, S.M. (1985) *Gene* **33**, 215-226.
4. Tuite, M.F., Dobson, M.J., Roberts, N.A., King, R.M., Burke, D.C., Kingsman S.M. and Kingsman, A.J. (1982) *EMBO J.* **1**, 603-608.
5. Mellor, J., Dobson, M.J., Roberts, N.A., Tuite, M.F., Emtage, J.S., White, S., Lowe, P.A., Patel, T., Kingsman A.J. and Kingsman, S.M. (1983) *Gene* **24**, 1-14.
6. Kingsman, S.M., Kingsman, A.J., Dobson, M.J., Mellor, J. and Roberts, N.A. (1985) *Biotech and Genetic Eng. Reviews* **3**, 377-416.
7. Guarente, L., (1984) *Cell* **36**, 799-800.
8. Giniger, E., Varnum, S.M. and Ptashne, M. (1985) *Cell* **40**, 767-774.
9. Hope, I.A. and Struhl, K. (1986) *Cell* **46**, 885-894.
10. Hope, I.A. and Struhl, K. (1985) *Cell* **43**, 177-188.
11. Arcangioli, B. and Lescure, B. (1985) *EMBO J.* **4**, 2627-2633.
12. Hommes, F.A. (1966) *Arch. Biochem. Biophys.* **114**, 231-233.
13. Maitra, P.K. and Lobo, Z. (1971) *J. Biol. Chem.* **246**, 475-488.
14. Holland, M.J. and Holland, J.P. (1978) *Biochemistry* **17**, 4900-4907.
15. Clifton, D. and Fraenkel, D.G. (1981) *J. Biol. Chem.* **256**, 13074-13078.
16. Miller, J.H., (1972) *Experiments in Molecular genetics*, p433. Cold Spring Harbour Laboratory. Cold Spring Harbour, N.Y.

-
17. Hawthorne, D.C. and Mortimer, R.K. (1960) *Genetics* **45**, 1085-1110.
 18. Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) *Molecular cloning, a laboratory manual*. Cold Spring Harbour Laboratory, Cold Spring Harbour, N.Y.
 19. Kim, S., Mellor, J., Kingsman, A.J. and Kingsman, S.M. (1986) *Mol. Cell. Biol.* **6**, 4251-4258.
 20. Hinnen, A., Hicks, J.B. and Fink, G.R. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 1929-1933.
 21. Dobson, M.J., Mellor, J., Fulton, A.M., Roberts, N.A., Kingsman, S.M. and Kingsman, A.J. (1984) *EMBO J.* **3**, 1115-1121.
 22. Cryer, D., Eccleshall, R. and Marmur, J. (1975) *Methods Cell Biol.* **12**, 39-44.
 23. Thomas, P., (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5201-5202.
 24. Southern, E.M. (1975) *J. Mol. Biol.* **98**, 503-517.
 25. Rigby, P.W.J., Dieckmann, N.M., Rhodes, C. and Berg, P. (1977) *J. Mol. Biol.* **113**, 237-251.
 26. Petes, T.D., Hereford, L.M. and Konstantin, K.G. (1978) *J. Bacteriol.* **134**, 295-305.
 27. Nelson, R.G. and Fangman, W.L. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 6515-6519.
 28. Bradford, M. (1976) *Anal. Biochem.* **72**, 248-254.
 29. Toliás, P.P. and DuBow, M.S. (1985) *EMBO J.* **4**, 3031-3037.
 30. Strauss, F. and Varshavsky, A. (1984) *Cell* **37**, 889-901.
 31. Galas, D.J. and Schmitz, A. (1978) *Nucleic Acids Res.* **5**, 3157-3170.
 32. Parker, C.S. and Topol, J. (1984) *Cell* **36**, 357-369.
 33. Maxam, A.M. and Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 560-564.
 34. Fried, M. and Crothers, D.M. (1981) *Nucleic Acids Res.* **9**, 6505-6525.
 35. Garner, M.M. and Revzin, A. (1981) *Nucleic Acids Res.* **9**, 3047-3060.
 36. Singh, H., Sen, R., Baltimore, D. and Sharp, P. (1986) *Nature* **319**, 154-158.
 37. Ptashne, M., (1986) *Nature* **322**, 697-701.
 38. Brent, R. and Ptashne, M. (1985) *Cell* **43**, 729-736.
 39. Keegan, L., Gill, G. and Ptashne, M. (1986) *Science* **231**, 699-704.