Volume 17 Number 22 1989 Nucleic Acids Research

Characterization of the transcriptional potency of sub-elements of the UAS of the yeast *PGK* gene in a *PGK* mini-promoter

Clive A.Stanway[1+], Alistair Chambers[1], Alan J.Kingsman[1,2] and Susan M.Kingsman[1]*

Department of Biochemistry, Oxford University, South Parks Road, Oxford OX1 3QU and [1]Department of Molecular Biology, British Biotechnology, Watlington Road, Cowley, Oxford OX4 5LY, UK

Received July 27, 1989; Revised and Accepted October 3, 1989

ABSTRACT

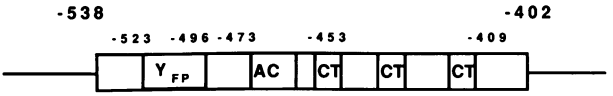
The upstream activator (UAS) of the yeast *PGK* gene comprises three different sequence elements. These are 1) a region of strong protein binding called the Y_{FP}, 2) three repeats of the motif CTTCC and 3) an essential activator core (AC) sequence that binds the protein RAP1. To assess the function of each of these elements in transcriptional activation we have inserted them individually and in various combinations into a *PGK* mini-promoter. This comprises only the transcription intiation elements from the *PGK* promoter and is inactive in the absence of activator sequences. None of the individual sequence elements was capable of activating the mini-promoter. However either the Y_{FP} or the CTTCC boxes in conjunction with the AC box resulted in efficient expression. Transcription levels were not however as high as when all three elements were inserted. These data suggest that the efficiency of *PGK* transcription depends upon the interactions between three different sequences. Furthermore while RAP1 *per se* is not a transcriptional activator it can associate promiscuously with other factors to create a functional transcription complex.

INTRODUCTION

The *PGK* gene is one of the most efficiently expressed genes in the yeast *Saccharomyces cerevisiae* (1) and its promoter has been widely used to direct high efficiency expression of 'foreign' genes in yeast (2,3). This high efficiency expression is likely to be a result of specific interactions of transcription factors with cognate sequences in the promoter. All yeast gene promoters contain an upstream activator sequence (UAS) that shares most of the features of mammalian enhancer sequences (4–6) and that is usually located several hundred bases upstream of the transcription start site (4,5). The initiation of transcription is directed by an AT rich element that is broadly analogous to the mammalian TATA box in both its sequence and in its protein binding interactions (7,8) although unlike mammalian TATA boxes it can function up to 120 nucleotides upstream of the RNA start (9). The precise requirements for accurate initiation in yeast have not yet been defined but it appears that the sequences around the initiation site (I site) are important (9,10). We have previously shown that deletion of the TATA box region does not affect the efficiency of transcription of the *PGK* gene (11). The most likely determinants of transcriptional efficiency are therefore the UAS binding proteins.

The *PGK* UAS was initially localised to a 136 bp fragment (coordinates −538 to −402; Figure 1a) that was essential for promoter function (11). Fine deletion analysis of this region (12) and protein binding studies (13) have revealed a number of different *cis* elements within the UAS. One of these that we have called the activator core (AC, coordinates −473 to −458; Figure 1a) binds the protein RAP1 (12,14, A.C., J Tsang, C.A.S., A.J.K. & S.M.K., manuscript in press, Mol. Cell. Biol.). This protein is probably the same as

a PGK UAS



-538 -520 -500
CCTCTTATCGAGAAAGAAATTACCGTCGCTCGTGATTTGTTTGCAAAA

-480 -460
AGAACAAAACTGAAAAAACCCAGACACGCTCGACTTCCTGTCTTCCTAT

-440 -420 -402
TGATTGCAGCTTCCAATTTCGTCACACAACAAGGTCCTAG

b PGK promoter mRNA initiation region

TATA BOX
-160
TTTACATATA TATAAACTTG CATAAATTGG TCAATGCAAG AAATACATAT

-110
TTGGTCTTTT CTAATTCGTA GTTTTTCAAG TTCTTAGATG CTTTCTTTTT

RIS
-60
CTCTTTTTTA CAGATCATCA AGGAAGTAAT TATCTACTTT TTACAACAAA

-10
TATAAAAACA ATGTCTTTA....

OLIGONUCLEOTIDES

R CTCGAGCTTTCTTTTTCTCTTTTTTACAGATCAAGGAAGTATAAAAGATCT

T GGATCCTACATATATATAAACTCTCGAG

T' GGATCCTACATATATATAAACTTGCATAAATTGGTCAATGCTCGAG

T'R
[-90] TATA BOX [-50]
5' GGATCCTACATATATATAAACTTGCATAAATTGGTCAATGCTCGAGCTTT-
[-30] RIS
CTTTTTCTCTTTTTTACAGATCAAGGAAGTATAAAAGATCT IFN coding sequence

GRF1 (15) and TUF (16). It binds numerous related sequences that are found in active promoters such as *PGK* (12) *ENO1, RP51* and *TEF2* (14,16,17), in inactive genes such as the mating type loci *HML* and *HMR* (14,17) and in telomeres (14,17). It has been proposed to act directly as a transcriptional activator (14,17) or as part of a transcriptional repressor (14). Recently it has been shown to mediate DNA loop formation between spatially distinct RAP1 binding sites possibly involving nuclear scaffold association (18). Although the AC box is essential for the *PGK* promoter to function efficiently it does not appear to be sufficient for full activity (12). Two other elements that have been identified are three repeats of the pentamer CTTCC that are located between coordinates −458 to −428 downstream of the AC box and a specific protein binding site called Y_{FP} (coordinates −523 to −496) located upstream of the AC box (Figure 1a). Deletion of the CTTCC boxes reduces transcription (12). Deletion of the Y_{FP} does not appear to affect transcription yet this is the site of a very strong protein interaction (13). Furthermore this DNA-protein interaction is carbon source regulated in the same fashion as the *PGK* promoter (13).

Deletion analyses suffer from the fact that the deletion alters the spatial relationship between different promoter elements and juxtaposes different sequences in different deletions. Some of these sequences may have transcriptional activity. The three hundred nucleotides between the functional UAS and the RNA start site in the *PGK* promoter are poorly characterised. They are known not to be essential for promoter activity but they do contain a functional heat shock response element (19,20). Comparisons of promoter efficiency between different deleted promoters can therefore be complicated. In attempt to remove some of these complications we have constructed a *PGK* mini-promoter that comprises only 91 nucleotides of the *PGK* gene that contains the TATA box, the I site and part of the untranslated leader region. Upstream of this we have placed a 720 nucleotide fragment from the *TRP1* 5′ non coding region that is known not to contain any transcriptionally active elements (21). The mini-promoter is activated by insertion of the 136bp entire *PGK* UAS fragment to produce a promoter with identical characteristics of efficiency and mRNA initiation as the full length *PGK* promoter. The ability of each of the UAS sub-elements to activate transcription individually and in various combinations was tested by insertion into this assay promoter where they are flanked by identical sequences and maintained at similar distances from the RNA start site.

MATERIALS AND METHODS

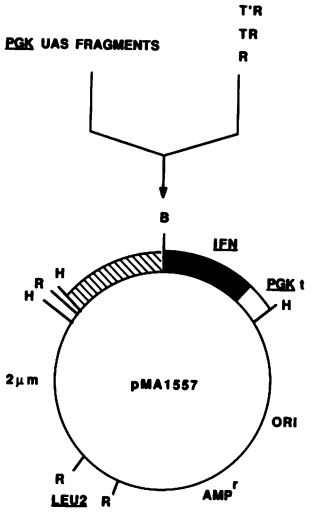
Strains, media and growth conditions.

Strains used were *Escherichia coli* MC1061 [F′*araD139* Δ(*lacIPOZYA*) *X74* Δ(*araABOIC-leu*) *7697 galk hsdR hsdM rpsL*] and *Saccharomyces cerevisiae* DBY745 (*adel-100 leu2-3*

Figure 1 a. Diagrammatic representation of the *PGK* UAS with its internal elements and their nucleotide co-ordinates indicated. The sequence of the *PGK* UAS containing fragment is shown underneath. The Y_{FP} sequence is underlined, the AC box is overlined and the CTTCC elements are boxed.

b. The sequence of the *PGK* promoter mRNA initiation region and oligonucleotides R T and T′. The positions of the TATA box and mRNA initiation site (RIS) are indicated. The artificial restriction sites in the oligonucleotides used in the plasmid constructions are boxed. R was linked to either T or T′ by the *XhoI* site shown at both the 5′ end of R and at the 3′ end of T or T′. The full T′R sequence is also listed, the co-ordinates, shown in brackets, relate to T′R, not to the authentic *PGK* promoter. The *BglII* site at the 3′ end of R is the point of fusion to the IFN coding sequence.

a



b

PGK				PLASMID
-538	to	-402		pKV550
-538	to	-402	+ R	pKV554
-538	to	-402	+ TR	pKV554T
-538	to	-402	+ T'R	pKV561
-------			+ T'R	pKV560
-531	to	-461	+ T'R	pKV562
-460	to	-402	+ T'R	pKV563
-473	to	-409	+ T'R	pKV564
-473	to	-453	+ T'R	pKV567
-453	to	-473	+ T'R	pKV568
-523	to	-496	+ T'R	pKV570
-523	to	-496		
+			+ T'R	pKV571
-473	to	-453		

leu2-112 ura3-52. *E. coli* cultures were grown on Luria broth (22) with 50 μg/ml ampicillin where necessary. Yeast cultures were grown in YEPD or defined minimal media with the necessary amino acid supplements at 30°C (23). Yeast transformations were performed following the procedure of Hinnen et al. (24) . Transformed yeast strains were grown for DNA/RNA isolation at 30°C for 16 to 24h until the culture had reached a cell density of 4 to 8×10^6 cells/ml.

DNA manipulations

All plasmid constructions were performed following standard procedures (25). DNA oligomer synthesis was conducted using an Applied Biosystems Inc. 380A synthesizer. All promoter constructions were confirmed by DNA sequencing.

The cloning, manipulation and *in vitro* transcription and translation of RAP1 are described elsewhere (A.C., J Tsang, C.A.S., A.J.K. & S.M.K., manuscript in press, Mol. Cell. Biol.).

Transcriptional analysis.

All constructions were analysed in yeast using DBY 745 as a recipient strain. Cultures were harvested in mid-exponential growth phase for RNA (3) and plasmid copy number DNA extraction (26). Efficiency of transcription was examined by Northern blotting (27) in comparison with plasmid copy number analysis by Southern blotting (28). The DNA preparations were digested with *Eco*RI prior to electrophoresis. In both analyses the nitrocellulose filters were probed with nick-translated (29) ribosomal or *PGK* and alpha 2 interferon (IFN) probes (30). In the Northern blots the ribosomal RNA or chromosomal *PGK* mRNA signal determines the relative loading of each track. The site of mRNA initiation was mapped by primer extension (31) using an IFN primer (30).

Nuclear protein extracts and retardation gel analysis.

Nuclear protein extracts were prepared as previously described (13,32) and DNA-protein binding reactions and retardation gels were performed as detailed by Chambers *et al.* (12, A.C., J Tsang, C.A.S., A.J.K. & S.M.K., manuscript in press, Mol. Cell. Biol.).

RESULTS

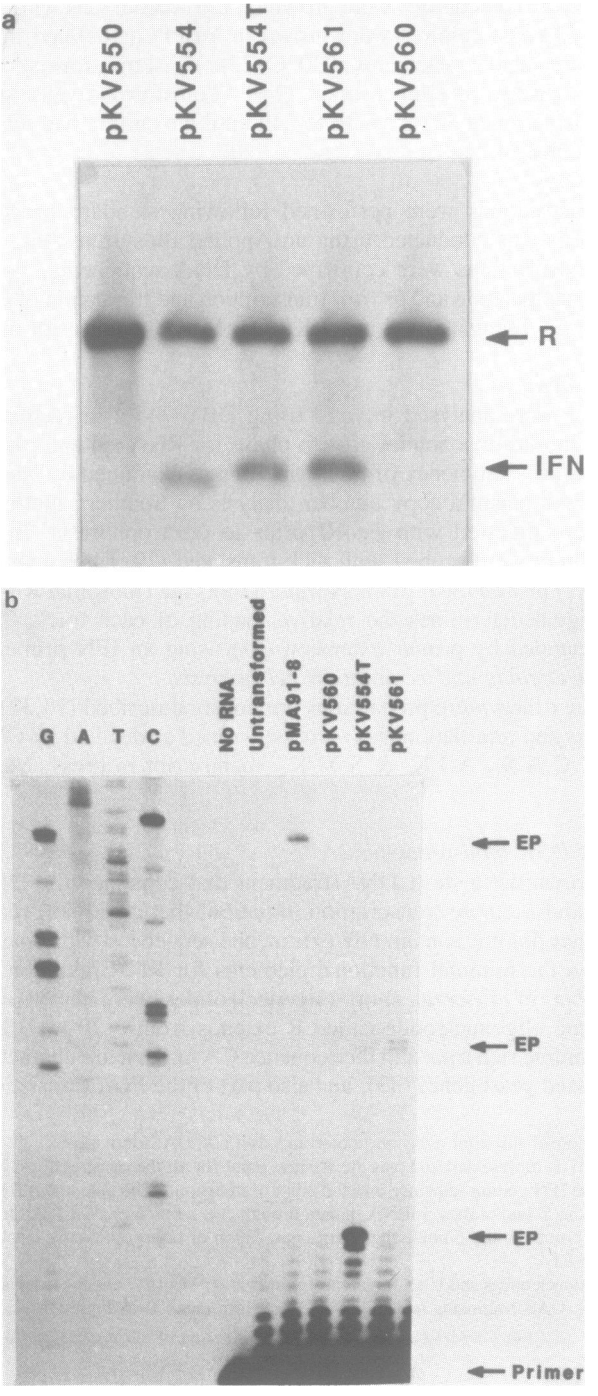
Construction of PGK mini-promoters.

We wished to construct a short DNA fragment that contained the DNA sequences that direct efficient and accurate transcription initiation, that could still respond to activation by a UAS but that did not contain any extraneous sequences with potential for activating transcription. As the minimal functional elements for RNA initiation have not yet been defined a number of different short oligonucleotides were tested individually and in combination. One oligonucleotide called R extends from −70 to −34/−10 to −4 and contains a pyrimidine rich tract and the sequence CAAG that are characteristic of a number of highly expressed yeast genes (33), and also part of the *PGK* translation leader. Another

Figure 2 Construction of the mini assay promoter and its *PGK* UAS derivatives.

a. Plasmid pMA1557 is represented and was the starting point for all the constructions. The unique *Bam*HI site immediately 5′ to the IFN coding sequence was the point of insertion of the potential mRNA initiation fragments T′R, TR and R. At the 5′ end of these mRNA initiation fragments a range of *PGK* UAS fragments were inserted, see b. The hatched box 5′ to the *Bam*HI site, represents 720bp of transcriptionally inactive DNA. B=*Bam*HI. H=*Hin*dIII, R=*Eco*RI.

b. Details of the oligonucleotides and UAS fragments inserted into pMA1557 and the resultant plasmid designations. The sequence of the UAS fragments and oligonucleotides are detailed in Figure 1.


a
pKV550
pKV554
pKV554T
pKV561
pKV560
R
IFN
b
G
A
T
C
No RNA
Untransformed
pMA91-8
pKV560
pKV554T
pKV561
EP
EP
EP
Primer

called T contains one of the TATA box motifs extending from −158 to −143. A third called T′ contains the same TATA motif but also an additional 18 bp downstream such that it extends from −158 to −125. The T and T′ oligonucleotides were each ligated to R to produce TR and T′R. These oligonucleotides and their location in the *PGK* promoter are indicated in Figure 1b.

The full length *PGK* UAS (cordinates −538 to −402) was inserted into the plasmid pMA1557 to produce pKV550. Plasmid pMA1557 contains a unique BamHI site flanked upstream by 720bp derived from the upstream region of the *TRP1* gene and downstream by an interferon-α2 reporter gene (IFN). The *TRP1* DNA has been shown previously to be transcriptionally inactive and serves to insulate any downstream promoter sequences from other plasmid encoded transcripts (21,32).

R, TR and T′R were inserted into the plasmid pMA1557 along with the complete *PGK* UAS, the latter being positioned upstream of the oligonucleotides and the interferon-α2 (IFN) reporter gene was downstream resulting in plasmids pKV554 (UAS-R-IFN), pKV554T (UAS-T-R-IFN) and pKV561 (UAS-T′R-IFN). In addition the plasmid pKV560 that contains only T′R-IFN and no UAS was also constructed (Figure 2). These constructions were compared with plasmid pMA91-8 (30) which possesses the whole *PGK 5′* non-coding region (ca. 1.5 kb) directing expression of the IFN reporter gene.

Characterisation of the PGK-mini promoters

Transcription directed by each of the mini-promoter combinations was analysed by Northern blotting (Figure 3a). As expected the *PGK* UAS alone in pKV550 and the initiation elements T′R alone in pKV560 did not direct transcription. However in the presence of the UAS each of the three oligonucleotides i.e R, TR and T′R directed transcription. Although the different oligonucleotides were equally efficient they differed in their ability to direct accurate transcription initiation as indicated by the different sizes of the transcripts. Primer extension was used to map the start sites in pKV561 and pKV554T (Figure 3b). Transcription directed by the UAS-TR promoter in pKV554T initiated at the A in the interferon initiating ATG i.e. 18 or 19 nucleotides downstream from the normal start site, (which is immediately 3′ to the end of the IFN primer, possibly explaining the intensity of the signal). Transcription directed by the UAS-T′R promoter in pKV561 however initiated at the same site as in the authentic *PGK* promoter, (the extension products of pMA91-8 and pKV561 only differ in size because of the reduction in the leader of pKV561; see Figure 1b and above). The only difference between the promoters of pKV554T and pKV561 is the presence of an additional 18 bp downstream of the TATA box in pKV561. The T′R promoter therefore fulfilled all the requirements of a mini-promoter for our present study. These were a) its activity was absolutely UAS dependent b) in the presence of the full UAS the transcription was efficient and c) the mRNA initiated at the correct site. The ability of various regions of the *PGK* UAS to activate transcription in derivatives of the mini-promoter in pKV560 was tested.

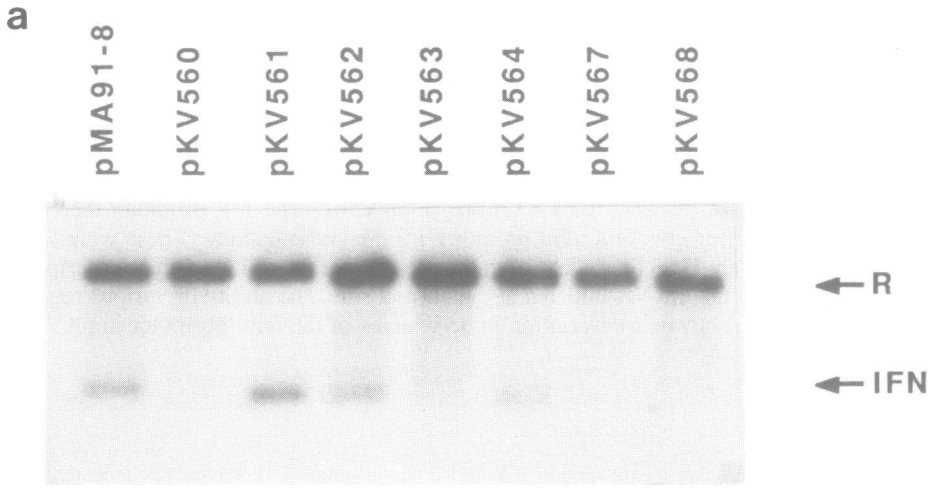
Figure 3 a. Northern blot showing the level of IFN mRNA expression, and the approximate transcript size, directed by the *PGK* UAS fused to the mRNA initiation fragments; R (pKV554), TR (pKV554T) and T′R (pKV561). R= ribosomal mRNA, IFN=IFN mRNA.

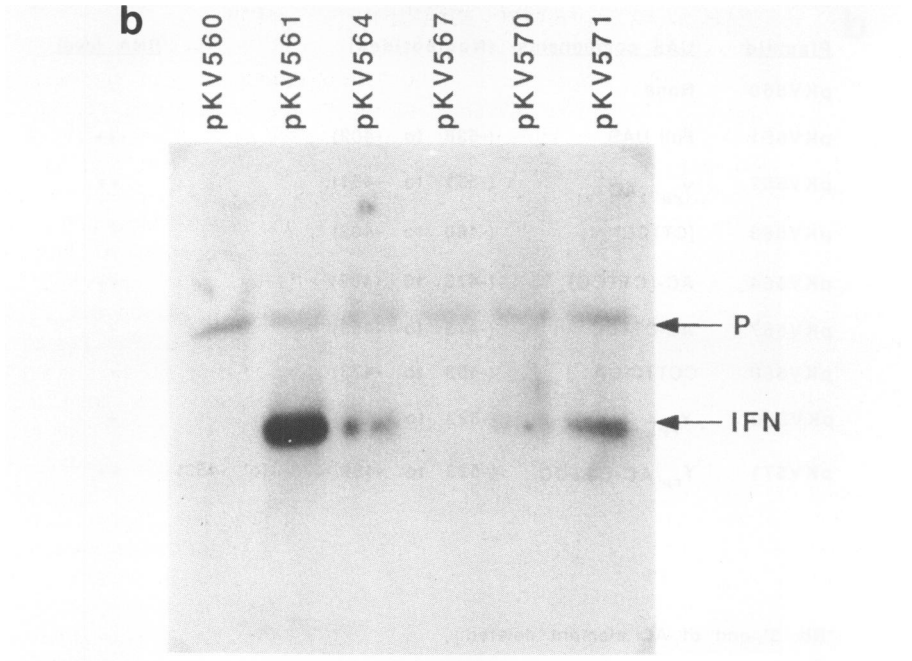
b. Primer extension analysis mapping the 5′ end of the transcripts encoded by the *PGK* UAS fused to the oligonucleotides TR (pKV544T) and T′R (pKV561) compared with the IFN transcript from pMA91-8. The four lanes on the left of the figure are a dideoxy sequencing reaction, using the same IFN primer, run as markers. EP= extension product.

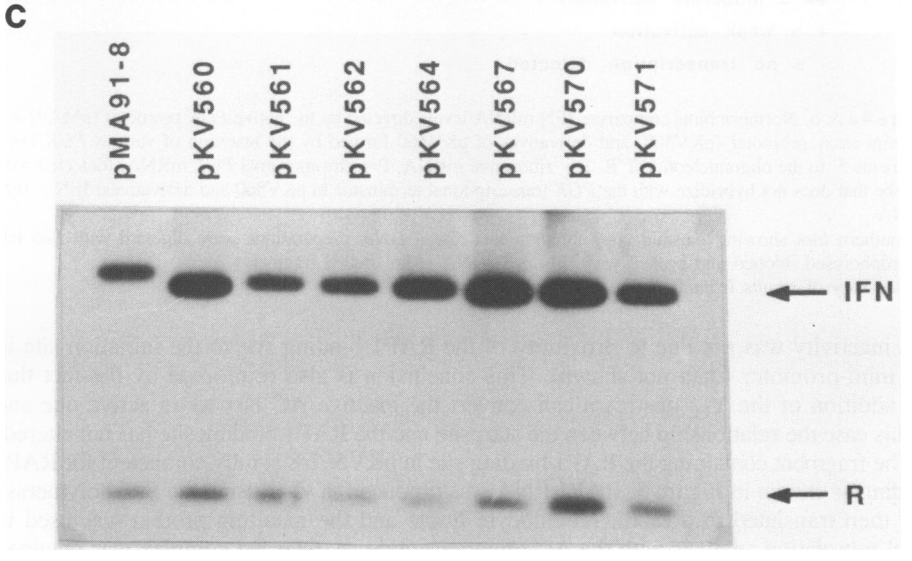
Analysis of the transcriptional activity of PKG UAS subfragments

Various restriction fragments and synthetic oligonucleotides that corresponded to functional UAS sub-elements previously defined by deletion and footprinting analyses were inserted into pKV560 (Figure 2). These UAS sub-fragments and the resulting plasmid designations are detailed in Figure 2b. Transcriptional potency was assessed by Northern blot analysis (Figure 4a, b) and confirmed by plasmid copy number analysis (Figure 4C and data not shown. pKV561 containing the entire UAS was as efficient as pMA91-8 that contains the entire *PGK* promoter. pKV 562 that contains only Y_{FP} and AC and lacks any of the CTTCC boxes was less active than the full UAS. pKV564 that contains the AC box and the three CTTCC boxes but no Y_{FP} was also significantly less active. pKV563 that contains only the three CTTCC boxes was inactive. The AC box plus a single CTTCC element in both orientations (pKV567 and pKV568) was also inactive. The Y_{FP} alone (pKV570) was barely active. When however the Y_{FP} was added to AC plus a single CTTCC (pKV571) there was significant activity. The plasmid copy number data (Figure 4c) shows that only when there is no promoter activity is there any significant increase in copy number, which indicates that the mRNA levels observed are a direct consequence of promoter capacity. These data are summarised in Figure 4d. They indicate firstly that no individual element within the *PGK* UAS is capable of producing high efficiency expression. Secondly, although the Y_{FP} and CTTCC regions have little or no individual activity either of them in combination with the AC box generate a moderately efficient promoter.

The finding that the AC box was not capable of activating transcription was somewhat surprising given that the RAP1 protein that binds to this region has been proposed to be a transcriptional activator protein. The same fragment was also inactive when placed in a UAS deletion of the *PGK* promoter spanning nucleotides −538 to −397 indicating that




b
pKV560
pKV561
pKV564
pKV567
pKV570
pKV571
P
IFN


c
pMA91-8
pKV560
pKV561
pKV562
pKV564
pKV567
pKV570
pKV571
IFN
R

d

Plasmid	UAS components	(Nucleotides)	RNA level
pKV560	None		-
pKV561	Full UAS	(-538 to -402)	+++
pKV562	Y_{FP}- AC*	(-531 to -461)	++
pKV563	$[CTTCC]_3$	(-460 to -402)	-
pKV564	AC-$[CTTCC]_3$	(-473 t0 -409)	++
pKV567	AC-CTTCC	(-473 to -453)	-
pKV568	CCTTC-CA	(-453 to -473)	-
pKV570	Y_{FP}	(-523 to -496)	+
pKV571	Y_{FP}/AC-CTTCC	(-523 to -496/-473 to -453)	++

*Nb. 3' end of AC element deleted.

+++ = strong activation

++ = moderate activation

+ = weak activation

- = no transcription detected

Figure 4 a & b. Northern blots comparing IFN mRNA levels directed by the native *PGK* promoter (pMA91-8), the mini assay promoter (pKV560) and derivatives of pKV560 formed by the insertion of various *PGK* UAS fragments 5' to the oligonucleotide T'R. R= ribosomal mRNA, P= chromosomal *PGK* mRNA, (detected with a probe that does not hybridize with the *PGK* transcriptional terminator in pKV560 and derivatives) IFN= IFN mRNA.
c. Southern blot showing plasmid copy number data. Total DNA preparations were digested with *Eco* RI, electrophoresed, blotted and probed with IFN and rDNA radiolabelled fragments.
d. Summary of results from Figs. 4a, b and c.

this inactivity was not due to proximity of the RAP1 binding site to the initiation site in the mini-promoter (data not shown). This conclusion is also reinforced by the fact that the addition of the Y_{FP} upstream can convert the inactive AC box to an active one and in this case the relationship between the start site and the RAP1 binding site has not altered.

The fragment containing the RAP1 binding site in pKV567/8 is fully competent for RAP1 binding as shown in Figure 5. RAP1 RNA was produced in vitro using the SP6 polymerase and then translated in a rabbit reticulocyte lysate and the resulting protein was used in a gel retardation analysis with the AC oligonucleotide. A retarded complex was obtained that was specific and that had identical mobility to a nuclear extract derived complex that has previously been proven to be due to RAP1 (Figure 5; 12; A.C., J Tsang, C.A.S., A.J.K. & S.M.K., manuscript in press, Mol. Cell. Biol.). This suggests that simple binding

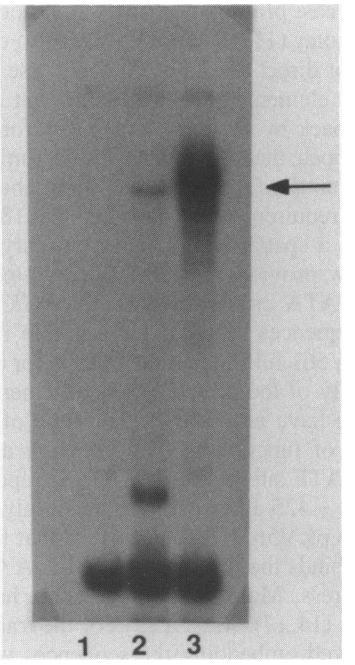


Figure 5 Retardation gel assay showing that RAP1 binds to the *PGK* UAS AC sequence.
The AC/CTTCC (nucleotides 473 to −453) element was radiolabelled. Lane1: fragment only. Lane2: fragment plus 200ng calf thymus DNA, plus 1 μg nuclear protein extract. Lane 3 : fragment plus 200ng calf thymus DNA, plus 10 μg RAP1 *in vitro* translation reaction.

of RAP1 to its cognate site should occur *in vivo* in the mini-promoter and therefore binding of RAP1 alone is not in itself sufficient to generate an active transcription complex. The interaction of the AC box with either Y_{FP} or CTTCC regions was sufficient to confer activity on the mini-promoter. This suggests that RAP1 is capable of indiscriminate association with DNA binding proteins to generate an active transcription complex.

DISCUSSION

We have shown that a short 91 bp DNA fragment derived from the *PGK* promoter is capable of directing highly efficient transcription when activated by the *PGK* UAS. The efficiency of transcription is equivalent to that directed by the whole *PGK* promoter and furthermore we have recently demonstrated that this *PGK* UAS mini promoter is carbon source regulated in the same way as the whole promoter (A.C., J Tsang, C.A.S., A.J.K. & S.M.K., manuscript in press, Mol. Cell. Biol.). This fragment contained the CT block-CAAG sequence that is a common feature of highly expressed yeast genes (33). In order to obtain accurate initiation as well as high efficiency it was essential to add a TATA box region upstream. When a short TATA box fragment was used (T) there was some initiation at the authentic site but significantly more initiation at a site 19 or18 base pairs downstream. A longer TATA fragment that had an additional downstream 18 bp (T′) resulted in accurate initiation. These data confirm some previous studies on the requirements for the TATA

box in the *PGK* and other yeast promoters. Firstly it is not important for transcriptional efficiency of the *PGK* promoter (11). Secondly it helps to constrain initiation to a single site (9,10). Thirdly it cannot direct the high efficiency use of a site that is within 40 bp of the 3′ end of the TATA element (9). The finding that spacing the TATA box back 18 bp moved the start site back by 18 bp was probably fortuitous and given the data of others (9,10) we would anticipate that moving the TATA further upstream would still effect initiation within the CAAG until a far upstream point at about 120 bp upstream from the I site had been reached. The requirement for the additional 18 bp downstream of the TATA element most likely reflects a spatial rather than a primary sequence requirement. This mini-promoter however now provides a well defined system for defining the 5′ and 3′ limits of the sphere of the TATA influence within the *PGK* promoter and for identifying any additional *cis* active sequences required for accurate initiation.

The mini-promoter in pKV561 fulfilled all the criteria for an assay system for screening for the transcriptional activity of the three UAS sub-elements, i.e. the Y_{FP}, the AC box and the CTTCC boxes. We have now shown that none of the three different elements within the UAS is capable of functioning independently as a transcriptional activator. Furthermore the potential yATF site in the *PGK* UAS, reported by Lin and Green (34), located between nucleotides −425 and −415, is apparently inactive even in conjunction with the CTTCC boxes (see pKV563). The discovery that the AC box was inactive was unexpected. This sequence binds the protein RAP1 (12, A.C., J Tsang, C.A.S., A.J.K. & S.M.K., manuscript in press, Mol. Cell. Biol.) which has been proposed to function as a transcriptional activator (14,17). Most assays of the transcriptional activity of RAP1 have however simply involved embedding the sequences within a larger promoter that has had its own UAS deleted (14,17,35). Clearly there is potential for interactions with other sequences in such analyses. In one study for example the RAP1 sequence would only function in the *CYC1* promoter when placed approximately 100bp upstream from the mRNA start site (35). Our data suggest that there is no spatial constraint upon the function of the RAP1 site and therefore we would interpret this finding as indicating a requirement for flanking sequences. Our data suggest that RAP1 is rather indiscriminate in its interactions with other sequences. The addition of either Y_{FP} or the CTTTC boxes to the RAP1 site constitutes a transcriptional activator. Other studies have suggested that the efficiency of activation by RAP1 and/or its propensity to function as either an activator or repressor are influenced by flanking sequences (14,17). Our data suggest that rather than an influence of flanking sequence there is an absolute requirement for some interaction for RAP1 to function within the *PGK* UAS. The nature of this interaction is at present obscure. Y_{FP} is known to bind a protein and the CTTCC boxes show a weak retardation complex under some conditions indicating the presence of a CTTCC binding factor (CBF) (J. Tsang, A.C., A.J.K. & S.M.K. manuscript in preparation). Dorsman *et al.* (36) have reported the existence of a transcription factor, GFI that binds to the promoters of various nuclear-encoded mitochondrial genes. The binding site consensus for GFI is compatible with the Y_{FP} sequence suggesting that they are related or even identical. It is not clear whether RAP1 is augmenting the transcriptional activation potential of these proteins or whether these proteins are augmenting the transcriptional activation potential of RAP1. Given our observations in the *PGK* promoter and the almost ubiquitous nature of RAP1 binding sites in yeast promoters and their presence in inactive genes and structural elements of chromosomes we suspect that RAP1 is in fact not a transcriptional activator in the classical mode of GAL4 (37) and GCN4 (38). This may be supported by recent data that indicate

that RAP1 may be involved in directing and/or anchoring DNA to the nuclear scaffold (18). There has however been no convincing demonstration that nuclear scaffold attachment is a prerequisite for transcriptional activation in yeast.

It may also be significant that *PGK* mini-promoter can be activated to very high efficiency by the insertion of the 12 bp recognition site (E2RE) for the papillomaviral protein E2. When E2 is expressed in yeast the E2RE mini-promoter is activated to the same levels as the full length *PGK* promoter (32). This then prompts two questions. Why is the *PGK* UAS composed of multiple elements that must cooperate to produce a highly efficient promoter when this can be achieved by a single element? and what are the features of the papillomavirus E2 protein that allow it to replace the combination of RAP1, Y_{FP} and CBF? It is possible that each of these proteins represent different essential functions of a transcriptional activator that can be provided by the multiple domains in a single viral protein. If this is the case then these functions are likely to be more than the simple provision of acidic regions that are all that is required to activate transcription in several other yeast genes (37,38).

ACKNOWLEDGEMENTS

This work was supported by the SERC LINK programme and AFRC. We would like to thank Elaine Byles for excellent technical assistance, Rob Docherty, Jimmy Tsang and Yves Henry for helpful discussions, and Ken Johnson for photographic work. C.A.S. gratefully acknowledges the award of the Florey Research Fellowship from Lady Margaret Hall.

*To whom correspondence should be addressed

+Present address: Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford OX3 9DU, UK

REFERENCES

1. Holland, M.J. and J.P. Holland. 1978. Biochemistry **17:** 4900–4907.
2. Tuite, M.F., M.J. Dobson, N.A. Roberts, R.M. King, D.C. Burke S.M. Kingsman and A.J. Kingsman. 1982. EMBO J. **1:** 603–608.
3. Dobson, M.J., J. Mellor, A.M. Fulton, N.A. Roberts, B.A. Bowen, S.M. Kingsman and A. J. Kingsman. 1984. EMBO J. **3:** 1115–1119.
4. Stanway, C.A., A.J. Kingsman and S.M. Kingsman. 1987. Bioessays **7:** 62–67.
5. Struhl, K. 1987. Cell **49:** 295–297.
6. Guarente, L. 1988. Cell **52:** 303–305.
7. Buratowski, S., S. Hahn, P.A. Sharp and L. Guarente. 1988. Nature **334:** 37–42.
8. Cavallini, B., J. Huet, J-L. Plasat, A. Sentenac, J-M. Egly and P. Chambon. 1988. Nature **334:** 77–80.
9. Chen, W. and K. Struhl. 1985. EMBO J. **4:** 3273–3280.
10. Nagawa, F. and G.R. Fink. 1985. Proc. Natl. Acad. Sci. USA. **82:** 8557–8561
11. Ogden, J.E., C. Stanway, S. Kim, J. Mellor, A.J. Kingsman and S.M. Kingsman. 1986. Mol. Cell. Biol. **6:** 4335–4343.
12. Chambers, A., C. Stanway, A.J. Kingsman and S.M. Kingsman. 1988. Nucl. Acids Res. **16:** 8245–8260.
13. Stanway, C., J. Mellor, J. Ogden, A.J. Kingsman and S.M. Kingsman. 1987. Nucl. Acids Res. **15:** 6855–6873.
14. Shore, D. and K. Nasmyth. 1987. Cell **51:** 721–732.
15. Buchman, A.R., W.J. Kimmerly, J. Rine and R.D. Kornberg. 1988. Mol. Cell. Biol. **8:** 210–225.
16. Huet, J. and A. Sentenac. 1987. Proc. Natl. Acad. Sci. **84:** 3648–3652.
17. Buchman, A.R., N.F. Lue and R.D. Kornberg. 1988. Mol Cell. Biol. **8:** 5086–5099.
18. Hofmann, J. F-X., T. Laroche, A.H. Brand and S.M. Gasser. 1989. Cell **57:** 725–737.

19. Piper, P.W., B. Curran, M.W. Davies, K. Hirst, A. Lockheart, J.E. Ogden, C.A. Stanway, A.J. Kingsman and S.M. Kingsman. Nucl. Acids Res. **16:** 1333–1348.
20. Tuite, M.F., P. Bossier, I.T. Fitch. 1988. Nucl. Acids Res. **16:** 11845.
21. Kim, S., J. Mellor, A.J. Kingsman and S.M. Kingsman. 1986. Mol. Cell. Biol. **6:** 4251–4258.
22. Miller, J.H. 1972. Experiments in molecular genetics. Cold Spring Harbour Laboratory, Cold Spring Harbour, N.Y.
23. Hawthorne, D.C. and R.K. Mortimer. 1960. Genetics **45:** 1085–1110.
24. Hinnen, A., J.B. Hicks and G.R. Fink. 1978. Proc. Natl. Acad. Sci. **75:** 1929–1933.
25. Maniatis, T., E.F. Fritsch and J. Sambrook. 1982. Cold Spring Harbour Laboratory, Cold Spring Harbour, N.Y.
26. Holm, C., D.W. Meeks-Wagner, W.L. Fangman and D. Botstein. 1986. Gene **42:** 169–173.
27. Thomas, P. 1980. Proc. Natl. Acad. Sci. USA 77: 5201–5202.
28. Southern, E.M. 1975. J. Mol. Biol. **98:** 503–517.
29. Rigby, P.W.J., N.M. Dieckmann, C, Rhodes and P. Berg. 1977. J. Mol. Biol. **113:** 237–251. 34.
30. Mellor, J., M.J. Dobson, N.A. Roberts, A.J. Kingsman and S.M. Kingsman. 1985. Gene **33:** 215–226.
31. Reddy, V.B., P.K. Gosh, P. Lebowitz, P. Piatak and S. Weissman. 1979. J. Virol. **30:** 279–296.
32. Stanway, C.A., M.P. Sowden, L.E. Wilson, A.J. Kingsman and S.M. Kingsman. 1989. Nucl. Acids Res. **17:** 2187–2196.
33. Dobson, M.J., M.F. Tuite, N.A. Roberts, A.J. Kingsman, S.M. Kingsman, R.E. Perkins, S.C. Conroy and L.A. Fothergill. 1982. Nucl. Acids Res. **10:** 2625–2637.
34. Lin,Y-S. and M.R. Green. 1989. Proc. Natl. Acad. Sci. USA. **86:** 109–113.
35. Woudt, L., W.H. Mager, R.T.M. Nieuwint, G.M. Wassenaar, A.C. van der Kuyl, J.J. Murre, M.F.M. Hoekman, P.G.M. Brockhoff and R.J. Planta. 1987. Nucl. Acids Res. **15:** 6037–6048.
36. Dorsman, J.C., W.C. van Heeswijk and L.A. Grivell. 1988. Nucl. Acids Res. **16:** 7287–7301.
37. Ma, J. and M. Ptashne. 1987. Cell **48:** 847–853.
38. Hope, I.A. and K. Struhl. 1986. Cell **46:** 885–894.

This article, submitted on disc, has been automatically converted into this typeset format by the publisher.