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A heat shock element in the phosphoglycerate kinase gene promoter of yeast

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ABSTRACT

The phosphoglycerate kinase (PGK) promoter is often employed in yeast expression vectors due to its very high efficiency. Its activity in unstressed cells has been shown to be due to an upstream activator site (UAS_{PGK}) at -402 to -479. Since levels of PGK mRNA can sometimes be elevated by heat shock of yeast cultures this investigation determined how specific deletions of PGK promoter sequences affect levels of PGK mRNA both before and after heat shock. A series of PGK promoter deletions was inserted on a high copy plasmid into cells having a TRP1 gene disruption of the solitary chromosomal PGK locus. This enabled PGK transcripts of plasmid and chromosomal origin to be distinguished by virtue of their different sizes. Certain deletions lacking UAS_{PGK} displayed activities that were very low in unstressed cells, but which increased fifty to one-hundred fold after heat shock. With UAS_{PGK} present heat shock had only a relatively small or negligible effect on PGK mRNA levels. Heat shock activation was abolished when the -256 to -377 region with homology to the heat shock element consensus of eukaryotes was deleted in addition to UAS_{PGK}, but was unaffected by the deletion of regions further downstream containing TATA- and CAAT- sequence motifs. This is the first demonstration of a heat shock element, an activator site normally found upstream of eukaryotic heat shock protein genes, as a natural constituent of a high efficiency glycolytic promoter. It is proposed that PGK may be one member of a small subset of yeast genes that are highly expressed in unstressed cells yet possess a heat shock element to ensure their continued transcription after heat shock.

INTRODUCTION

Much of the interest in the phosphoglycerate kinase (PGK) gene of *Saccharomyces cerevisiae* stems from its very high level of expression, this single gene contributing no less than 1-5% of the total protein of the cell [1]. Because of this efficient expression the PGK promoter is frequently employed in yeast expression vectors as a sequence which will direct high levels of transcription of heterologous genes carried on these vectors [2-5]. Detailed deletion analysis of PGK 5' noncoding sequences has revealed some of the requirements for efficient functioning of this promoter. An upstream activator site (UAS_{PGK}) located between -402 and -479 nucleotides relative to

the initiation codon of the protein is needed for full transcriptional activity in unstressed cells [6,7]. UAS_{PGK} functions efficiently in either orientation and can also stimulate transcription of a TRP1-IFN gene fusion comprising the promoter of the yeast TRP1 gene fused to the coding region of human α-2 interferon [6,7].

This paper reports evidence that the yeast PGK promoter also possesses, unexpectedly for a wild-type glycolytic promoter, a functional heat shock element (HSE). This lies adjacent to UAS_{PGK} at -359 to -372, and represents the second PGK activator element to be identified so far. Its effect on PGK mRNA levels is marked only when UAS_{PGK} is deleted. HSEs are the upstream elements of heat shock protein genes to which heat shock transcription factor is bound as it activates the transcription of these genes in heat stressed cells [8-11]. They have previously been demonstrated in genes expressed in unstressed cells, such as the human hsp70 gene (expressed at a low basal level [12]) or the rat hsc73 gene (expressed at a higher level in the absence of heat shock [13]). However this is the first time a naturally-occurring HSE has been shown functioning within a gene that is not heat shock protein encoding, or a member of one of the families of hsp70-related genes [8].

The presence of this HSE in a glycolytic promoter poses questions with regard to its function. We propose that this function may be to sustain transcription of the yeast PGK gene after heat shock at a time when the transcription of non-heat shock genes is terminated abruptly. A similar role might be attributable to the HSEs identified by Sorger and Pelham [13] in hsc73, a rat hsp70-cognate gene.

MATERIALS AND METHODS

Strains and media

The haploid *S. cerevisiae* strains used were MD40-4c (α ura2, trp1, leu2-3, leu2-112, his3-11, his3-15) and DBY747 (a ura3-52, trp1, leu2-3, leu2-112, his3). The diploid BC1 was derived by mating MD40-4c and DBY747 and selected as a uracil prototroph. Yeast cultures were grown in shaking flasks at 25°C in rich (1% yeast extract, 2% peptone) medium containing 2% (w/v) glucose (YEPD); 3% (v/v) glycerol (YEPG) or 3% glycerol plus 2% ethanol (YEPGE) or complete minimal (CMD) medium (0.67% yeast nitrogen base, 2% glucose, plus when necessary uracil (20 mg/L), histidine (20 mg/L), tryptophan (20 mg/L) and leucine (30 mg/L)). Yeast mating, sporulation and tetrad dissection were as in Hawthorne & Mortimer [14]. *Escherichia coli* JA221 (recA1, leuB6, trp E5, $hsdM^+$, $hsdR^-$, lacY, C600) was grown on 2xYT. For

plasmid propagation 50 μg/ml ampicillin was added, or minimal medium supplemented with tryptophan but not leucine [15] was employed.

Heat shock of yeast and RNA analysis

The procedure adopted for heat shocking yeast cells employed cells in the maximal rate of exponential growth at 25°C. This was because earlier experiments had indicated that the heat shock elevation of PGK mRNA levels was greatest with the most rapidly dividing cultures [16]. A fresh CMD plate culture of the transformant under study was used to innoculate a 10 ml CMD medium minus leucine culture (conditions selective for plasmid maintenance) which was then grown at 25°C overnight to 3-5x10^6 cells ml^{-1}. These cells were subcultured at 25°C in 60 ml YEPD (initial density 0.5-1.5x10^5 cells ml^{-1}) for 7-8 generations. 30 ml of the culture was then harvested for RNA preparation (the minus heat shock sample), and the remaining 30 ml placed at 38°C for 40 min before RNA (the plus heat shock sample) was prepared. Total nucleic acid was prepared by glass bead vortexing and PGK mRNA to Ty mRNA ratios were measured as in Piper et al [17].

Transformation of E. coli and yeast

E. coli was transformed by standard procedures [15]. Yeast was transformed by the method of Hinnen et al [18]. S. cerevisiae MD40-4c transformants are designated as in ref. 6 as T followed by the number of the pMA series plasmid used in their derivation (e.g. T767 is MD40-4c transformed with pMA767). The 3.4 kb double HindIII fragment used to transform the diploid BC1 in the derivation of BC2.2 was from a clone of this sequence in pBR322 (plasmid pMA958; gift of M. Dobson). TRP^+ haploid segregants of BC2.2 transformed with pMA-series plasmids are designated T/TRP, followed by the number of the plasmid they contain (e.g. T/TRP767 is a TRP^+ LEU^+ segregant of BC2.2 transformed with pMA767).

Construction of plasmids containing two internal deletions in the PGK promoter

The internal deletions in the 5' noncoding region of PGK that were employed have been described by Ogden et al [6]. For this study the UAS_{PGK} deletion of pMA767 was placed in the same plasmid as deletions removing one or more of the TATA- and CAAT- region sequences that lie much nearer to the coding region. This was performed as summarised in Fig. 6, exploiting the PvuI restriction site that lies in the promoter between UAS_{PGK} and these other sequences. Plasmid pMA767, possessing a deletion of UAS_{PGK}, was digested with PvuI and the 2.4 kb and 2.6 kb LEU2d gene-bearing fragments isolated. Plasmids pMA773, pMA774, pMA775, pMA776 and pMA777 with deletions on the

opposite side of the PvuI site of the promoter were digested with PvuI and their 5.7 kb PvuI products isolated. This was followed by ligation of each 5.7 kb fragment to the two smaller fragments of pMA767, followed by selection for ampicillin resistance and complementation of the leuB⁻ mutation in E. coli JA221 (Fig. 6). The pMA-plasmid derived in this way was numbered so to indicate its derivation (e.g. pMA767/773 was derived from both pMA767 and pMA773 (Fig. 1) and contains the promoter deletions of both these plasmids).

Plasmid copy number determination

RNA for Northern analysis and DNA for plasmid copy number determination were always prepared from the same yeast pellet. Total nucleic acid was prepared from each cell pellet by the glass bead vortexing procedure [17]. Approximately 40 µg of this material was subjected to ribonuclease A digestion (4.0 µg enzyme, 40 µl 10 mm Tris-HCl pH7.5, 1 mM EDTA, 30 min 37°C), followed by extraction with phenol and precipitation of the aqueous phase with 2.5 volumes ethanol. The resulting pellets were redissolved in 10 µl EcoRI digestion buffer, digested 1 h 37°C with 4U EcoRI restriction endonuclease, electrophoresed on 1% agarose gels and transferred to Biodyne filters according to standard procedures [15]. The filters were hybridised to a probe comprising sequences of the 2.95 kb HindIII fragment insert of plasmid pMA1, labelled by nick-translation [17]. The bands corresponding to the plasmid PGK gene and to the chromosomal PGK gene (Fig. 2) were cut out of the blots and counted in toluene-based scintillant. The ratio of their respective labellings was a measure of the number of plasmid copies in the transformed cells at the time of heat shock experiments.

RESULTS

The effects of promoter deletions on the response of PGK mRNA levels to heat shock, measured in transformants of strain MD40-4c

The effects of the deletions in Fig. 1a on the PGK mRNA levels of strain MD40-4c are described in ref. 6 for 30°C growth conditions that ensure maintenance of the deleted PGK gene on a multicopy plasmid. In this study we measured initially the effects of 25°C to 38°C heat shock on PGK mRNA levels in the same transformants (Fig. 1a). Unlike in the study of Ogden et al [6] these measurements were made after 7 to 8 generations growth in rich glucose medium (YEPD), conditions nonselective for plasmid maintenance (Materials and Methods). This caused a reduction in plasmid copy number (see below). However it also minimised cell doubling time and was adopted because the heat shock effect on PGK mRNA levels in MD40-4c is greatest in the most rapidly

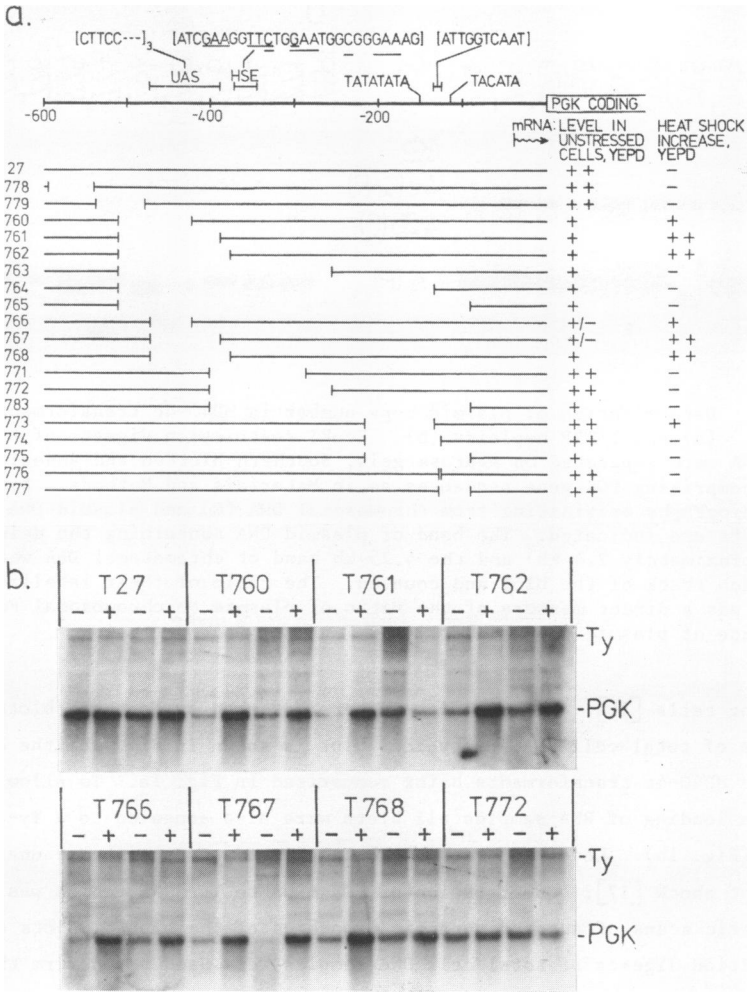


Fig. 1 (a) The effect of heat shock on PGK mRNA levels in MD40-4c transformants after growth for 7-8 generations at 25°C on YEPD. The 25°C expression values are represented, similar to Ogden et al [6] by : ++, levels comparable to wild type promoter of T27; +/-, levels 10% or less of wild type; +, intermediate levels. The heat shock expression values, measured 40 min after 25°C to 38°C shift are : - less than 2-fold increase in PGK mRNA relative to unstressed level; +, 2-6 fold increase relative to unstressed level; ++, more than a 6-fold increase. At the top of this figure the limits of the UAS, and the homology to HSE and TATA consensus sequences are shown.

(b) Sample Northern blot of the RNA of individual MD40-4c transformants before (-) and after (+) heat shock probed for PGK and Ty mRNAs. For each transformant four samples are shown, the two on the left being from a culture grown on glucose (YEPD) and the two on the right from a culture grown on glycerol (YEPG).

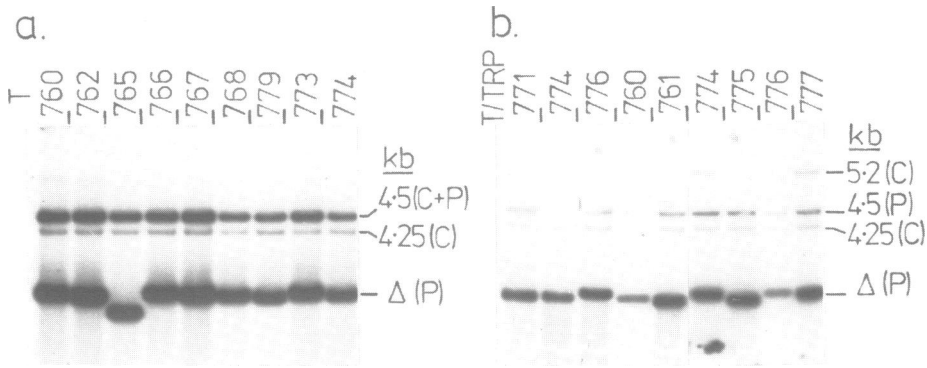


Fig. 2 Determination of plasmid copy number in MD40-4c transformants (a) and T/TRP haploids (b). EcoRl restriction digests of total cell DNA were separated on agarose gels, Southern blotted and annealed to a probe comprising PGK gene sequences as in Materials and Methods. Bands on the autoradiographs originating from chromosomal DNA (C) and plasmid DNA (P) fragments are indicated. The band of plasmid DNA containing the deletion (Δ, approximately 2.4 kb) and the 4.25 kb band of chromosomal DNA were excised from each track of the blot and counted. The ratio of their labelling in each sample was a direct measure of the ratio of plasmid to chromosomal PGK loci and hence of plasmid copy number.

dividing cells [16]. PGK mRNA levels were measured by Northern blotting of samples of total cell RNA. A typical blot is shown in Fig. 1b, the data for all the MD40-4c transformants being summarised in Fig. 1a. To allow for non-uniform loading of RNA samples all blots were also annealed to a Ty-specific probe (Fig. 1b). Levels of the 5.7 kb Ty element transcript are unaffected by the heat shock [17]. Quantitation of PGK mRNA to Ty mRNA ratios was by densitometric scans of autoradiographic exposures of the blots. Blots of EcoRl restriction digests of total cell DNA samples were used to confirm that each of the MD40-4c cultures had retained its plasmid at the time of RNA preparation, and that there had been no integration of plasmid-derived PGK sequences into chromosomal DNA (Fig. 2a).

The relative levels of PGK mRNA after 25°C YEPD growth of these MD40-4c transformants are listed under "level in unstressed cells" in Fig. 1a. They were essentially identical to the results of Ogden et al [6] except that T775 and T783 both gave intermediate levels of expression. This earlier study had defined as "Class 1" deletions those which lack the UAS_{PGK} (-479 to -402) essential for high transcription in the absence of heat shock, and as "Class 2" deletions those which retain UAS_{PGK} and which consequently give levels of

transcription similar to that of the wild type gene. All Class 2 deletions yielded either no increase in PGK mRNA with heat shock, or else a relatively small increase. In contrast some Class 1 deletions (T760, T761, T762, T766, T767, T768) showed a relatively strong heat shock activation, while others displayed none (T763, T764, T765). The PGK mRNA levels of T760, T761, T762, T766, T767 and T768 were low in unstressed cells but almost as high 40 min after heat shock as the extraordinarily high PGK mRNA level produced by the wild type PGK gene on the same vector (T27, Fig. 1b). Therefore heat shock restores to the promoters of T760, T761, T762, T766, T767 and T768 high, approximately wild type levels of promoter activity, presumably through the action of an activator element other than UAS_{PGK}. That this is a heat shock element (HSE_{PGK}) is also indicated since the -256 to -377 region was required for a marked heat shock response with Class 1 deletions, and it contains at -359 to -372, the only good (7/8 bp) agreement to the HSE consensus of eukaryotes in the 770 bp upstream of the coding region (Fig. 1a). Heat shock can increase PGK mRNA levels dramatically in cells bearing several copies of the PGK gene on a multicopy plasmid only when the basal activity of the promoter is low through the absence of UAS_{PGK}. A response similar to that of a normal heat shock promoter (low basal expression, high expression after heat shock) can then ensue provided the -256 to -377 region is present. It can be observed in both fermentative (glucose) and aerobically respiring (glycerol) cultures, as shown in Fig. 1b.

Among the MD40-4c transformants displaying virtually no increase in PGK mRNA with heat shock was T27 (Fig. 1b). We have consistently found an increase of up to 6 to 7-fold in PGK mRNA with heat shock in strains with a single chromosomal PGK gene [16,17] but a negligible or less than 2-fold increase in transformants overexpressing the PGK gene through possession of the wild type gene (T27) or, with one exception (T773), Class 2 promoter deletions on a multicopy plasmid. T773 showed an increase of approximately fourfold. The reasons why overexpression of the PGK gene results in an apparent reduction in the heat shock effect on levels of its transcripts have yet to be determined.

Plasmid copy number of MD40-4c transformants at the time of PGK mRNA measurements

In medium minus leucine MD40-4c transformants carrying plasmids derived from the vector pMA3a have 100-120 plasmid copies per cell (Mellor et al, 1985). It is thought that this high copy number stems in part from weak expression of the LEU2-d gene due to its truncated promoter [4]. The MD40-4c

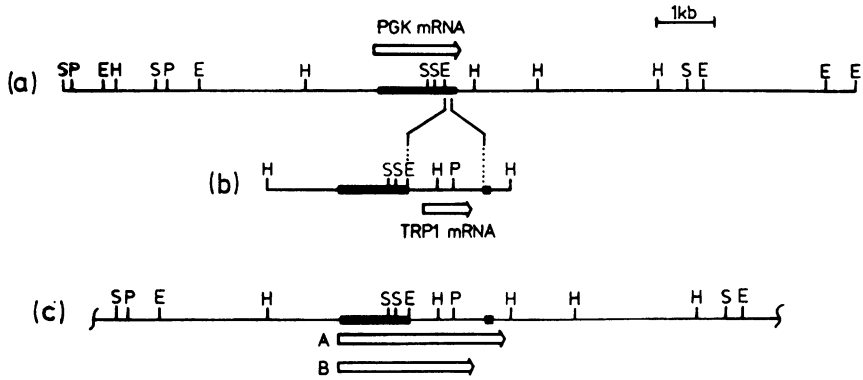


Fig. 3 Derivation of the diploid strain BC2.2. (a) shows a restriction map of the region of yeast chromosome III around the PGK gene. This map was derived by λ mapping [20] applied to a λ isolate picked from a library of yeast sequences in a Charon 30/λ1059 hybrid vector [21]. There is one additional HindIII site that has not been mapped. (b) shows the 3.4 kb double HindIII fragment used to transform the diploid strain BC1 to tryptophan prototrophy and (c) the structure of the disrupted PGK allele of the TRP$^+$ transformant BC2.2. Restriction sites : H = HindIII, E = EcoR1, P = Pst1, S = Sal1. PGK coding sequences are represented by a thicker line. The transcripts unique to BC2.2, A and B in the Northern blots of Fig. 4b, probably originate from the disrupted PGK allele as shown in (c).

transformant cultures used to obtain the data in Fig. 1 had been grown on rich medium for 7-8 generations. When gels of EcoR1 restriction digests of their DNA were stained, plasmid bands were considerably less intense than bands originating from chromosomal ribosomal RNA genes (data not shown), the latter being reiterated 100-120 times in the genome. Plasmid copy number had therefore fallen during nonselective growth. It was determined as 20-30 copies per cell from the relative labelling of plasmid and chromosomal PGK bands on blots as shown in Fig. 2a, and was approximately the same for cultures of each transformant.

The introduction of plasmids containing PGK promoter deletions into cells having a disruption of the chromosomal PGK locus

The transformants of strain MD40-4c used for the PGK mRNA measurements in Fig. 1 still possess a single wild type chromosomal copy of the PGK gene. The contribution to these measurements of the transcripts of this chromosomal gene will be significant in transformants whose plasmid-borne promoter deletions yield only a small percentage of the activity of the wild-type PGK promoter. To obtain more precise measurements of the effects of these promoter deletions on expression it was necessary to distinguish PGK transcripts of plasmid and

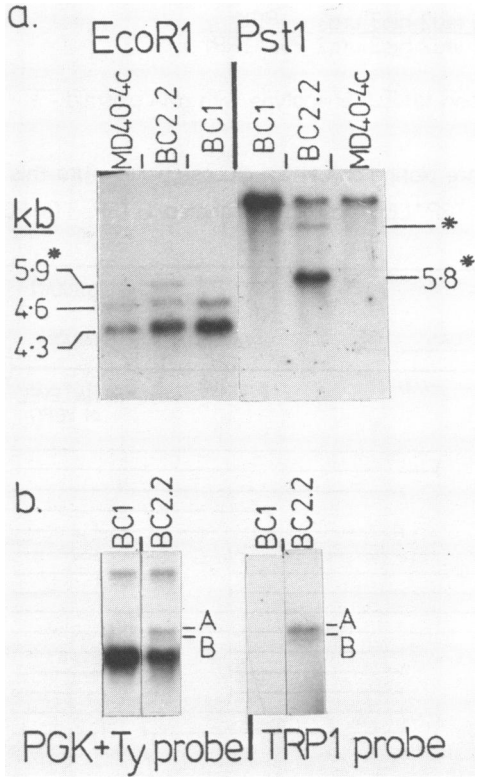


Fig. 4 (a) EcoR1 and Pst1 restriction digests of the DNA of MD40-4c, BC2.2 and BC1 (the strain from which BC2.2 was derived) were Southern blotted and annealed to a probe comprising PGK gene sequences as in Materials and Methods. Additional bands resulting from the disruption of one PGK allele in BC2.2 (see Fig. 3c) are indicated by an asterisk.

(b) Northern blots of the total RNA of BC1 and BC2.2 grown at 25°C on YEPD. These were annealed to the same PGK probe as the blots n (a) plus a Ty-specific probe; or to a probe comprising sequences of the 1.45 kb EcoR1 fragment of the TRP1 gene from plasmid YRp7 [22]. The major (A) and minor (B) bands unique to BC2.2 detected by the PGK & TRP1 probes are indicated.

chromosomal origin. To achieve this the deletion-carrying plasmids were placed in strains that had a chromosomal PGK locus with a TRP1 fragment inserted.

Cells expressing a *pgk*$^-$ phenotype are difficult to manipulate. They grow only on glycerol plus ethanol as carbon source [24] are transformed to PGK$^+$ at only very low frequencies by plasmids carrying the PGK gene, and *pgk*$^-$ spores generally show less than 10% viability (ref. 19, and B.C. unpub-

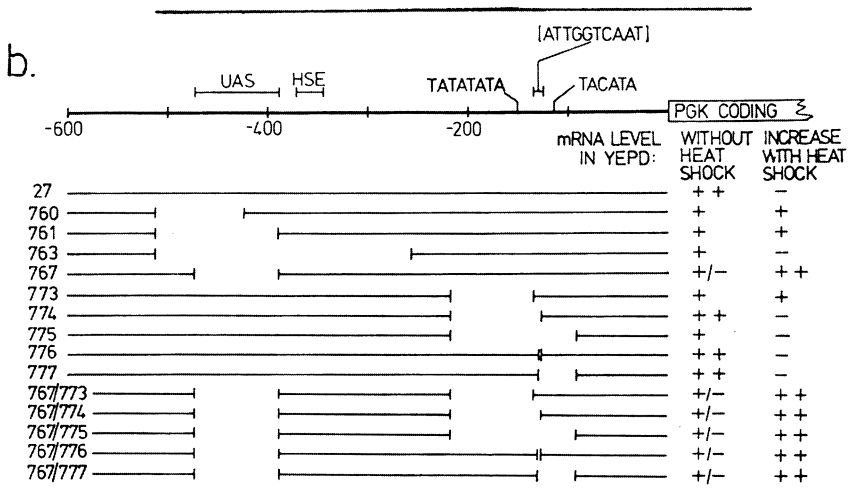
a. BC2.2 $\frac{a}{\alpha}$ $\frac{\text{trp1 leu2 his3 ura3}}{\text{trp1 leu2 his3 ura2}}$ $\frac{\text{PGK}}{\text{PGK::TRP1}}$

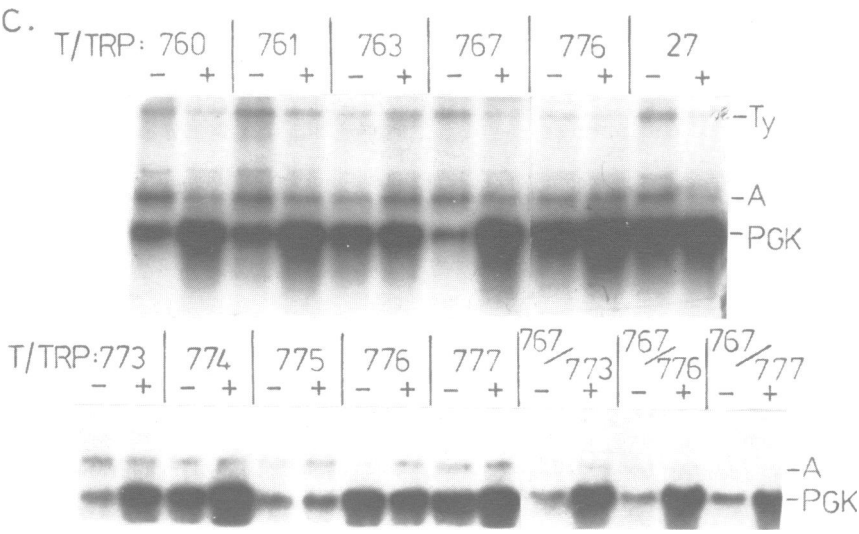
(i) Transformation to LEU$^+$ phenotype with pMA plasmid

(ii) Sporulation

(iii) Random spore plating on CM 2% glucose plates +ura +his

(iv) Selection of TRP$^+$ LEU$^+$ haploid (designated T/TRP...) for PGK expression study





lished results). Because of this we adopted the strategy in Fig. 5a to transfer plasmids to strains disrupted in the chromosomal PGK gene. The diploid BC1 (homozygous for leu2-3,112 and trp1, see Materials and Methods) was initially transformed to tryptophan prototrophy using the 3.4 kb double HindIII restriction fragment in Fig. 3b, in which a fragment containing the TRP1 gene replaces a small EcoR1-Bg1II restriction fragment of the PGK coding region. Southern blot analysis of the DNA of the TRP^+ transformant BC2.2 and its BC1 parent (Fig. 4a) showed that the former had had the TRP1 gene inserted in one of its PGK alleles by homologous recombination. This insertion inactivates this gene since when BC2.2 cells were sporulated more than 90% of spores germinating on YEPGE plates were $trp1^-$ and PGK^+ and the tiny proportion that were TRP^+ all expressed a pgk^- phenotype. Northern blots of the RNA of BC1 and BC2.2 showed two additional RNA bands containing PGK sequences in BC2.2 but not BC1 RNA (A and B, Fig. 4b). The size of the more intense of these bands (A, approximately 1.9 ± 0.1 kb) is consistent with a transcript directed by the promoter of the disrupted PGK allele and terminating in the PGK terminator region (Fig. 3c). The weaker band (B, 1.7 ± 0.1 kb) may indicate that a small proportion of these transcripts terminate at the normal termination site of the TRP1 gene (Fig. 3c). Both are of identical size to RNAs hybridising to a TRP1 gene probe in BC2.2 but not BC1 (Fig. 4b), these RNAs being much larger and more abundant than normal [22,23] TRP1 mRNAs. Also these transcripts are in much lower level than transcripts of the undisrupted PGK allele of BC2.2, yet measurements (not shown) of their disappearance in BC2.2 following the addition of the transcription inhibitor 1,10-phenanthroline indicate that they are not unstable RNAs. These results indicate that the disruption of the PGK coding region in Fig. 3c is causing, like many other

Fig. 5 (a) Steps in the derivation of T/TRP haploid strains having a disrupted chromosomal PGK locus and a variant PGK gene on a multicopy plasmid.

(b) PGK gene expression in both unstressed and heat shocked cells of different T/TRP haploids. The PGK mRNA of cells grown for 7-8 generations at 25°C on YEPD, and its elevation with 25°C to 38°C heat shock are summarised as described in the legend to Fig. 1a.

(c) Typical Northern blots of the RNA of T/TRP haploids before (-) and after (+) heat shock. Note that band A; the presumptive major transcript of the disrupted PGK gene (Figs. 3c, 4b) is showing no appreciable increase with heat shock as judged by its level in relation to the Ty transcript in the upper blot. This disrupted gene has a wild-type PGK promoter and, in common with the wild type PGK gene when on a multicopy plasmid in the same cells (as T/TRP27), shows virtually no activation with heat shock.

disruptions of PGK coding sequences [3,5] an appreciable reduction in transcription from the adjacent PGK promoter.

Strain BC2.2 was transformed to leucine prototrophy with several of the plasmids in Fig. 1a, plus similar plasmids containing double promoter deletions described below. Each transformant was then sporulated, the equiproportional germination of *trp*$^-$ to TRP$^+$ spores in random spore platings indicating that the PGK gene on each plasmid had restored high viability to the TRP$^+$ spores. A LEU$^+$ TRP$^+$ spore colony from each sporulation was selected for further study, this haploid with a disrupted chromosomal PGK gene being designated T/TRP-followed by the number of the pMA series plasmid used in the BC2.2 transformation step of its derivation (e.g. T/TRP27; T/TRP776, etc.).

The responses of PGK mRNA levels to heat shock in T/TRP haploids

The effects of different promoter deletions on the response of PGK mRNA levels to heat shock were measured in T/TRP haploid strains, exactly as in the previous experiments (Fig. 1) that employed MD40-4c transformants. Measurements were again made after 7 to 8 generations of YEPD growth so as to maximise growth rate. A summary of the results is given in Fig. 5b, and typical Northern blots are shown in Fig. 5c. DNA analysis was also performed, as in Fig. 2b, and showed the plasmid copy number per cell to be 20-30 at the time of PGK mRNA measurement for each culture.

The level of PGK transcripts in non-heat-shocked cultures of T/TRP haploids was lowest in the case of T/TRP767 (Fig. 5b,c), although other class 1 deletions tested gave levels not more than five-fold greater (T/TRP760, T/TRP761, T/TRP763). Unstressed T/TRP767 cells yielded transcripts from the plasmid PGK gene (20-30 copies per cell) at only a slightly higher level than transcripts of the solitary disrupted chromosomal PGK gene (Fig. 5c). Since we estimate transcription of the latter to be 15-20% of the level from the wild type PGK gene (from the blot in Fig. 4b), this shows that the promoter of pMA767 has 0.5% to 1% of the activity of the wild type PGK promoter in non-heat-shocked cells. Such low transcription of multiple plasmid sequences is sufficient to restore high viability to *pgk*$^-$ spores, as was exploited in the isolation of T/TRP767. Also T/TRP767 does not grow more slowly on glucose than other T/TRP haploids isolated (data not shown).

The effects of heat shock on PGK mRNA levels in T/TRP haploids are summarised in Fig. 5b. These results are essentially similar to those given by MD40-4c transformants (Fig. 1) but, in view of the absence of transcripts of a wild type chromosomal PGK gene, the measurements of low PGK mRNA levels are a more accurate representation of the activity of promoter deletions.

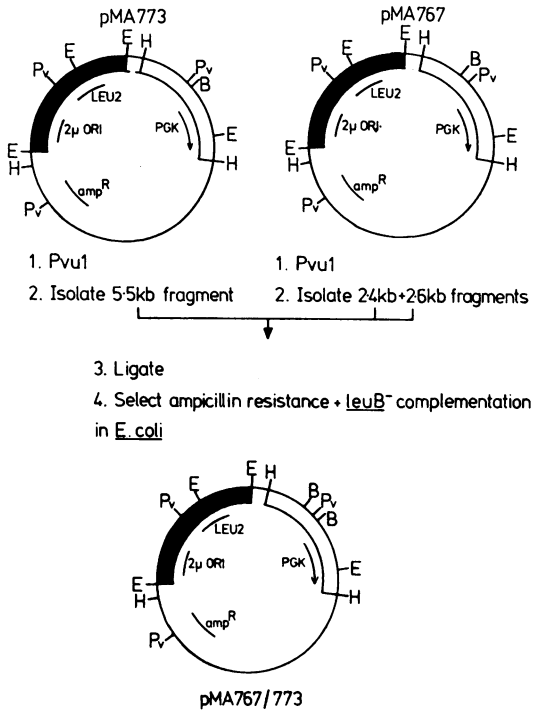


Fig. 6 The procedure for constructing plasmids containing the UAS_{PGK} deletion of pMA767 in common with promoter deletions nearer to the coding region. Construction of pMA767/773 is shown, but that of pMA767/774, pMA767/775, pMA767/776 and pMA767/777 was essentially identical (see Materials and Methods). The position of each promoter deletion corresponds to the BAM restriction site [6]. Open box, HindIII fragment carring the PGK gene with one or two promoter deletions; thin line, pBR322; solid line, 2 μm origin of replication plus LEU2 gene double EcoRl fragment. Restriction sites : Pv = Pvul; B = BAM; E = EcoRl; H = HindIII.

Also the fluctuations induced by heat shock were more marked than with MD40-4c transformants and therefore easier to detect by Northern blotting. Class 1 deletions again gave either a dramatic increase in PGK mRNA with the shock (T/TRP760, 761, 767) or no increase (T/TRP763), while Class 2 deletions gave increases which were negligible or only small (less than two-fold except for T/TRP773 which showed an increase of about five-fold, Fig. 5c). Class 1 deletions responding in the manner expected for a heat shock promoter gave levels of PGK mRNA similar to T/TRP27 after the shock. This showed, as with the results obtained with MD40-4c transformants (Fig. 1), that heat shock can restore to these promoters lacking UAS_{PGK} approximately wild type levels of

promoter activity. For T/TRP767 this represents at least a 50 to 100-fold increase in activity.

The role of TATA- and CAAT- region sequences in the heat shock activation of the PGK gene

Although individual TATA- and CAAT- region sequences downstream of UAS_{PGK} (Fig. 1a) are not essential for the high basal activity of the PGK promoter in unstressed cells [6], it remained possible that they are required for heat shock activation rather than UAS_{PGK} operation. Since with wild type or Class 2 deletion PGK promoters on the plasmid vector there was relatively little if any effect of heat shock on PGK mRNA levels, it was necessary to study the effects of TATA- or CAAT- region deletions on HSE_{PGK} function in promoters lacking UAS_{PGK}. For this purpose the pMA767 deletion was placed in plasmids that also had the deletions of pMA773, pMA774, pMA775, pMA776 or pMA777 (Fig. 6, see Materials and Methods). The resulting plasmids were inserted into the diploid BC2.2 by transformation and a T/TRP haploid segregant containing each one isolated as in Fig. 5a. The effect of these double deletions on the response of PGK mRNA levels to heat shock was essentially unchanged as compared to T/TRP767 (Fig. 5b,c). Therefore the deletion of the -217 to -91 region containing the TATA- and CAAT- sequence elements has little effect on the operation of HSE_{PGK}. This region also contains, at -159 to -172, a 6/8 bp homology to the HSE consensus positioned just upstream of TATA sequences, a positioning similar to the HSEs of many eukaryotic promoters [11]. However this HSE homology can be removed without detriment to the heat shock activation of the pMA767 promoter (Fig. 5b,c), and cannot substitute for deletion of the region containing 7/8 bp homology to the HSE consensus as shown by T/TRP763.

DISCUSSION

HSEs act as the binding sites on DNA for heat shock transcription factor in eukaryotic cells as diverse as HeLa and yeast [8-11]. Many of the most strongly heat-induced genes have two or more HSE-like sequences in their promoters. Yeast may differ from higher eukaryotes in that the binding of the yeast transcription factor to HSE sequences seems not to be sufficient for transcriptional activation by heat shock, a prerequisite phosphorylation state of this factor also being necessary [10]. This study has shown that the -256 to -377 region of the PGK promoter, a region with 7/8 bp homology to the HSE consensus, determines the effect of heat shock on PGK transcription. PGK may not be the only glycolytic gene in yeast with a HSE in its promoter since

one of the three glyceraldehyde-3-phosphate dehydrogenase isoenzymes (hsp35) is also induced by heat shock [8]. PGK and hsp35 are two of the very small number of proteins made as efficiently as heat shock proteins after a 25°C to 38°C heat shock of yeast [16,17]. Only with more severe heat shock to 40-42°C is their synthesis less than that of heat shock proteins [16]. The HSE within the wild type PGK promoter evidently acts differently from HSEs in heat shock protein genes since it maintains or even slightly enhances an already high level of transcription, at a time that transcription of most non heat shock genes stops and heat shock genes are switched from low to high levels of expression. The benefit to heat-stressed cells of a HSE in a glycolytic promoter may be to help ensure continued transcription after heat shock. It has been suggested that the synthesis of certain glycolytic mRNAs in heat stressed cells may be of some assistance in subsequent recovery when more favourable conditions prevail, particularly in fermentative cultures which are devoid of functional mitochondria and therefore highly dependent on glycolysis [16]. HSEs may yet be uncovered in other genes exhibiting high expression levels in unstressed yeast cells, genes which like PGK continue to be transcribed after heat shock. The operation of such HSEs in genes not induced strongly by the shock, and therefore not normally thought of as heat shock protein encoding, may determine a not inconsiderable fraction of the transcription occurring in heat shocked yeast.

This study has shown that the HSE of the PGK promoter has a marked effect on the PGK mRNA levels of heat shocked cells only when the other activator element, UAS_{PGK}, is absent. Sorger and Pelham [13] reported similar findings with the HSEs and other activator elements of the rat hsc73 gene. Although we suggest that the UAS_{PGK} of the PGK promoter becomes inoperative in heat shocked cells and that PGK transcription is then directed by HSE_{PGK}, it would not have been revealed from the effects of specific deletions on PGK mRNA levels whether UAS_{PGK} is still active after the shock. This is because this RNA has a halflife of more than 30 min at 38°C [17] so that PGK mRNA present at the time of the 25°C to 38°C shift will be substantially undegraded 40 min later. The results presented have however confirmed that the deletion of UAS_{PGK} lowers basal promoter activity, shown that the selective removal of this site causes a dramatic effect of heat shock on PGK mRNA levels, and revealed that the PGK promoter has no requirement for specific TATA or CAAT sequences for its heat shock activation. The -126 to -153 region of the PGK promoter has two TATA- box like sequences (TACATA at -116 and TATATATA at -153); also a small region of CAAT dyad symmetry at -134 (Fig. 1a).

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