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Isolation and molecular analysis of the phosphoglucose isomerase structural gene of *Saccharomyces cerevisiae*

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Summary. The *PGI1* gene of *Saccharomyces cerevisiae* coding for the glycolytic enzyme phosphoglucose isomerase has been cloned by complementation of a mutant strain (*pgi1*) with a strongly reduced phosphoglucose isomerase activity. A genomic library constructed in the yeast multicopy vector YEp13 (Nasmyth and Tatchell 1980) was used. Four plasmids containing an overlapping region of 4.1 kb were isolated and characterized by restriction endonuclease mapping. Southern analysis of genomic digests prepared with different restriction enzymes confirmed the same pattern for the chromosomal sequences. Transformants with the isolated plasmids had a phosphoglucose isomerase activity increased by a factor of 7. The cloned sequence hybridized to a constitutively synthesized 2.2 kb RNA in Northern analysis. The coding region includes a 2.05 kb *Eco*RI fragment common to all four inserts. A fragment including part of the *PGI1* region was subcloned into vector YRp7 and used to induce integration at the *PGI1* locus. Genetical and Southern analysis of stable transformants showed that single as well as tandem integration took place at this locus. This showed that the *PGI1* gene had been isolated. Finally, and in contrast to the results of Kempe et al. (1974a, b) who reported three isoenzymes in yeasts, only one copy of the *PGI1* gene per genome was found in several laboratory strains tested by Southern analysis.

Key words: Phosphoglucose isomerase – Glycolysis – *S. cerevisiae* – Gene cloning – Molecular analysis

Introduction

Phosphoglucose isomerase is the enzyme that catalyses the interconversion of glucose-6-phosphate and fructose-6-phosphate in yeasts and in bacteria (for reviews see Fraenkel and Vinopal 1973; Fraenkel 1982). The phosphoglucose isomerase reaction should be needed for growth on sugars whose metabolism proceeds via glucose-6-phosphate and, for growth on other carbohydrates, for the synthesis of glucose-6-phosphate (Fraenkel 1982).

The enzyme is a dimer composed of two identical subunits with a molecular weight of 60,000 (Kempe et al. 1974b). Although the presence of three isoenzymes has been reported (Kempe et al. 1974a) genetical evidence has shown that a single protein is responsible for phosphoglucose isomerase activity in *Saccharomyces cerevisiae* (Maitra and Lobo 1977). Several mutants with reduced phosphoglucose isomerase activity have been selected for their inability to grow on glucose as the sole carbon source (Maitra 1971; Clifton et al. 1978; Ciriacy and Breitenbach 1979). These mutants are affected in the gene *PGI1*, that appears to be the structural gene for phosphoglucose isomerase (Maitra and Lobo 1977). The role of the reported isoenzymes remains unknown.

Although most of the glycolytic genes, including *PGI*, have been cloned in a multicopy vector (Kawasaki and Fraenkel 1982), only increases in enzymatic activities and protein quantities have been reported in the transformants. In order to study the molecular organization of yeast phosphoglucose isomerase, we cloned the *PGI1* structural gene by complementation of a *pgi1* mutant with very low residual activity. Enzymatic and genetic evidence showed that we had isolated the structural gene. The coding region for *PGI1* was identified by hybridization with mRNA, nuclease S1 protection and subcloning experiments. The genomic organization of the *PGI1* region in the chromosome and the cloned fragments was the same, as identified by restriction enzyme analysis. Only one copy of this gene per genome was found in several strains tested. Integration of the cloned fragment at the *PGI1* gene was taken as conclusive evidence that it contained this gene.

Materials and methods

Strains. Strains UTL-7A (*MATa leu2-3,112 trp1 ura3-52*), SMC-19A (*MATα leu1 MAL2-8^c MAL3 SUC3*), A1327A (*MATa ade2-1 leu1 MAL1*) and GR1.2-1A (*MATα ilv1 MAL4 SUC4*) were used as wild types as well as sources for genomic DNA and RNA. DMA4 (*MATa pgi1 leu2-3,112 trp1 ura3-52*), DMA7 (*MATa pgi1 leu2-3,112 trp1 ura3-52*) and the diploid DD1 (*MATα/MATa leu2-3,112/leu2-3,112 trp1/trp1 ura3-52/ura3-52 PGI1/pgi1*) were used as recipient strains for transformation. Strain RR1 of *Escherichia coli* was used for propagating plasmids.

Plasmids The yeast-*E. coli* shuttle vectors used were YRp7 (Struhl et al. 1979) and YEp13 (Broach et al. 1979). The *PGI1* gene was isolated from a genomic library constructed in YEp13 by Nasmyth and Tatchell (1980).

Media and growth conditions. YEP medium, 2% peptone (Difco, Detroit, MI, USA), 1% yeast extract (Oxoid, Basingstoke, England) supplemented with 2% glucose (YEPD),

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2% fructose (YEPF) or 2% glycerol and 2% ethanol (YEPGE) as carbon sources, was used as rich medium. The synthetic medium was based on 1.6 g/l Difco yeast nitrogen base without amino acids and ammonium sulphate, supplemented with $(NH_4)_2SO_4$, 5 g/l, and amino acids and bases as required. Media were solidified with 15 g/l agar (Oxoid). Liquid cultures were grown with shaking at 28° C in metal-capped tubes or flasks. Classical genetical analysis was performed by published procedures (Sherman et al. 1979).

Preparation of crude extracts and enzyme assays. Crude extracts were prepared as described by Ciriacy and Breitenbach (1979) using ballotini glass beads (Sigma, St. Louis, MO, USA) for breaking the cells. For the assay of phosphoglucose isomerase activity according to Ciriacy and Breitenbach (1979) 50 mM imidazole buffer, pH 7.0, containing 10 mM Mg^{++}or 0.1 M phosphate buffer, pH 7.0, containing 0.1 mM EDTA was used. Specific activities were computed as mU based on protein determination according to Zahmenhoff (1957).

Transformation experiments. Transformation of *E. coli* RR1 was as described by Cohen et al. (1972). Yeast cells were transformed according to Beggs (1978). The recipient strains were pregrown in fructose medium. Sphaeroplasts were prepared using glucuronidase/arylsulphatase (Boehringer, Mannheim, FRG) or zymolyase 20T (Miles GmbH, Frankfurt, FRG) at a concentration of 50 µg/ml. Tryptophan or leucine were omitted from the transformation media to select for markers present on vectors YRp7 and YEp13. Transformants complementing the *pgi* mutation were isolated on synthetic media with glucose as the sole carbon source.

Nucleic acid preparations. Yeast chromosomal DNA was prepared according to Ciriacy and Williamson (1981). Plasmid DNA was prepared from yeast transformants according to Nasmyth and Reed (1980) and from *E. coli* according to Bolivar and Backman (1979) for small scale preparations or Birnboim (1983) for larger amounts. RNA was extracted from exponentially growing yeast cells as described by Denis et al. (1981) with the modification of Schmitt et al. (1983) and enriched for poly(A^+)RNA by oligo (dT)-cellulose chromatography (Aviv and Leder 1972).

Electrophoresis of DNA and RNA and hybridization. RNA was glyoxylated prior to electrophoresis according to McMaster and Carmichael (1977). Electrophoresis of DNA was performed in 0.83% agarose, 0.09 M Tris-H_3BO_3, pH 8.3, and of RNA in 1.2% agarose, 10 mM potassium phosphate, pH 7.0. DNA and RNA were transferred to nitrocellulose filters as described by Wahl et al. (1979) and Thomas (1980), respectively. Plasmid DNA, prepared from *E. coli* according to Clewell (1972), was radioactively labelled by nick translation using (α^{35}S)dATP (New England Nuclear, Boston, MA, USA) and DNA polymerase I (Bethesda Research Laboratories, Gaithersburg, MD, USA) as described by Rigby et al. (1977). DNA-DNA hybridizations were performed in 0.6 M NaCl, 0.5 M sodium phosphate, pH 6.2, 3 mM EDTA, 1% sarcosyl and 0.1 mg/ml sonicated salmon sperm DNA at 65° C, and DNA-RNA hybridizations in 50% formamide, 0.75 M NaCl, 0.075 M Na_3 citrate, 10 mM sodium phosphate, pH 6.5, 0.25 mg/ml sonicated salmon sperm DNA and 10 mM dithiothreitol at 42° C for 48 h.

Preparation of DNA fragments and ligation into vector DNA. DNA restriction fragments were electrophoresed in 0.6% agarose gels with 0.09 M Tris and acetic acid adjusted to pH 8.3. Bands of interest were cut out of the gel, equilibrated with 0.3 M sodium acetate, pH 7.0, containing 1 mM EDTA and DNA fragments and removed from the gel by the optimized freeze-squeeze method of Tautz and Renz (1983). This DNA was either radioactively labelled and used for hybridization or ligated to vector DNA according to Mertz and Davis (1972), using T4 DNA ligase (Boehringer, Mannheim, FRG).

Nuclease S1 protection experiments. S1 mapping of transcriptional termination was carried out basically as described by Sharp et al. (1980). DNA fragments were 3′-end labelled with α^{35}S-dATP and DNA polymerase I, Klenow fragment, according to Maniatis et al. (1982). They were mixed with 15 µg of poly (A)-enriched RNA, precipitated and the pellet resuspended in 40 mM Pipes, 1 mM EDTA, 0.4 M NaCl and 80% formamide. After denaturating at 78° C for 15 min, the probes were renaturated at 45° C, allowing DNA-RNA hybrids to form. After 3 h of hybridization the samples were diluted ten fold with S1 digestion buffer containing 400 U/ml S1 nuclease (Boehringer, Mannheim, FRG) and incubated at 37° C for 30 min. After stopping the reaction with 4 M ammonium acetate, 0.1 M EDTA, the samples were phenol-chloroform extracted and then isopropanol precipitated and applied to a neutral agarose gel. Kodak XR-5 film was applied directly onto the dried gel.

Results

Isolation of recombinant plasmids containing the gene PGI1

Recombinant plasmids containing the gene *PGI1* were isolated from a genomic library, prepared by Nasmyth and Tatchell (1980) in the multicopy vector YEp13, by selecting for their ability to complement a *pgi1* mutation in *S. cerevisiae* strains DMA4 and DMA7. Forty-six glucose-fermenting transformants were obtained. Concomitant loss of the LEU^+ and PGI^+ phenotypes was taken as an indication of autonomously replicating plasmids bearing the *LEU2* and *PGI1* genes (Nasmyth and Reed 1980). Plasmid DNA was prepared from each transformant and used to transform *E. coli* to ampicillin resistance. Plasmid DNA was prepared from 60 different *E. coli* transformants and digested with *Eco*RI. Gel electrophoresis of DNA revealed that most of these plasmids contained a common *Eco*RI fragment of 2.05 kb. Five of these plasmids were purified on a large scale and used for back-transformation into yeast. A high frequency of transformation to PGI^+ (5×10^2–10^3 transformants per µg DNA) was obtained, indicating that each plasmid contained a sequence able to complement the *pgi* mutation in yeast.

Thus far, complementation had been assayed on the basis of carbon source utilization. Next, enzymatic activity was tested in the transformants and for this the wild-type strain for *PGI1*, UTL-7A, was also transformed to leucine prototrophy with the plasmids isolated. Table 1 shows an

Table 1. Phosphoglucose isomerase activity in yeast transformants and wild-type strains

Strain	Plasmid	Media	mU/mgP[a]	Relative[b] activity
DMA4	–	YEPF	9	0.01
UTL-7A	–	YEPD	973	1.00
UTL-7A	–	SD	725	1.00
DMA4	pPGI1-4	YEPD	8133	8.40
DMA4	pPGI1-3	YEPD	4702	4.80
DMA4	pPGI1-5	YEPD	7786	8.00
UTL-7A	pPGI1-2	SD	5273	7.30
UTL-7A	pPGI1-1	SD	4370	6.00

[a] mU per mg protein
[b] Wild-type activity taken as 1.00

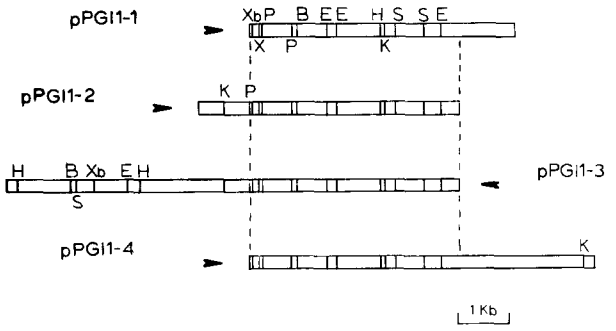

Fig. 1. Restriction maps of sequences cloned into the *Bam*HI site of the YEp13 vector. Plasmids were digested with the following restriction endonucleases and the location of the sites determined: *Eco*RI (E); *Hin*dIII (H); *Bgl*II (B); *Sal*I (S); *Xba*I (Xb); *Xho*I (X); *Kpn*I (K); *Pst*I (P) and *Bam*HI (no site found). The orientation of the inserted fragments was determined relative to the *Sal*I site of pBR322 as indicated by *arrows*

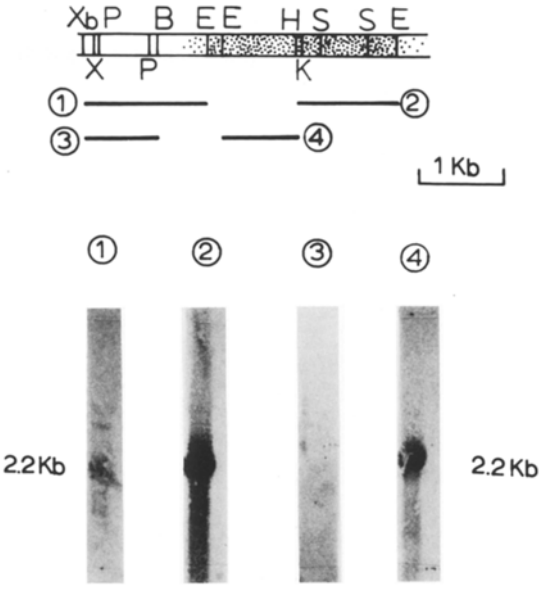

Fig. 2. Northern analysis of the *PGI* region. Total RNA from transformants DMA4 (pPGI1-3) and DMA7 (pPGI1-4) was extracted from cells grown overnight in YEPD. Poly(A)-enriched RNA 15 μg was applied to an agarose gel, electrophoresed, transferred to nitrocellulose and hybridized to (α^{35}S)dATP nick translated fragments 1, 2, 3 and 4 isolated from agarose gels by the freeze-squeeze method of Tautz and Renz (1983). The *stippled region* shows the region hybridizing to mRNA; rRNAs from *Escherichia coli* were used as size standards. For restruction sites see Fig. 1 legend

increase in specific activity of seven- to eight fold over the wild-type level in all the transformants tested. These data correspond to the results expected if a glycolytic structural gene had been cloned (Kawasaki and Fraenkel 1982).

Four different plasmids were used for restriction analysis (Fig. 1). All of them showed an overlapping DNA fragment of 4.1 kb which should contain the cloned gene.

An mRNA of 2.2 kb is constitutively synthesized and hybridizes to the cloned DNA

Kempe et al. (1974b) determined the number of amino acid residues of the dimeric protein to be about 1,230. Consequently, the *PGI1* mRNA must be longer than 1.8 kb, including the flanking 5′- and 3′-regions and a poly(A) tail. Northern blot experiments were performed to detect this mRNA and also to localize the coding region of the gene to the 4.1 kb region common to all cloned sequences.

For this purpose, total RNA was extracted from different transformants and enriched by passages through an oligo-dT cellulose column. Different DNA fragments from the overlapping region were used as radioactive probes to determine which of them hybridized to mRNA (Fig. 2). An mRNA sequence of about 2.2 kb hybridized very clearly with probes 2 and 4. Only much weaker hybridization was detected with probe 1. This means that the coding region of *PGI1* is located mainly in the *Eco*RI-*Eco*RI fragment of 2.05 kb.

In order to localize the mRNA more accurately and to determine the direction of transcription, S1 nuclease protection experiments were performed (Berk and Sharp 1977). Plasmid pPGI1-2 was digested with endonucleases *Bgl*II and *Hin*dIII, the latter cleaving in the coding sequence (see Fig. 2). The 1.55 kb *Bgl*II-*Hin*dIII fragment (probe A in Fig. 3) as well as the 1.95 kb *Hin*dIII-*Hin*dIII fragment (probe B in Fig. 3) were isolated from agarose gels and 3′-end labelled with α^{35}S-dATP and Klenow polymerase. After hybridization with poly(A)-enriched RNA and S1 nuclease treatment, fragments were separated on a neutral agarose gel. The autoradiogram (Fig. 3) shows that at 45° C (the renaturation temperature used) it is possible to get some DNA-DNA hybrids appearing as very weak bands of 1.55 and 1.95 kb with probes A and B, respectively. However, a new and very strongly hybridizing band of 0.75 kb appears when probe A is used. This band, only detected when RNA is used in the renaturation mix, corresponds to the DNA-RNA hybrid. Therefore, we can conclude that transcription terminates near the right-hand side of the middle *Eco*RI site and starts no further than 2.2 kb upstream (Fig. 3). The weak hybridization obtained with probe 1 in Fig. 2 can be explained as an artefact, produced by incomplete separation of the fragment used as probe 1 from adjacent DNA sequences, that we frequently obtain with probes prepared after freeze-squeeze.

Because phosphoglucose isomerase is a constitutive enzyme (Maitra and Lobo 1971) Northern blot experiments were carried out in order to test this at the transcriptional level. Poly(A)-enriched RNAs from transformant, mutant and wild-type strains grown on different carbon sources

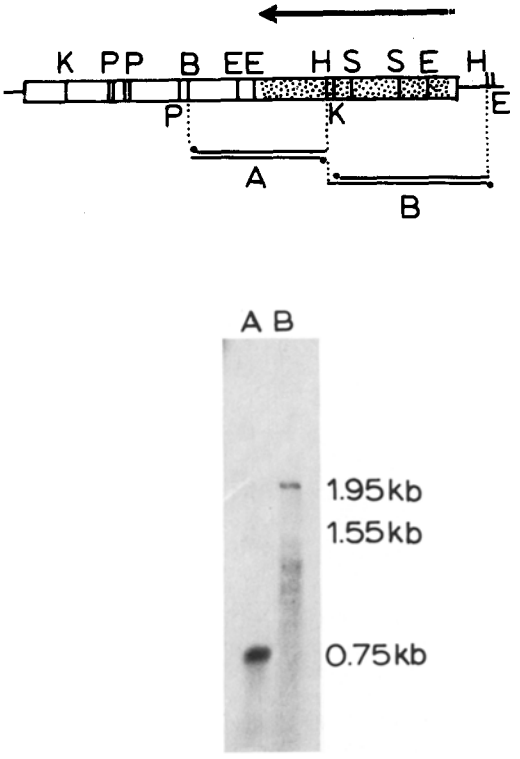

Fig. 3. S1 nuclease protection experiment of *PGI1* transcripts. Fragments A and B were isolated from plasmid pPGI1-2 digested with endonucleases *Hin*dIII and *Bgl*II. They were recovered from an agarose gel by the freeze-squeeze method and 3′-end labelled with (α^{35}S)dATP and Klenow polymerase (Maniatis et al. 1982). Poly(A)-enriched RNA (15 μg) isolated from transformant UTL-7A (pPGI1–1) was used for hybridization with the DNA probes followed by S1 nuclease treatment. The *upper arrow* indicates the *PGI1* transcript (see Results). The *dots* indicate the 3′-ends. Restriction sites as in Fig. 1. λ digested with *Eco*RI and *Hin*dIII was used as size standards

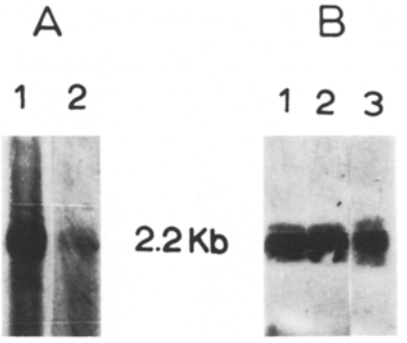

Fig. 4. A Northern analysis of wild-type UTL-7A transformed with plasmid pPGI1–1 (1) and untransformed wild-type UTL-7A (2). Total RNA was extracted from cells grown overnight in YEPD after a previous growth period of 3 days on selective media without leucine in order to enhance the plasmid copy number; 12 μg of poly(A)-enriched RNA was applied. **B** Northern analysis of *pgi* mutant strain DMA4 grown on YEPF (1) and wild-type UTL-7A grown on YEPD (2) and YEPGE (3); 15 μg of poly(A)-enriched RNA was applied. For **A** and **B** (α^{35}S)dATP nick-translated fragment *SalI-BglII*, isolated from pPGI1-1 plasmid according to Tautz and Renz (1983), was used as probe. For more details see Fig. 2 legend

(glucose, fructose and glycerol-ethanol) were prepared for Northern analysis. 1.8 kb *SalI-BglII* fragment containing part of the coding region was used as a radioactive probe. Figure 4 shows that mRNA was overproduced in the transformants relative to the wild type and similar levels of transcripts were detected in the cells growing on non-fermentable and fermentable sugars. This demonstrated the constitutive expression of the cloned gene as expected for phosphoglucose isomerase. Finally, the *pgi* mutant used as recipient produced an RNA of wild-type size.

Subcloning the PGI region into YRp7

Shuttle vector YEp13 contains the replication origin of the circular endogenous 2 μ DNA plasmid. Yeast chromosomal genes in such plasmids can be integrated into chromosomes but this integration makes the chromosome unstable (Falco et al. 1982). Therefore, the *PGI* region was subcloned into a shuttle vector without 2 μ DNA. Plasmid pPGI1-3 was partially digested with *SalI* and the 6.2 and 6.7 kb fragments, containing only part of the *PGI1* gene, were isolated and ligated into the vector YRp7 which had been opened up at the *SalI* site in the tetracycline resistance gene of pBR322 (Fig. 5). Then *E. coli* was transformed to ampicillin resistance and plasmids pB and pC were recovered from different bacterial transformants. These plasmids were then

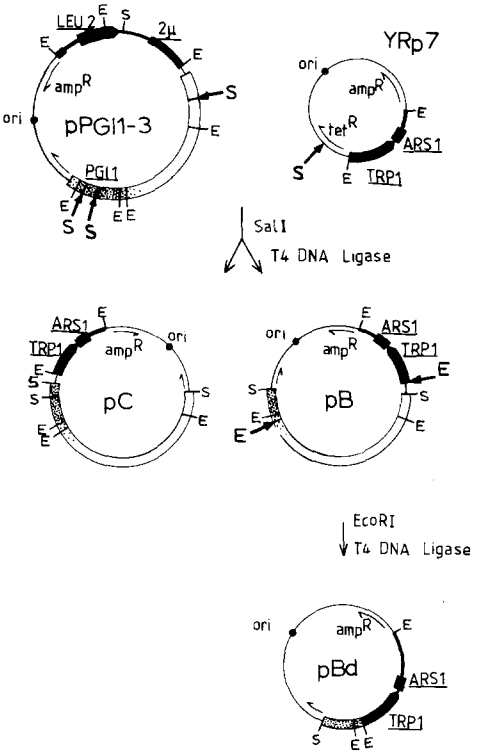

Fig. 5. Subcloning of PGI sequences into YRp7. Plasmid pPGI1-3 was partially digested with *SalI* and after electrophoresis fragments of 6.2 and 6.7 kb, containing part of the *PGI1* coding region, were recovered according to Tautz and Renz (1983). These fragments were ligated into the YRp7 vector, digested with *SalI* according to Mertz and Davis (1972), using T4 ligase. Plasmids pB and pC were recovered from *Escherichia coli amp*^{r} *tet*^{s} transformants. In the same way, plasmid pB was partially digested with *Eco*RI and the 6.8 kb fragment containing the *PGI* region was isolated, religated and used to transform *E. coli* to ampicillin resistance as described before. This led to the recovery of plasmid pBd. For restriction sites see Fig. 1 legend. Ba represents the *Bam*HI site and *dark arrows* the sites used for opening the plasmids

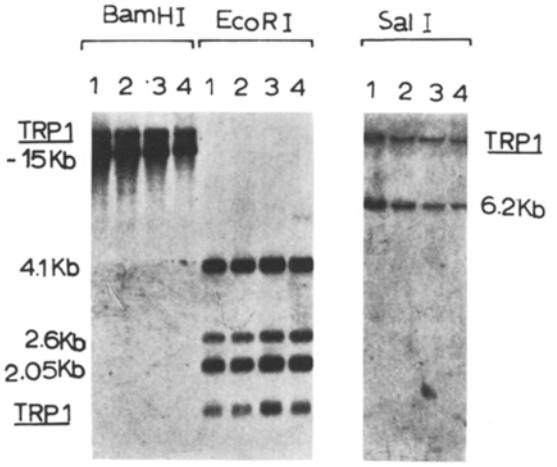

Fig. 6. Southern analysis of region *PGI1* in different laboratory wild-type strains. Total DNA was prepared from wild-type strains UTL-7A (1), A1327A (2), SMC-19A (3) and GR1.2-1A (4) after growth on YEPD according to Ciriacy and Williamson (1981). It was digested with *Eco*RI, *Bam*HI or *Sal*I and applied to an agarose gel, electrophoresed and transferred to a nitrocellulose filter. For hybridization (α^{35}S)dATP nick-translated total plasmid pC (*Bam*HI- and *Eco*RI-digested DNA) or pBd (*Sal*I) were used as probes

used to transform *S. cerevisiae* DMA4 to tryptophan independence. A high transformation frequency was obtained when selecting for the TRP$^+$ phenotype, but none of the TRP$^+$ transformants tested were PGI$^+$. This result clearly showed that the sequence on the right-hand side of the two *Sal*I sites of the *PGI1* region, absent from the newly constructed plasmids, is an essential part of this gene (Fig. 2).

Following this, plasmid pB was partially digested with *Eco*RI and the fragment containing the *PGI1* region, pBR322 and the *TRP1* marker was isolated, religated and used to transform *E. coli* to ampicillin resistance. Plasmid pBd was recovered from the *E. coli* transformants (Fig. 5).

Southern analysis of genomic DNA

Total genomic DNA from different wild-type yeast strains was isolated and digested with different restriction endonucleases for Southern analysis. The probes used were plasmid pC for the *Bam*HI and *Eco*RI digests and plasmid pBd for the *Sal*I digest. The results are shown in Fig. 6. The two bands of the *Bam*HI digests hybridizing to the probe are the typical fragment carrying *TRP1* and a shorter fragment of about 15 kb which corresponds to the *PGI1* gene. Four bands hybridized in the *Eco*RI digests: the 1.45 kb fragment carrying the *TRP1* gene and three other bands corresponding to the 4.1 and 2.05 kb bands found in the sequences inserted in plasmid pC and the 2.6 kb band which reflects the leftmost part of the insertion (see Fig. 1, plasmid insert pPGI1-3). The expected band of about 200 bp flanked by *Eco*RI sites was not detected because it ran out of the gel. The *Sal*I digest was hybridized with plasmid pBd which contained only the central part of the gene flanked by the central *Sal*I and the leftmost *Eco*RI site. This hybridized to only one 6.2 kb fragment (see Fig. 1, plasmid insert pPGI1-3) in addition to the band containing the *TRP1* gene. This indicated that the cloned sequence reflected a continuous sequence on the chromosome. It is also important to note that only one chromosomal region was found, reflecting the formal genetic data of Maitra and Lobo (1977) who found only one *PGI1* gene.

Table 2. Genetic linkage between TRP$^+$ and PGI$^+$ phenotypes in the stable transformants DD1-K and DD1-L

Strain	Number of spores with phenotype			
	PGI$^+$TRP$^+$	PGI$^+$trp$^-$	pgi$^-$-trp$^+$	pgi$^-$trp$^-$
DD1-K	18	0	0	22
DD1-L	20	0	0	20

Integration of the cloned gene into the PGI locus

The final proof that the *PGI1* gene had been isolated would be its integration at the *PGI1* locus. This usually occurs by homologous recombination (Hinnen et al. 1978). Orr-Weaver et al. (1981) reported that linear DNA molecules targeted integration into homologous regions at the site of cleavage. Plasmid pBd was cut open at the unique *Kpn*I site. The insertion in this plasmid lacks the right-hand part of the *PGI1* gene. A diploid recipient strain, heterozygous for *PGI1*/*pgi1* and homozygous for *trp1*, was constructed and used as a recipient. Transformants of the phenotype TRP$^+$ PGI$^+$ were selected and finally five stable clones were established. Tetrad analysis was performed with two such clones. As shown in Table 2, TRP$^+$ and PGI$^+$ segregated together indicating that integration had taken place in the wild-type *PGI1* gene.

Southern analysis of genomic digests was performed with the recipient strain, the two transformants DD1-K and DD1-L and the four spores of one tetrad of each (Fig. 7). The probe for the Southern blots of the diploids was plasmid pBd which carries only the *Sal*I-*Eco*RI fragment, which in fact hybridized only to the typical 2.05 kb *PGI1* band and the 1.45 kb *TRP1* band of the *Eco*RI digest of the recipient strain. A third band was visible in the blots with DNA of the two transformants digested with *Eco*RI. In the case of a *Bam*HI digest there was a band in addition to the typical *PGI1* and *TRP1* fragments. DNA from the spores of the two tetrads was digested with *Bam*HI and the probe used was a 1.55 kb fragment flanked by the central *Hin*dIII and the next *Bgl*II site to the left (see Figs. 1 and 2). The normal length *Bam*HI fragment was found in those spores which were *pgi1* whereas the longer band segregated with the active *PGI1* allele. It was striking that the new bands in the transformant DD1-L (lanes 2 in Fig. 7) were longer and showed much higher radioactivity than transformant DD1-K (lanes 3) or the regular *PGI* band. This suggested that tandem integration of the plasmid pBd had taken place in stable transformant DD1-L.

Discussion

The phosphoglucose isomerase structural gene *PGI1* was isolated from a genomic library by complementation of a *pgi1* mutation. Evidence for the isolation of *PGI1* was obtained by classical genetic procedures and Southern analysis of stable transformants according to Nasmyth and Reed (1980). Moreover, the cloned DNA sequence hybridized very strongly to an mRNA species of 2.2 kb. This result is consistent with the expected minimum length of 1.8 kb

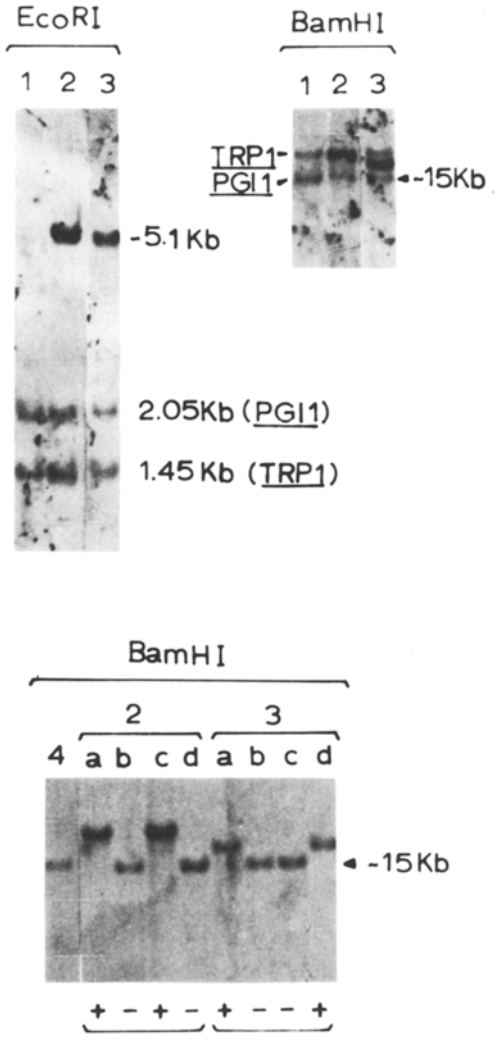

Fig. 7. Southern analysis of the *PGI1* and *TRP1* region in different stable diploid transformants and their tetrads. Total DNA was prepared from the diploid recipient strain DD1 (1), diploid stable transformants DD1-L (2) and DD1-K (3), haploid *pgi*$^-$ mutant DMA4 (4) and the four spores of one complete tetrad (a–d) of each of the stable diploid transformants DD1-L (2) and DD1-K (3). DNA was digested with *Eco*RI or *Bam*HI, applied to an agarose gel, electrophoresed and transferred to a nitrocellulose filter. For hybridization (α^{35}S)dATP nick-translated total plasmid pBd (for the Southern blot with the diploid strain DNAs) or the 1.55 kb *Hin*dIII-*Bgl*II fragment from the *PGI1* region isolated by the freeze-squeeze method (for the Southern blot with the haploid spore DNAs) were used as probes. The presence or absence of phosphoglucose isomerase in the individual spores is indicated by + or –

of the *PGI* gene, based on the study of amino acid residues and the molecular weight of the dimeric protein (Kempe et al. 1974b). Phosphoglucose isomerase mRNA is longer than the direct coding region of the protein, as found with other glycolytic genes such as *PDC1* (Schmitt et al. 1983) or *PFK1* and *PFK2* (J. Heinish, personal communication).

The hybridization intensities with *PGI* mRNA, from cells growing in media containing ethanol or glucose as carbon sources, were also consistent with constitutive expression of phosphoglucose isomerase as expected from biochemical and genetical data (Maitra and Lobo 1971). The increase in enzymatic activity, as well as an overproduction of mRNA in transformants, served as further evidence that the *PGI1* structural gene had been isolated. The data provided clearly show that *PGI1* is the only structural gene required for the constitutive expression of phosphoglucose isomerase and that only one copy of this gene exists per haploid genome. The three different phosphoglucose isomerase isoenzymes initially reported by Noltmann (1972) and studied by Kempe et al. (1974b) for an industrial yeast strain of unknown genetic composition may have been due to the presence of different allelic forms of the gene *PGI1* or caused by limited proteolytic degradation.

Finally, we deduce that the coding region is at the right-hand side of the middle *Eco*RI site (Fig. 3). The fact that plasmids pB and pC lack the ability to complement the *pgi1* mutation and that integration of plasmid pBd at the *PGI1* locus does not produce a Pgi$^-$ phenotype agrees with the results of Northern blot and S1 nuclease protection experiments, showing that sequences to the left of the *Eco*RI site nearest *Bgl*II are not required for coding a functional *PGI* transcript.

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