The *cdc30* Mutation in *Saccharomyces cerevisiae* Affects Phosphoglucose Isomerase, the Cell Cycle and Sporulation

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Spontaneous revertants of the *cdc30* mutation in *Saccharomyces cerevisiae* simultaneously regained the ability to grow and divide at 36.5 °C on glucose-containing media along with a more thermostable phosphoglucose isomerase (PGI). An independently isolated allele of *cdc30* gave a similar phenotype to that previously described including temperature-sensitivity of PGI. Isoelectric focussing allowed the separation of two isoenzymes of PGI. These results all support the idea that two genes – *PGII* and *CDC30* – are responsible for PGI activity in yeast. Diploid strains homozygous for the *cdc30* mutation sporulated poorly in potassium acetate irrespective of whether the cells had previously been cultured at a temperature that was permissive or restrictive for cell cycle progression. This was not surprising because a strain defective in PGI would not be expected to be able to complete the gluconeogenic events of sporulation.

INTRODUCTION

Control of the cell cycle and cell development are unsolved problems in biology. In the yeast *Saccharomyces cerevisiae* the isolation and study of cell division cycle (*cdc*) mutants has provided much genetic and physiological information. Biochemical understanding of regulation and control of the cell cycle has lagged behind genetic and physiological descriptions because the identities of the products of most *CDC* (wild-type) genes remain unknown. However the following gene/enzyme relationships have been established: *CDC7* – protein kinase (Patterson *et al.*, 1986); *CDC8* – thymidylate kinase (Jong *et al.*, 1984; Sclafani & Fangman, 1984); *CDC9* – DNA ligase (Johnston & Nasmyth, 1978; Barker *et al.*, 1985); *CDC19* – pyruvate kinase (Kawasaki, 1979); *CDC21* – thymidylate synthetase (Game, 1976); *CDC28* – protein kinase (Reed *et al.*, 1985); *CDC30* – high K_m ('isoenzyme I') PGI (Dickinson & Williams, 1987); and *CDC35* – adenylate cyclase (Mason *et al.*, 1984; Caspersen *et al.*, 1985).

We realized that our view that PGI activity in *S. cerevisiae* requires the products of both the *CDC30* and the *PGII* genes (Dickinson & Williams, 1987) differs from that of several other workers who have shown that mutation in the single nuclear gene *PGII* (Clifton *et al.*, 1978; Herrera & Pascual, 1978; Ciriacy & Breitenbach, 1979; Maitra, 1971; Maitra & Lobo, 1977) or that deletion of the *PGII* gene (which had been isolated by complementation of a *pgil* mutation) (Aguilera, 1986) gives a complete phenotype, although earlier work of a purely biochemical nature had shown that yeast has three isoenzymes of PGI which differ in net charge but not in M_r (Nakagawa & Noltmann, 1967; Kempe *et al.*, 1974*a, b*).

In order to confirm that the *CDC30* gene encodes isoenzyme I of PGI we isolated revertants of *cdc30* for further study. We analysed the PGI isoenzyme complement of wild-type and *cdc30* mutant strains using isoelectric focussing gels. This report also describes a strain carrying a newly isolated allele of *cdc30* and the effect of the *cdc30* mutation on sporulation in yeast.

Abbreviation: PGI, phosphoglucose isomerase.

Table 1. *Genotypes and origins of strains used*

Strain	Genotype	Origin*
A364A	<i>MATa ade1 ade2 ura1 his7 lys2 tyr1 gal1</i>	YGSC
23015	<i>MATa ade1 ade2 ura1 his7 lys2 tyr1 gal1 cdc30-1</i>	YGSC
59.4A	<i>MATa HO arg4-1 cyh1 spd1</i> <i>MATα HO arg4-1 cyh1 spd1</i>	I. Dawes
JPT99	<i>MATa cdc30-3</i>	J. Pringle
WD1	<i>MATa arg4-1 URA3 CDC30-3</i> <i>MATα ARG4-1 ura3 cdc30-3</i>	This work
WD2	<i>MATa ade1 ade2 ura1 his7 lys2 tyr1 gal1 URA3 cdc30-1</i> <i>MATα ADE1 ADE2 URA1 HIS7 LYS2 TYR1 GAL1 ura3 cdc30-3</i>	This work
WD3	<i>MATa arg4-1 HIS3 LEU2 URA3 TRP1 cdc30-3</i> <i>MATα ARG4-1 his3 leu2 ura3 trp1 CDC30-3</i>	This work
WD4	<i>MATa arg4-1 HIS3 LEU2 URA3 TRP1</i> <i>MATα ARG4-1 his3 leu2 ura3 trp1</i>	This work
WD6	<i>MATa ade1 ade2 ura1 his7 lys2 tyr1 gal1 ARG4-1 cdc30-1</i> <i>MATα ADE1 ADE2 URA1 HIS7 LYS2 TYR1 GAL1 arg4-1 cdc30-3</i>	This work

* YGSC, Yeast Genetic Stock Center, Berkeley, California, USA; I. Dawes, University of Edinburgh, UK; J. Pringle, University of Michigan, Ann Arbor, Michigan, USA.

METHODS

Strains, growth conditions and genetic procedures. The strains used are described in Table 1. Cells were grown with shaking in conical flasks filled to 40% total volume in YEPD medium, which comprised 1% (w/v) yeast extract, 2% (w/v) peptone, 2% (w/v) glucose and 0.01% (w/v) of both adenine and uracil. Standard genetic techniques were used (Sherman, 1975; Mortimer & Hawthorne, 1975). To study the effect(s) of *cdc30* mutations on sporulation cells were grown in YEPD medium at 25 °C to mid exponential phase. Each culture was then divided into three equal portions. Portion 'a' was incubated for 2.5 h at 25 °C when the cells were harvested by centrifugation and transferred to sporulation medium (2%, w/v, potassium acetate). Portion 'b' was incubated at 36.5 °C for 5 h, by which time all cells bearing the temperature-sensitive *cdc30* mutation will have cell cycle arrested (Dickinson & Williams, 1987), and then transferred to sporulation medium in the same way as portion 'a'. Portion 'c' was incubated at 25 °C for 2.5 h after which time the cells were harvested by centrifugation and resuspended in fresh YEPD medium. These cells were incubated at 30 °C for a further 6 h during which period samples were removed and fixed in formalin/saline (40%, w/v, formaldehyde/1%, w/v, NaCl; 0.5:100, v/v) for subsequent cell counting using a Coulter counter. Cells were incubated in sporulation medium at 30 °C for 48 h after which time sporulation was assessed by scoring at least 400 cells. The permissive temperature for the *cdc30* mutants is 25 °C, hence portion 'a' is a control. Portion 'c' is also a control to check whether the temperature of 30 °C used to induce sporulation causes cell cycle arrest in *cdc30* mutants: it does not (J. R. Dickinson & A. S. Williams, unpublished result).

Isolation of revertants. The *cdc30* mutation is expressed on YEPD medium at 36.5 °C but not at 25 °C. Spontaneous revertants of 23015 were obtained by plating cells on YEPD at 36.5 °C. After 4–5 d papillae were present. These were collected, checked for clonal purity and retested.

Assay of PGI activity. Cell extracts were prepared and enzyme activity determined as described previously (Dickinson & Williams, 1987). In experiments to determine the effect of heating on PGI activity cell extracts were heated and shaken rapidly in a water-bath at 55 °C with vigorous vortexing at 0, 30 and 60 min before being used immediately in the assay.

Isoelectric focussing. Cell extracts prepared as described previously (Dickinson & Williams, 1987) and containing 1% (w/v) phenylmethylsulphonyl fluoride to prevent proteolytic breakdown were analysed on ultra-thin polyacrylamide gels. The gels were cast according to the method of Caswell *et al.* (1986), and were composed of 5% (w/v) acrylamide, 0.17% (w/v) *N,N'*-methylene bisacrylamide, 20% (v/v) glycerol, 10 µl *N,N,N',N'*-tetramethylethylenediamine (TEMED), 5% (w/v) carrier ampholytes (pH range 3–10) and 0.038% ammonium persulphate, made up to a final volume of 10 ml. Prefocussing was at 2 W for 30 min and focussing was at 4.5 W constant power or 2 kV maximum voltage for 170 min. Gels were stained using PAGE blue G-90 (BDH) as described by Blakesley & Boezi (1977). Quantification was achieved using a LKB 2202 Ultrosan laser densitometer and Ultrosan computer program. Purified yeast PGI of known concentration was used for calibration.

Table 2. *Effect of heat treatment on PGI activity*

A typical experiment in which the wild-type strain (A364A), mutants 23015 and JPT99 and the revertants derived from 23015 (1A, 1B, etc.) were grown at 25 °C to mid exponential phase. Cells were then harvested, washed, broken and cell extracts prepared. Enzyme activity was determined (in duplicate) in unheated controls and in samples which had been heated at 55 °C for 1 h. PGI specific activity in unheated extracts of strain A364A was 1175 nmol min⁻¹ (mg protein)⁻¹.

Strain	PGI activity remaining after heat treatment (%)	Strain	PGI activity remaining after heat treatment (%)
A364A	29.97	1G	22.21
23015	0.0	2A	17.89
JPT99	0.0	2B	7.10
1A	28.17	4G	14.48
1B	24.90	6A	9.87
1C	32.69	6G	19.90
1D	17.99	7G	14.84
1E	32.94	8B	31.14
1F	19.83	8C	4.25

RESULTS AND DISCUSSION

Revertants of 23015 have recovered PGI activity

We obtained 51 spontaneous revertants of 23015 which were able to grow and divide at 36.5 °C. In order to test between intragenic and extragenic reversion events outcrosses were made to spores of the homothallic strain 59.4A and the resulting diploids sporulated. Analysis of the spore progeny merely required inspection for *cdc30*-bearing (YEPD-negative at 36.5 °C) clones. These could only arise from strains carrying the original *cdc30* mutation and an extragenic suppressor. The majority (18 out of 21 examined) were the result of intragenic reversion events (i.e. no *cdc30*-bearing clones recovered from the cross described).

The *cdc30* mutation in strain 23015 results in a thermolabile PGI which is unable to survive the heating regime (Table 2). In the wild-type (A364A) approximately 30% of the PGI activity remained after the heat treatment. PGI activity from all of the revertants was more thermostable than that from strain 23015, and in two cases (revertants 1C and 1E) even more stable than that from the original wild-type parent. This experiment used a more aggressive heat-treatment than the one described previously (Dickinson & Williams, 1987), as it simultaneously highlights thermolability and simplifies the handling of extracts from a larger number of strains. These results are consistent with the idea that the *CDC30* gene codes for isoenzyme I of PGI.

Table 2 also shows that the independently isolated strain JPT99 has a temperature-sensitive PGI. The mutation in this strain had been tentatively assigned as *cdc30* by the Michigan group on the basis of failure to complement a *cdc30-1* mutation. We confirmed this result and observed that the mutation in strain JPT99 behaved like the *cdc30-1* allele in crosses. Furthermore, strain JPT99 and the progeny derived therefrom were like strain 23015 in that they were all capable of growth and cell division at 36.5 °C on non-fermentable carbon sources. On the basis of these criteria we feel confident in assigning the mutation present in strain JPT99 as '*cdc30-3*', for, to our knowledge, this is only the third allele of *cdc30* that has been described. To be consistent with convention '*cdc30*' should be re-named '*pgi2*', hence this new allele of *cdc30* ought to be called '*pgi2-3*'.

Physical separation of PGI isoenzymes I and II

Fig. 1 shows that isoelectric focussing physically resolved isoenzymes I and II of PGI which had been deduced previously from kinetic data (Dickinson & Williams, 1987). From Table 3 it is clear that the wild-type and mutant strains contained similar amounts of isoenzyme II, but that the mutant strain 23015 contained only half the amount of isoenzyme I that was present in the wild-type. This result could be taken as evidence that *CDC30* is a regulator of the amount of PGI isoenzyme I rather than the structural gene, though this would contradict the majority of the

Fig. 1. Isoelectric focussing gel showing isoenzymes of PGI. Cells of the wild-type strain (A364A) and *cdc30*-bearing mutant (23015) were grown to mid exponential phase at 25 °C. Cell extracts were then prepared and analysed as described in Methods. Lane 1, purified PGI; lane 2, extract from wild-type A364A; lane 3, extract from mutant strain 23015.

Table 3. *Relative amounts of PGI isoenzymes I and II determined by scanning densitometry of isoelectric focussing gels*

Values are means $\pm$ SEM of three determinations (four in the case of A364A).

Source of PGI	Relative amount of protein in isoenzyme (percentage of total protein)		Ratio (I/II)
	I	II	
Purified enzyme	66.80 $\pm$ 0.20	33.20 $\pm$ 0.20	2.01
A364A	6.86 $\pm$ 0.68	2.93 $\pm$ 0.33	2.34
23015	3.64 $\pm$ 0.23	3.15 $\pm$ 0.22	1.15

evidence presented earlier and in this report. Perhaps there is increased proteolysis of the mutant protein and we are currently examining this possibility using a range of protease mutants.

Effect of cdc30 mutations on sporulation

Simchen (1974) was the first to examine whether *cdc* mutations affect sporulation. He divided cell cycle mutations into three classes depending upon their effect on sporulation (Simchen 1974): (i) mutations permitting normal sporulation; (ii) mutations giving no sporulation at the restrictive temperature but normal sporulation at the permissive temperature; and (iii) mutations producing no sporulation at either temperature. The results of many different investigations have been reviewed (Pringle & Hartwell, 1981; Esposito & Klapholz, 1981). We were interested to examine the effect of *cdc30* mutations on sporulation for three reasons. Firstly, because diploids can only switch from vegetative growth and mitosis to the alternative developmental pathway of meiosis and sporulation when in the G1 portion of the cell cycle whereas at 36.5 °C the *cdc30* mutation causes arrest late in nuclear division. Secondly, because it is known that an intact sequence of gluconeogenic enzymes is required for sporulation

Table 4. Effect of *cdc30* mutations on sporulation

Cells were grown at 25 °C to mid exponential phase. The cultures were then divided and the cells treated as indicated before being transferred to sporulation medium. The results are means $\pm$ SEM of three experiments.

Strain	Relevant genotype	Sporulation (percentage asci)	
		After prior culture at 25 °C for 2.5 h	After prior culture at 36.5 °C for 5 h
WD1	<i>CDC30-3</i> <i>cdc30-3</i>	71.0 $\pm$ 2.5	58.2 $\pm$ 3.9
WD2	<i>cdc30-1</i> <i>cdc30-3</i>	12.6 $\pm$ 2.0	10.0 $\pm$ 0.5
WD3	<i>cdc30-3</i> <i>CDC30-3</i>	55.3 $\pm$ 3.1	52.3 $\pm$ 3.4
WD4	<i>CDC30</i> <i>CDC30</i>	72.2 $\pm$ 0.35	54.2 $\pm$ 3.4
WD6	<i>cdc30-1</i> <i>cdc30-3</i>	20.5 $\pm$ 0.5	24.2 $\pm$ 0.85

(Dickinson & Williams, 1986) and thirdly, because *pgi1* homozygous diploids are asporogenous on potassium acetate (Aguilera, 1986).

After preincubation at 36.5 °C the wild-type (WD4) and *cdc30* heterozygous diploids (WD1 and WD3) sporulated equally well compared with the homozygous diploids (WD2 and WD6) which had significantly reduced sporulation (Table 4). Thus it seems that *cdc30* mutations do affect sporulation and hence one might conclude that cell cycle blockade prior to transfer to sporulation medium was the reason for this. Notice, however, that in the case of WD2 and WD6 there was almost no difference between sporulation after preincubation at 25 °C or 36.5 °C. In other words, the *cdc30* mutation is temperature-sensitive with respect to the cell cycle but absolute with respect to sporulation. Our explanation for this is that *S. cerevisiae* requires PGI to be able to complete the gluconeogenic events of sporulation. In *cdc30*-bearing strains grown glycolytically the activity of this enzyme is reduced to about two-thirds of the level in the wild-type, even at 25 °C (Dickinson & Williams, 1987). Furthermore, under the gluconeogenic conditions of acetate utilization that are involved in sporulation PGI activity is further reduced (Maitra & Lobo, 1971), presumably in *cdc30* homozygous diploids to the point where the flux of carbon into glucose 6-phosphate is inadequate.

It is interesting to note that Southern analysis has revealed only one copy of the *PGII* gene per haploid genome in *S. cerevisiae* (Aguilera & Zimmermann, 1986). The analogous experiment will be possible with the cloned *CDC30* gene. If the *PGII* and *CDC30* genes do not have significant nucleotide homology this raises the interesting possibility that the isoenzymes of PGI are an example of convergent evolution at the molecular level.

There will undoubtedly be further interest in PGI beyond its role as an essential catalyst in a major metabolic pathway due to the very recent reports that the neurotrophic factor neuroleukin is actually PGI (Chaput *et al.*, 1988; Faik *et al.*, 1988). The availability of the cloned yeast *PGII* gene facilitated this discovery (Faik *et al.*, 1988). It might be that there is also a mammalian homologue of *CDC30*.

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