Responsiveness to Exogenous cAMP of a *Saccharomyces cerevisiae* Strain Conferred by Naturally Occurring Alleles of *PDE1* and *PDE2*

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Manuscript received February 24, 1993

Accepted for publication June 18, 1993

ABSTRACT

The *Saccharomyces cerevisiae* strain P-28-24C, from which cAMP requiring mutants derived, responded to exogenously added cAMP. Upon the addition of cAMP, this strain showed phenotypes shared by mutants with elevated activity of the cAMP pathway. Genetic analysis involving serial crosses of this strain to a strain with another genetic background revealed that the responsiveness to cAMP results from naturally occurring loss-of-function alleles of *PDE1* and *PDE2*, which encode low and high affinity cAMP phosphodiesterases, respectively. In addition, P-28-24C was found to carry a mutation conferring slow growth that lies in *CYR1*, which encodes adenylate cyclase, and the slow growth phenotype caused by the *cyr1* mutation was suppressed by the *pde2* mutation. Therefore P-28-24C is fortuitously a *pde1 pde2 cyr1* triple mutant. Responsiveness to cAMP conferred by *pde* mutations suggests that *S. cerevisiae* cells are permeable to cAMP to some extent and that the apparent absence of effect of exogenously added cAMP on wild-type cells is due to immediate degradation by cAMP phosphodiesterases.

GENETIC study of the cAMP pathway in the yeast *Saccharomyces cerevisiae* was initiated by the isolation of cAMP-requiring mutants, which depend on exogenously added cAMP for growth (MATSUMOTO *et al.* 1982b). These mutants were isolated from a strain carrying the *cam1*, *cam2* and *cam3* mutations, which enable cells to utilize exogenous cAMP as an adenine source and were considered to facilitate cAMP uptake (MATSUMOTO *et al.* 1982a). The *cam* mutations, however, were found not to be necessary for cAMP-dependent growth of *cyr1-1* mutants with the P-28-24C genetic background (MATSUMOTO *et al.* 1982b). Here I report that the responsiveness to exogenously added cAMP of strain P-28-24C results from mutations in the cAMP phosphodiesterase genes.

MATERIALS AND METHODS

Strains: Yeast strains used in this study are listed in Table 1. A *cyr1-230* strain, HM110-3B, was constructed by five serial crosses of HM57-2C to YPH252. Gene disruptions of *PDE1* and *PDE2* were carried out by one-step gene replacement (ROTHSTEIN 1983) using plasmids *ppde1::URA3* and *ppde2::HIS3*, respectively (NIKAWA *et al.* 1987). Strains carrying a deletion allele of *CYR1* were constructed by transplacement (WINSTON, CHUMLEY and FINK 1983). A *URA3* plasmid carrying a *CYR1* gene with an internal deletion (MORISHITA, MATSUURA and UNO 1993) was integrated at the *CYR1* locus of *ura3* strains after digestion with *Cla*I, yielding strains carrying *URA3* flanked by intact and deletion alleles of *CYR1*. 5-Fluoroorotic acid resistant derivatives from these strains were screened for cAMP-dependent growth, yielding strains carrying only the deletion allele of *CYR1*.

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Media: Complete medium (YPD), synthetic minimal medium (SD) supplemented with nutrients, and minimal sporulation medium were prepared as described (SHERMAN, FINK and HICKS 1986; SHERMAN 1991). Synthetic complete medium lacking uracil was prepared by supplementing SD with tryptophan, adenine, and 0.5% casamino acids. YPD medium containing 1 or 5 mM cAMP was prepared by adding a filter-sterilized 50 mM cAMP solution, the pH of which was adjusted to 5.6 with NaOH. Medium containing 5-fluoroorotic acid was prepared as described (BOEKE *et al.* 1987). Cells were grown at 30° unless noted otherwise.

General genetic methods and transformation: Standard methods were used for genetic analysis (SHERMAN, FINK and HICKS 1986; SHERMAN 1991). Yeast transformation was carried out by the lithium acetate method (ITO *et al.* 1983).

Assay of responsiveness to cAMP: Since mutants with elevated activity of the cAMP pathway lose viability rapidly, colonies or patches of such mutants show a red stain on a YPD plate containing 20 µg/ml phloxine B, which stains dead cells (CANNON, GIBBS and TATCHELL 1986). The ability of cells to respond to exogenously added cAMP was tested by phloxine B staining. A red stain with the dye upon the addition of cAMP indicates responsiveness to cAMP. Incubation at 30° for 2 days on YPD medium containing phloxine B did not allow the development of red color by *ade2*.

Assay of glycogen accumulation: Relative glycogen levels were measured by iodine staining with a solution of 0.2% I₂, 0.4% KI (CHESTER 1968).

RESULTS

Cyclic AMP-responsive strains: Strains with the P-28-24C genetic background, unlike strains with other genetic backgrounds, responded to exogenously added cAMP. Upon the addition of cAMP, strain P-28-24C showed phenotypes shared by mutants with elevated activity of the cAMP pathway, including low glycogen accumulation and rapid loss of viability (data

TABLE 1
Yeast strains

Strain	Genotype or cross	Strain	Genotype or cross
P-28-24C ^a	<i>MATa eca1 eca2 eca3 pho3-1</i>	HM128-27B	<i>MATa eca3 lys2-801 trp1-Δ1</i>
YPH252 ^b	<i>MATα lys2-801 leu2-Δ1</i> <i>trp1-Δ1 ura3-52 ade2-101 his3-Δ200</i>	HM128-37D	<i>MATa eca1 eca2 eca3 lys2-801</i> <i>trp1-Δ1 ura3-52 ade2-101 his3-Δ200</i>
HM57-2C ^c	<i>MATa ras1::HIS3 cyr1-230</i> <i>leu2 trp1 ura3 met4 his3</i>	HM132-1A	<i>MATα eca3 lys2-801 leu2-Δ1</i> <i>trp1-Δ1 ura3-52 ade2-101 his3-Δ200</i>
HM110-3B	<i>MATa cyr1-230 lys2-801</i> <i>leu2 trp1 ura3 his3</i>	HM129	YPH252 × HM128-10B
HM120-15C ^d	<i>MATa eca1 eca2 eca3 lys2-801</i> <i>trp1-Δ1 ura3-52 ade2-101</i>	HM130	HM128-6D × HM128-10B
HM127-2D	<i>MATa eca1 eca2 eca3 lys2-801</i> <i>trp1-Δ1 ura3-52 ade2-101 his3-Δ200</i>	HM132	YPH252 × HM128-27B
HM128-6B	<i>MATα eca1 lys2-801 leu2-Δ1</i> <i>trp1-Δ1 ura3-52 ade2-101 his3-Δ200</i>	HM138	HM128-10B × HM128-10D
HM128-6D	<i>MATα eca1 eca2 lys2-801</i> <i>leu2-Δ1 trp1-Δ1 ura3-52 ade2-101 his3-Δ200</i>	HM139	P-28-24C × HM132-1A
HM128-9B	<i>MATa lys2-801 trp1-Δ1</i> <i>ura3-52 ade2-101 his3-Δ200</i>	HM142	YPH252 carrying <i>pde2::HIS3</i> × HM128-9B
HM128-10B	<i>MATa eca1 lys2-801 trp1-Δ1</i> <i>ura3-52 ade2-101 his3-Δ200</i>	HM143	YPH252 carrying <i>pde2::HIS3</i> × HM128-10B
HM128-10D	<i>MATα eca3 lys2-801 leu2-Δ1</i> <i>trp1-Δ1 ura3-52 ade2-101 his3-Δ200</i>	HM144	HM128-6B × HM128-10B carrying <i>pde1::URA3</i>
HM128-13B	<i>MATα eca1 eca3 lys2-801</i> <i>leu2-Δ1 trp1-Δ1 ura3-52 ade2-101 his3-Δ200</i>	HM145	HM128-6D × HM128-10B carrying <i>pde1::URA3</i>
		HM146	HM128-6D × HM128-27B
		HM147	HM128-6D carrying $\Delta cyr1$ × HM128-9B
		HM148	HM128-6D carrying $\Delta cyr1$ × HM128-27B
		HM149	YPH252 carrying <i>CYR1-URA3</i> × HM128-27B
		HM150	HM128-13B carrying <i>pde1::URA3</i> × HM128-37D

Haploid parents are given for diploids. On the basis of the allelism, *eca1*, *eca2* and *eca3* have been renamed *pde2-28*, *pde1-28* and *cyr1-28*, respectively, for P-28-24C. The genotype at the *PHO3* (formerly *PHOC*) locus was not determined during strain constructions.

^a TOH-E, KAKIMOTO and OSHIMA (1975). See DISCUSSION.

^b SIKORSKI and HIETER (1989). This strain is congenic with S288C.

^c MITSUZAWA *et al.* (1989).

^d H. MITSUZAWA, unpublished.

not shown). I constructed a cAMP-responsive strain (HM120-15C) for cell cycle analysis by crossing P-28-24C three times to YPH252, which has the S288C genetic background and was unable to respond to exogenously added cAMP (unpublished results). Responsiveness to cAMP was routinely assessed by phloxine B staining (see MATERIALS AND METHODS). HM120-15C stained dark red with phloxine B in the presence of 1 mM cAMP. Preliminary analysis to identify gene(s) responsible for the responsiveness to cAMP suggested that HM120-15C carries three unlinked mutations. By crossing HM120-15C three times to YPH252, a cAMP-responsive strain that was presumed to carry the postulated three mutations (HM127-2D) was constructed for further genetic analysis. HM127-2D was crossed to YPH252 to yield HM128, in which the genetic contribution of YPH252 is >99%.

Three unlinked mutations that derive from P-28-24C: Figure 1 shows meiotic progeny from HM128.

FIGURE 1.—Occurrence of slow growing spore clones that suggests segregation of a mutation conferring slow growth and its unlinked suppressor. A diploid that resulted from seven serial crosses of P-28-24C to YPH252 (HM128) was sporulated, and the tetrads were dissected onto YPD medium and allowed to grow at 30° for 2 days. The four spores from each tetrad are vertically aligned. Segregations of 2s:2n, 1s:3n and 0s:4n were observed for slow to normal growth in 8, 37 and 9 tetrads, respectively.

Segregations of 2s:2n, 1s:3n, and 0s:4n were observed for slow (s) to normal (n) growth. Approximately one-fourth of the segregants showed slow growth, although neither of the parent strains grew slowly. The result suggests that a mutation conferring slow growth

TABLE 2

Segregation of cAMP responsiveness and slow growth

Phloxine B staining ^a		Eca phenotype	No. of spore clones	
1 mM cAMP	5 mM cAMP		Normal	Slow growing
—	—	Eca [—]	55	53
—	+	Eca ⁺	53	0
++	++	Eca ⁺⁺	55 ^b	0

HM128 was sporulated, and the tetrads were dissected. Spore viability was 88%. Segregants were tested for responsiveness to cAMP by phloxine B staining. Results of 54 tetrads with four viable spores are shown. Segregations of 2Eca[—]:2Eca⁺, 2Eca[—]:1Eca⁺:1Eca⁺⁺, and 2Eca[—]:2Eca⁺⁺ were observed in 9, 35 and 10 tetrads, respectively.

^a Cell suspensions were spotted on YPD medium containing 20 µg/ml phloxine B, and the plates were incubated at 30° for 2 days. —, white or pale pink; +, red; ++, dark red.

^b About half of the Eca⁺⁺ spore clones (31 of 55) stained pink in the absence of cAMP.

and its suppressor segregate independently. The segregants were tested for responsiveness to cAMP by phloxine B staining. In all of the 54 tetrads examined, two spore clones stained red or dark red with phloxine B in the presence of 5 mM cAMP. The ratio of red to dark red spore clones was approximately 1:1. Spore clones that stained dark red in the presence of 5 mM cAMP also showed a dark red stain in the presence of 1 mM cAMP. In contrast, spore clones that stained red in the presence of 5 mM cAMP showed no stain in the presence of 1 mM cAMP. Therefore the spore clones were divided into three classes (Table 2): spore clones that did not stain in the presence of cAMP (this phenotype will be referred to as Eca[—] for exogenous cyclic AMP), spore clones that stained red in the presence of 5 mM cAMP but not in 1 mM cAMP (referred to as Eca⁺), and spore clones that stained dark red in the presence of 5 mM cAMP and in 1 mM cAMP (referred to as Eca⁺⁺). These results suggest that two unlinked mutations are involved in the responsiveness to cAMP: a mutation that confers responsiveness to 5 mM cAMP (designated *eca1*) and a mutation that confers responsiveness to 1 mM cAMP only in an *eca1* background (designated *eca2*). Eca[—] spore clones can be inferred to be either *ECA1 ECA2* or *ECA1 eca2*; Eca⁺ spore clones were *eca1 ECA2*; Eca⁺⁺ spore clones were *eca1 eca2*. None of the Eca⁺ and Eca⁺⁺ spore clones, both of which are postulated to carry *eca1*, showed slow growth (Table 2), suggesting that *eca1* itself is the suppressor of the mutation conferring slow growth (designated *eca3*). Taken together, these results suggest that P-28-24C carries three unlinked mutations.

***eca1* segregates as a single nuclear mutation:** A presumed *eca1* strain (HM128-10B, which was an Eca⁺ spore clone from a tetrad with two slow growing spore clones) was crossed to a wild-type (that is, Eca[—]) strain (YPH252). The resulting diploid (HM129) showed an Eca[—] phenotype, indicating that *eca1* is recessive. Te-

FIGURE 2.—2:2 segregation for responsiveness to cAMP conferred by *eca1* or *eca1 eca2*. Cell suspensions were spotted, and the plates were incubated at 30° for 2 days. When photographs were taken, a green filter was used to increase the contrast between white and red. Tetrads are shown in rows. (A) Tetrads from a diploid heterozygous for *eca1* (HM129) on YPD medium containing phloxine B and 5 mM cAMP. All of the 24 tetrads examined showed 2 white:2 red segregation. (B) Tetrads from a diploid homozygous for *eca1* and heterozygous for *eca2* (HM130) on YPD medium containing phloxine B and 1 mM cAMP. All of the 24 tetrads examined showed 2 white:2 dark red segregation on this medium and 2 red:2 dark red segregation on YPD medium containing phloxine B and 5 mM cAMP.

trad analysis of the diploid showed 2:2 segregation for responsiveness to 5 mM cAMP (Figure 2A), indicating that *eca1* segregates as a single nuclear mutation, as expected.

***eca2* segregates as a single nuclear mutation:** A presumed *eca1 eca2* strain (HM128-6D, which was an Eca⁺⁺ spore clone from a tetrad with two slow growing spore clones) was crossed to an *eca1* strain (HM128-10B). The resulting diploid (HM130) showed an Eca⁺ phenotype, indicating that *eca2* is recessive. Tetrad analysis of the diploid showed 2:2 segregation for responsiveness to 1 mM cAMP (Figure 2B), indicating that *eca2* segregates as a single nuclear mutation, as expected.

***eca3* segregates as a single nuclear mutation and is suppressed by *eca1*:** A presumed *eca3* strain (HM128-27B, which was a slow growing spore clone from a tetrad that consisted of two Eca⁺⁺ and two slow growing spore clones) was crossed to a wild-type strain (YPH252). The resulting diploid (HM132) showed normal growth, indicating that *eca3* is recessive. Tetrads from the diploid showed 2:2 segregation for slow growth (Figure 3), indicating that *eca3* segregates as a single nuclear mutation, as expected. Analysis of this cross involving the centromere-linked marker *leu2* showed that *eca3* is linked to a centromere (13 parental ditype:14 nonparental ditype:3 tetratype for *eca3* and *leu2*).

An *eca3* strain (HM128-10D) was crossed to an *eca1* strain (HM128-10B), and the resulting diploid (HM138) was sporulated and subjected to tetrad analysis. Segregations of 2s:2n, 1s:3n, and 0s:4n were observed for slow to normal growth. All tetrads

FIGURE 3.—2:2 segregation for slow growth conferred by *eca3*. A diploid heterozygous for *eca3* (HM132) was sporulated, and the tetrads were dissected onto YPD medium and allowed to grow at 30° for 2 days. The four spores from each tetrad are vertically aligned. All of the 30 tetrads examined showed 2:2 segregation.

showed 2Eca⁺:2Eca[−] segregation, and none of the Eca⁺ spore clones showed slow growth. These results confirm that *eca1* suppresses slow growth conferred by *eca3*.

To confirm that the *eca* mutations derived from P-28-24C, I crossed P-28-24C to an *eca3* strain (HM132-1A). The resulting diploid (HM139), unlike P-28-24C, showed slow growth, indicating that P-28-24C does carry *eca3* and its recessive suppressor *eca1*.

Cyclic AMP-dependent growth of *eca* Δ *cyr1* strains: Responsiveness to exogenously added cAMP of strains carrying the *eca* mutations was confirmed from the observation that *eca* Δ *cyr1* strains showed cAMP-dependent growth. An *eca1* Δ *cyr1* strain (HM128-6B carrying Δ *cyr1*) and an *eca1 eca2* Δ *cyr1* strain (HM128-6D carrying Δ *cyr1*) were unable to grow on YPD medium. The *eca1* Δ *cyr1* strain was able to grow on YPD medium containing 5 mM cAMP but not on 1 mM cAMP, and the *eca1 eca2* Δ *cyr1* strain was able to grow on YPD medium containing 1 mM cAMP.

Phenotypes of *eca* strains suggest that the *ECA* genes are involved in the cAMP pathway: Table 3 summarizes phenotypes of *eca* strains. It should be noted that *eca1 eca2* strains stained pink with phloxine B even in the absence of cAMP. In addition, *eca1 eca2* strains failed to accumulate glycogen, as judged by iodine staining. These phenotypes were suppressed by *eca3*; *eca1 eca2 eca3* showed no stain with phloxine B in the absence of cAMP and showed glycogen accumulation intermediate between those of *eca1 eca2* and wild-type strains. *eca3* strains showed more glycogen accumulation than did wild-type strains. The phenotypes observed for *eca1 eca2* strains are shared by mutants with elevated activity of the cAMP pathway, and the phenotypes observed for *eca3* strains are shared by mutants with reduced activity of the cAMP pathway (reviewed in BROACH 1991). These results suggest that *ECA1* and *ECA2* act negatively and *ECA3* acts positively in the cAMP pathway. Consistent with this, tetrad analysis of a cross between an *eca1* strain (HM128-6B) and a *cyr1-230* strain (HM110-3B) showed that *eca1* partially suppresses the temperature-sensitive growth conferred by *cyr1-230*. The responsiveness to cAMP conferred by *eca1* or *eca1 eca2* and

the centromere linkage of *eca3* suggest the possibility that *eca1*, *eca2* and *eca3* are alleles of *PDE2*, *PDE1* and *CYR1*, respectively.

***eca1* is allelic to *pde2*:** To determine whether *eca1* is an allele of *PDE2*, a *pde2* strain (YPH252 carrying *pde2::HIS3*) was constructed and tested for Eca phenotype. Like *eca1* strains, the *pde2* strain showed an Eca⁺ phenotype. A *pde2/PDE2* strain (HM142), like an *eca1/ECA1* strain (HM129), showed an Eca[−] phenotype, while a *pde2/eca1* strain (HM143) showed an Eca⁺ phenotype, indicating that *eca1* and *pde2* fail to complement each other. The *pde2/eca1* cells were sporulated and subjected to tetrad analysis. All of the 24 tetrads examined showed 4Eca⁺ segregation. These results indicate that *eca1* is a loss-of-function allele of *PDE2*, which encodes high affinity cAMP phosphodiesterase (SASS *et al.* 1986; WILSON and TATCHELL 1988). Therefore *eca1* has been renamed *pde2-28*. The resemblance of *pde2-28* strains to a *pde2::HIS3* strain in phloxine B staining suggests that the *pde2-28* product has little or no activity.

***eca2* is allelic to *pde1*:** To determine whether *eca2* is an allele of *PDE1*, an *eca1 pde1* strain (HM128-10B carrying *pde1::URA3*) was constructed and tested for Eca phenotype. Like *eca1 eca2* strains, the *eca1 pde1* strain showed an Eca⁺⁺ phenotype. An *eca1 pde1/eca1 PDE1* strain (HM144), like an *eca1 eca2/eca1 ECA2* strain (HM130), showed an Eca⁺ phenotype, while an *eca1 pde1/eca1 eca2* strain (HM145) showed an Eca⁺⁺ phenotype, indicating that *eca2* and *pde1* fail to complement each other. Since the *eca1 pde1/eca1 eca2* cells were found to be unable to sporulate (presumably because of elevated activity of the cAMP pathway), an *eca1 pde1 eca3/eca1 eca2 eca3* strain (HM150) was constructed and subjected to tetrad analysis. All of the 24 tetrads examined showed 4Eca⁺⁺ segregation. These results indicate that *eca2* is a loss-of-function allele of *PDE1*, which encodes low affinity cAMP phosphodiesterase (NIKAWA, SASS and WIGLER 1987). Therefore *eca2* has been renamed *pde1-28*. The resemblance of *pde1-28 pde2-28* strains to a *pde1::URA3 pde2-28* strain in phloxine B staining suggests that the *pde1-28* product has little or no activity.

***eca3* is allelic to *cyr1*:** An *eca3/ECA3* strain (HM146) and a Δ *cyr1/CYR1* strain (HM147) showed normal growth, while an *eca3/ Δ cyr1* (HM148) strain, like *eca3* strains, showed slow growth, indicating that Δ *cyr1* fails to complement *eca3*. A *ura3 eca3* strain was crossed to a *ura3* strain carrying a *CYR1* allele marked with *URA3*, and the resulting diploid (HM149) was subjected to tetrad analysis. In all of the 30 tetrads examined, the Ura[−] and slow growing phenotypes cosegregated 2:2. These results indicate that *eca3* is an allele of *CYR1*, which encodes adenylate cyclase (KATAOKA, BROEK and WIGLER 1985). Therefore *eca3* has been renamed *cyr1-28*. Since *CYR1* is an essential gene, the *cyr1-28* product must have residual activity.

TABLE 3
Phenotypes of *eca* strains

Genotype ^a	Phloxine B staining ^b			Eca phenotype	Growth ^c
	Without cAMP	1 mM cAMP	5 mM cAMP		
<i>ECA1 ECA2 ECA3</i>	—	—	—	Eca [—]	Normal (1.43 ± 0.04)
<i>ECA1 ECA2 eca3</i>	—	—	—	Eca [—]	Slow (2.66 ± 0.06)
<i>ECA1 eca2 ECA3</i>	—	—	—	Eca [—]	Normal
<i>ECA1 eca2 eca3</i>	—	—	—	Eca [—]	Slow
<i>eca1 ECA2 ECA3</i>	—	—	+	Eca ⁺	Normal
<i>eca1 ECA2 eca3</i>	—	—	+	Eca ⁺	Normal
<i>eca1 eca2 ECA3</i>	±	++	++	Eca ⁺⁺	Normal
<i>eca1 eca2 eca3</i>	—	++	++	Eca ⁺⁺	Normal

^a Strains with each genotype could be identified among segregants from HM128. For example, an *eca1 eca2 eca3* strain was identified as an Eca⁺⁺ spore clone from a tetrad with no slow growing spore clones, and an *eca1 eca2 ECA3* strain was identified as an Eca⁺⁺ spore clone from a tetrad with two slow growing spore clones.

^b Responsiveness to cAMP was assessed by phloxine B staining as described in the legend to Table 2. —, white or pale pink; ±, pink; +, red; ++, dark red.

^c For wild-type and *eca3* strains, doubling time in liquid YPD medium at 30° was determined by measuring OD₆₆₀. Mean doubling time ± SD in hours of four segregants with each genotype from HM132 are given in parentheses. *eca3* strains were able to grow on a YPD plate at 37°.

DISCUSSION

To identify gene(s) responsible for the responsiveness to exogenously added cAMP of strains with the P-28-24C genetic background, I crossed P-28-24C serially to YPH252, which has another genetic background. P-28-24C has been shown to carry mutations in the *PDE1*, *PDE2* and *CYR1* genes. The *pde* mutations confer the responsiveness to cAMP. P-28-24C derived from a *pho3* mutant isolated from H-42, O8-M3 (TOH-E *et al.* 1973). Strains H-42 and T-1074-77D (TOH-E *et al.* 1973) contribute approximately 87.5% and 12.5%, respectively, of the gene pool of P-28-24C (A. TOH-E, personal communication). The origins of the mutant alleles of *PDE1*, *PDE2* and *CYR1* in P-28-24C are unknown. Slow growth conferred by *cyr1-28* was suppressed by *pde2-28*, and rapid loss of viability conferred by *pde1-28* *pde2-28* was suppressed by *cyr1-28*. Thus the mutations in *CYR1* and *PDE* compensate for each other. *pde2-28* or *pde2::HIS3* was necessary and sufficient for responsiveness to 5 mM cAMP, and *pde1-28* or *pde1::URA3* had no effect on responsiveness to cAMP in a *PDE2* background but conferred increased responsiveness (that is, responsiveness to 1 mM cAMP) in a *pde2* background. A similar effect of a mutation in *PDE2* (*SRA5*) was reported by WILSON and TATCHELL (1988); a *pde2* null mutation causes rapid loss of viability upon nitrogen starvation in the presence of cAMP. Responsiveness to cAMP conferred by *pde* mutations suggests that *S. cerevisiae* cells are permeable to cAMP to some extent and that the apparent absence of effect of exogenously added cAMP on wild-type cells is due to immediate degradation by cAMP phosphodiesterases. Responsiveness to cAMP can be explained simply by defects in degradation, although the possibility cannot

be ruled out that the *PDE* products play a role in uptake and/or excretion of cAMP.

MATSUMOTO *et al.* (1982a) isolated mutations that enable cells to utilize exogenous cAMP as an adenine source: *cam1*, *cam2* and *cam3*. Since the *cam* mutations were considered to facilitate cAMP uptake, a strain carrying the *cam* mutations was used as the parent strain for the isolation of cAMP-requiring mutants (MATSUMOTO *et al.* 1982b). The functions of the CAM products remain unknown. Other mutations conferring responsiveness to cAMP have been reported. The *rca1* allele (BOY-MARCOTTE, GARREAU and JACQUET 1987) was identified through a cross between a *cyr1-1* strain with the P-28-24C genetic background and a strain with another genetic background. Therefore *rca1* is most likely to be identical to *pde2-28*. The *cas1* mutation (BOULET, PETITJEAN and HILGER 1985), which enables *cdc35* mutants to grow at the restrictive temperature in the presence of exogenous cAMP, may also be an allele of *PDE2*.

In this study crosses between strains with different genetic backgrounds have uncovered three mutations. Laboratory strains of *S. cerevisiae* are known to show genetic heterogeneities including allele variations that were introduced in crosses of the initial breeding stocks, induced by mutagenesis, or arose spontaneously. In fact, the analyses of the *CHC1*, *SIT4* and *SNF3* genes unexpectedly revealed allele variations among strains at the *SCD1*, *SSD1* and *RGT1* loci, respectively (LEMMON and JONES 1987; SUTTON, IMMANUEL and ARNDT 1991; MARSHALL-CARLSON *et al.* 1991). Genetic heterogeneity among laboratory strains can be useful for the identification of new genes. A difference in phenotype between strains with different genetic backgrounds (this study, for example) or a difference in phenotype conferred by a

mutation between strains with different genetic backgrounds could lead to the identification of gene(s) involved in the phenotype.

A cAMP-responsive strain can be constructed simply by disrupting the *PDE2* gene. However, *pde2* strains did not respond to 1 mM cAMP. In fact, higher concentrations of cAMP (4–5 mM) were added to strains carrying a disruption of *PDE2* to examine their effects on sensitivity to nitrogen starvation (WILSON and TATCHELL 1988) and trehalose metabolism (FRANÇOIS, NEVES and HERS 1991). Responsiveness to a lower concentration of cAMP (1 mM) was conferred by *pde1* in a *pde2* background. However, *pde1 pde2* strains stained pink with phloxine B even in the absence of exogenous cAMP. In contrast, *pde1-28 pde2-28 cyr1-28* strains did not stain with phloxine B in the absence of cAMP, although these strains have slightly elevated activity of the cAMP pathway, as judged by iodine staining. *pde1-28 pde2-28 cyr1-28* strains show responsiveness to 1 mM cAMP and have many auxotrophic markers useful for molecular genetics. Thus *pde1-28 pde2-28 cyr1-28* strains would be useful to examine the effects of the activation of the cAMP pathway by adding cAMP exogenously.

I thank J. NIKAWA and A. MATSUURA for plasmids and Y. OHYA and S. HARASHIMA for strains. I am grateful to A. TOH-E for providing information on a pedigree of P-28-24C.

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Communicating editor: E. W. JONES