Cloning of the pka1 Gene Encoding the Catalytic Subunit of the cAMP-dependent Protein Kinase in Schizosaccharomyces pombe*

(Received for publication, October 4, 1993, and in revised form, November 18, 1993)

Tatsuya Maeda†, Yoshinori Watanabe, Hirofumi Kunitomo, and Masayuki Yamamoto‡

From the Department of Biophysics and Biochemistry, School of Science, University of Tokyo, Hongo, Tokyo 113, Japan

We have isolated Schizosaccharomyces pombe genes that strongly hybridized to a probe carrying the open reading frame of Saccharomyces cerevisiae TPK1, which encodes a catalytic subunit of the cAMP-dependent protein kinase (protein kinase A). This S. pombe gene, named pka1, has a coding potential of 512 amino acids, and the deduced gene product is 60% identical with the S. cerevisiae Tpk1 protein in the C-terminal 320 amino acids. Disruption of pka1 slows cell growth but is not lethal. The resultant cells, however, are highly derepressed for sexual development, readily undergoing conjugation and sporulation in the absence of nutrient starvation. They are, thus, phenotypically indistinguishable from the adenylyl cyclase-defective (cyr1) cells previously characterized, except that the pka1 spores are retarded in germination, whereas the cyr1 spores are not. Disruption of pka1 is epistatic to a defect in cgs1, which encodes the regulatory subunit of protein kinase A. These results strongly suggest that the product of pka1 is a catalytic subunit of protein kinase A and, furthermore, that S. pombe has only one gene encoding it. This situation contrasts with the case of S. cerevisiae, in which three genes encode the catalytic subunits.

Cyclic AMP (cAMP) plays an important role in the regulation of sexual development in fission yeast Schizosaccharomyces pombe. Addition of cAMP to the medium inhibits sexual development (1, 2). Analysis of cAMP-related mutants isolated in S. pombe has confirmed that the level of intracellular CAMP is a critical factor in the initiation of mating and meiosis in this organism. S. pombe cells defective in the cgs2/pde1 gene, which encodes cAMP phosphodiesterase, have a high level of cAMP and can scarcely mate and sporulate (3, 4). In contrast, cells defective in the cyr1 gene, which encodes adenylyl cyclase (5, 6), do not have a detectable level of cAMP and readily initiate sexual development in the absence of nutritional deprivation (7, 8).

Analysis of S. pombe cells defective in cgs1, the gene for the regulatory subunit of the cAMP-dependent protein kinase (protein kinase A), has been done (5). The activity of the enzyme is constitutive in these cells, and they scarcely mate and sporulate.
periments of *S. pombe* (2, 17). SSA medium (18) was used to induce mating and sporulation in *S. pombe*. Standard techniques for molecular cloning were adopted from Sambrook *et al.* (19).

*S. pombe Genomic Library—* *S. pombe* genomic DNA was digested partially with Sau3AI. DNA fragments of 3.5–10 kb in length were recovered from an agarose gel after electrophoresis and ligated to the BamHI site of the vector pDB248′ (20). About 25,000 independent clones were pooled.

**Amplification of Probe DNA by Polymerase Chain Reaction—** We prepared a pair of primers to amplify a DNA fragment carrying the *S. cerevisiae* *TPKI* open reading frame (ORF) sequence using the polymerase chain reaction (PCR). Each primer consisted of 27 nucleotides. One had 18 nucleotides corresponding to the N-terminal 6 amino acids of Tpk1, whereas the other had the same number of nucleotides corresponding to the C-terminal 6 amino acids. Both primers had an additional nine nucleotides at their 5’ end, which provided an EcoRI cutting site. The primers were used as the membrane to accept DNA. Hybridization was done in 5 M LiCl, 0.075 M NaCl, 0.03 M sodium citrate, and 3% sodium dodecyl sulfate.

**Blotting Analysis—** Blotting analysis of DNA was performed according to Southern (22). The stringency conditions employed in this study are as follows. Either hybrid- 

**DNA Sequence Determination—** The nucleotide sequence of two adjacent HindIII fragments that in combination cover the entire *pha1* gene was determined by the dideoxy chain termination method (24) using an automated DNA sequenator (Applied Biosystems). Subclones for sequencing were generated by progressive deletion with exonuclease III and S1 nuclease (Takara Shuzo Co., Kyoto), according to Henikoff (25). To rule out the possibility that a small HindIII fragment is located between the two fragments, sequencing across the joint was carried out using the original clone pAK1 and two synthesized sequencing primers. All parts of the sequence (see Fig. 2) were determined in both directions at least once.

**Disruption of *pha1*—** One-step gene disruption (26) of *pha1* was carried out as follows. A 0.9-kb *PspoII* fragment within the cloned *pha1* ORF was replaced by a 1.8-kb *S. pombe ural* cassette (27). A 2.9-kb HindIII fragment carrying this disrupted *pha1* gene was used to transform both a haploid strain, *JY742*, and a diploid strain, *JY765*. Stable *Ura*+ transformants were selected in each case. A successful replacement of the wild-type *pha1* allele by the disrupted gene was confirmed by PCR and Southern blot analysis.

The abbreviations used are: kb, kilobase(s); ORF, open reading frame; PCR, polymerase chain reaction.

### Table I

*S. pombe* strains used

| Strain | Description |
|--------|-------------|
| L975   | *h* prototroph |
| JY450  | *h* ade6-M216 leu1 |
| JY476  | *h* ade6-M210 leu1 |
| JY742  | *h* ade6-M216 leu1 *ura4-D18* |
| JY765  | *h* ade6-M210 ade6-M216 leu1 *ura4-D18* |
| ZJ099  | *h* ade6-M216 leu1 *pat1-114* |
| ZJ633  | *h* ade6-M216 leu1 *ura4-D18* pAK1::*ura4* |
| ZJ634  | *h* ade6-M210 ade6-M216 leu1 *ura4-D18* pAK1::*ura4* |

### Table II

*S. cerevisiae* strains used

| Strain | Description |
|--------|-------------|
| KYM208-3C | α ade2 *ura3* trpl *ura3* cdc25-1 |
| RAY3A-1  | α his3 *ura3* trpl *ura3* |

--

**Expression of *pha1* in *S. cerevisiae—***To express *S. pombe* *pha1* in *S. cerevisiae*, we used an *S. cerevisiae* expression vector pKT10, which carries the *ARS* sequence of 2-mic DNA, *URA3*, and the promoter and terminator regions of the glyceraldehyde-phosphate dehydrogenase gene (28). The ORF sequence of *pha1* was amplified by PCR using a pair of primers carrying an EcoRI cutting site at one end, and the obtained fragment was inserted into the EcoRI site between the glyceraldehyde-phosphate dehydrogenase promoter and terminator of pKT10. The resultant plasmid was named pAR1.

**RESULTS**

**Isolation of Plasmids That Confer Sterility to the Wild-Type *S. pombe* Cells—** An *S. pombe* genomic library based on a multicyclic vector pDB248′ (20) was introduced into mating-proficient homothallic strains *JY450* and *JY476*. Altogether 50,000 transformants were tested by iodine staining of the colony for their ability to mate and sporulate (13). Nearly 300 transformants were judged to have lost the ability, and plasmid-segregation analysis suggested that one-third of them were sterile due to the plasmids they retained. Plasmids were recovered from them, and those which hybridized to either *pac1* or *pac2*, as mentioned in the Introduction, were eliminated from the following analysis. We obtained 61 novel plasmids that are able to endow sterility to the host cell upon transformation. Preliminary restriction mapping of these plasmids suggested that at least four different genes are responsible for the sterility (data not shown).

**Identification of the *pha1* Gene That Encodes a Putative Catalytic Subunit of Protein Kinase A—** The 61 plasmids obtained in the above screening were tested for their ability to hybridize to a probe carrying the *S. cerevisiae* *TPKI* ORF, which encodes a catalytic subunit of protein kinase A. One of the plasmids, which we hereafter call pAK1, hybridized strongly to the probe (data not shown). Cells transformed with pAK1 were defective in mating and assumed a long rodlike shape under nitrogen starvation (Fig. 1), like cells that have a high level of intracellular cAMP or protein kinase A activity (2, 3, 7, 8).

The genomic DNA of *S. pombe* inserted in pAK1 was about 5.5 kb in length. Subcloning of this insert suggested that a HindIII site is within the region essential for expression of the inhibitory activity (data not shown). We, therefore, sequenced the two HindIII fragments flanking this site, which were 2.0 and 0.9 kb in length, respectively. An ORF encompassing the essential region was carried by these two fragments, and we call this gene *pha1* hereafter.
The nucleotide sequence of the pkal gene and its deduced amino acid sequence are shown in Fig. 2. There is no evidence of introns in the gene structure, and the ORF can encode 512 amino acids. The deduced Pkal protein has unambiguous homology to catalytic subunits of protein kinase A. It is missing in the other proteins. The meaning of this result is unclear, but the N terminus of Pkal agrees with both the amino acid sequence and the intron structure. The deduced amino acid sequence is shown in Fig. 2.

**Fig. 2. Nucleotide sequence of the pkal gene and its deduced amino acid sequence.** The sequence of two adjacent HindIII fragments that in combination carry the entire pkal gene is shown. The contiguity of the two fragments has been confirmed by sequencing across the HindIII site between them, which is underlined. Numbering of the amino acid residues begins with the first methionine codon of the possible ORF.

The nucleotide sequence of the pkal gene and its deduced amino acid sequence are shown in Fig. 2. There is no evidence of introns in the gene structure, and the ORF can encode 512 amino acids. The deduced Pkal protein has unambiguous homology to catalytic subunits of protein kinase A. It is missing in the other proteins. The meaning of this result is unclear, but the N terminus of Pkal agrees with both the amino acid sequence and the intron structure. The deduced amino acid sequence is shown in Fig. 2.

**Fig. 2. Nucleotide sequence of the pkal gene and its deduced amino acid sequence.** The sequence of two adjacent HindIII fragments that in combination carry the entire pkal gene is shown. The contiguity of the two fragments has been confirmed by sequencing across the HindIII site between them, which is underlined. Numbering of the amino acid residues begins with the first methionine codon of the possible ORF.
described under "Experimental Procedures." Either a haploid strain JY742 (h' ade6-M216 leu1 ura4-D18) or a diploid strain JY765 (h'/h' ade6-M210/adde-M216 leu1/leu1 ura4-D18/ura4-D18) was used as the parent. JY763, derived from JY742, was proved to be a proper disruptant by PCR and Southern blot analysis of the chromosome structure (data not shown). For an unclear reason, the mating type of JY763 was changed to h' (homothallism).

Several diploid strains having one pkal allele properly disrupted were obtained from JY765. One of them, named J633, was subjected to tetrad analysis. Dissection of ascospores produced by J633 gave two pkal-disrupted haploid strains. Because these pkal disruptants exhibited essentially the same phenotypes as J633, we used J633 as a representative in the following analysis.

J633 forms colonies very slowly even on the complete medium YPD, and many zygotes and ascii can be seen in these colonies. When J633 is cultured in liquid medium, cells that give rise to colonies do so very slowly, and the colony size varies greatly from cell to cell. The colony size was not due to differential timing of germination, but rather due to differential growth rate of the cells after germination. The transformant carrying pDB248' failed to grow at 32 or 37 °C, being committed to ectopic sexual development due to the overexpression of pkal (see Table III). The phenotype of this strain is exactly the same as the pkal disruptant. The null cgsl allele in this strain was proven by genetic and Southern analysis. Thus, pkal is epistatic to cgsl, again confirming that it encodes the catalytic subunit of protein kinase A.

Expression of pkal—Expression of pkal mRNA in various strains under either nitrogen-rich or nitrogen-depleted conditions was measured by Northern blot analysis. A single RNA species of 2.9 kb in length was detected as the pkal transcript in all conditions examined. Neither the mating type of the cell, the abundance of a nitrogen source, nor the status of the cyrl allele greatly affected the level of expression of the pkal gene (data not shown).

Genetic Interaction of pkal with S. pombe cgsl and patl—
The cgsl gene of S. pombe encodes the regulatory subunit of protein kinase A (5). Mutants defective in cgsl are sterile because of the constitutive activation of protein kinase A. We constructed a strain in which both cgsl and pkal are disrupted. The phenotype of this strain is exactly the same as the pkal disruptant (data not shown). The null cgsl allele in this strain was proven by genetic and Southern analysis. Thus, pkal is epistatic to cgsl, again confirming that it encodes the catalytic subunit of protein kinase A.

Loss of function of the patl (also called ranl) gene derepresses sexual development ectopically in S. pombe (30–32). An increase in the level of intracellular cAMP can suppress this uncontrolled sexual development by repressing the expression of key genes essential for sexual development (2, 11, 17). Because pkal has not been isolated as a multicopy suppressor of patl, while pac1 and pac2 have (12, 17), we examined whether overexpression of pkal could inhibit ectopic sexual development driven by loss of patl function. To do this, a patl' strain JZ409 was transformed with either pAKl or the vector pDB248'. At 25 °C, both transformants could grow vegetatively. The transformant carrying pDB248' failed to grow at 32 or 37 °C, being committed to ectopic sexual development due to loss of patl function. However, the transformant carrying pAKl could grow at the restrictive temperature (Table III). Thus, overexpression of pkal has an ability to suppress patl.

Effects of Expression of pkal in S. cerevisiae—The activity of protein kinase A is essential for cell growth in S. cerevisiae (29, 33). The level of cAMP is regulated by Ras proteins in this yeast (34). The product of S. cerevisiae CDC25 is a positive regulator of Ras (35–37). Hence, the activity of CDC25 is essential for cell growth.
growth, and a cdc25\(^{c}\) strain KMY208-3C fails to grow at the restrictive temperature because the cells do not have enough cAMP and protein kinase A activity to support growth. To examine whether pka1 can generate protein kinase A activity in S. cerevisiae, we expressed it in KMY208-3C cells from a multicopy plasmid pAKS1. This plasmid carries the coding region of pka1 under the transcriptional control of the S. cerevisiae glyceraldehyde-phosphate dehydrogenase promoter. The KMY208-3C cells were converted to Ts\(^{c}\) by pAKS1 but not by the parental vector pKT10 (Fig. 5). This again strongly sug-

**TABLE III**

| JZ409 cells transformed with | 25° C | 32° C | 37° C |
|-----------------------------|-------|-------|-------|
| pDB248\(^{c}\) (vector)     | 5/5   | 0/5   | 0/5   |
| pAK1 (pka1\(^{c}\))         | 5/5   | 5/5   | 5/5   |

\(^{c}\) Five independent transformants were replica-plated and tested for their growth at the respective temperature indicated. Results were scored after 3 days of incubation. The number of grown transformants/total is given.

**Fig. 4. Southern blot analysis of S. pombe genomic DNA.** DNA prepared from a wild-type S. pombe strain L975 was digested with restriction enzymes, separated in agarose gel electrophoresis, and analyzed by Southern blotting as described under "Experimental Procedures." A, probed by DNA carrying part of the pka1 ORF (Ala\(^{c}\)-Ala\(^{c}\)); B, probed by DNA carrying the entire TPK1 ORF. These probes were prepared by PCR as described under "Experimental Procedures." E lanes, EcoRI digest; H lanes, HindIII digest; and B lanes, BamHI digest. Roughly equal amounts of probe DNA, labeled under the same protocol, were used in A and B. A\(^{c}\) and B\(^{c}\) are longer exposures of A and B, respectively. Bars on the left represent size markers, from top to bottom, 23, 9.4, 6.6, 2.3, and 2.0 kilobases.

**Fig. 5. Suppression of an S. cerevisiae cdc25\(^{c}\) mutation by pka1.** S. cerevisiae RAY3A-1 (cdc25) and KMY208-3C (cdc25\(^{c}\)) were transformed with either pAKS1, which can express S. pombe pka1 in S. cerevisiae, or the parental vector pKT10 (28). Patches of the transformed cells were replicated and grown for 2 days at the permissive (25° C) and the restrictive (37° C) temperature. A, RAY3A-1 carrying the vector; B, RAY3A-1 carrying pAKS1; C, KMY208-3C carrying the vector; and D, KMY208-3C carrying pAKS1. Two independent isolates were tested for each kind of transformant.

suggests that S. pombe Pka1 has protein kinase A activity.

S. cerevisiae cells become heat shock sensitive if they have a high level of either cAMP or protein kinase A activity. We, therefore, examined heat shock sensitivity of a wild-type S. cerevisiae strain (RAY3A-1) transformed with pAKS1. The parental and the transformed strains showed no significant difference in the sensitivity to heat shock (data not shown). This result may suggest that the expression of pka1 from pAKS1 was sufficient to suppress cdc25 but not high enough to make S. cerevisiae cells sensitive to heat shock. Alternatively, S. pombe protein kinase A and S. cerevisiae protein kinase A may have somewhat different substrate specificities.

**DISCUSSION**

This study has shown that S. pombe pka1 most likely encodes the catalytic subunit of cAMP-dependent protein kinase and, furthermore, that pka1 appears to be the only gene encoding it in the fission yeast. This situation contrasts with that in S. cerevisiae, where three genes encode the subunit. The redundancy of the genes in S. cerevisiae may reflect that the activity of protein kinase A is indispensable for cell growth in this organism (29). In S. pombe, however, although protein kinase A has a pivotal role in the developmental choice, its loss does not result in growth arrest, as shown in this study.

Including the results of this study, four major components of the cAMP cascade are cloned in S. pombe, namely adenyl cyclase (5, 6), cAMP phosphodiesterase (3, 4), the regulatory subunit of protein kinase A (3), and the catalytic subunit of it. Disruption and overexpression of these genes have given consistent results. Any genetic manipulation that increases the level of intracellular cAMP or the activity of protein kinase A makes S. pombe cells incapable of sexual development, whereas any manipulation that decreases the cAMP level or the protein kinase A activity propels cells toward ectopic sexual development (Refs. 3, 4, 7, 8, and this study). This agrees very well with the formula we proposed previously that cAMP serves as the
second messenger in the signaling pathway that controls gene expression for sexual development in response to the nutritional conditions (4, 7).

The cAMP cascade in *S. pombe* is involved also in the regulation of expression of the glucose-repressible gene *fbp1*, which encodes fructose-1,6-bisphosphatase (38). This gene is expressed in the absence of glucose, where the level of intracellular cAMP is decreased. Hoffman and Winston (39) isolated mutants in which transcription of *fbp1* is constitutive and named them *glt* (glucose-insensitive transcription). These mutations identified 10 genes, and one of them, *git2*, has been shown to be the same as *cyr1*, the gene for adenyl cyclase (38). The *pka1* and *gpa2* genes, the latter of which possibly regulates the activity of adenyl cyclase (10), are also found among the *glt* genes. However, *fbp1* is not regulated by *steI1* (see below). Thus, it is an interesting question how the two pathways, one for sexual development and the other for *fbp1* expression, are differentially regulated by protein kinase A.

This study predicts that the Pkal protein bears an extra N-terminal region not found in other protein kinase A. This region is not essential for the catalytic activity of the enzyme, because a domain carrying the kinase consensus alone apparently has the activity (data not shown). Whether this N-terminal region is involved in regulation of the enzyme activity unique to *S. pombe* remains unsolved.

Although the phenotypes of the *pka1*-defective and the *cyr1*-defective mutants are quite similar, only *pka1* spores display apparent germination disability. This may mean that a low activity of protein kinase A in the *cyr1* mutant, due either to leakage of the enzyme activity in the absence of cAMP or to a trace of cAMP in the medium, can accelerate the germination process.

The major substrate(s) of protein kinase A in *S. pombe* is yet unclear, as is true with many other eukaryotes. The most significant physiological function of *S. pombe* protein kinase A appears to be in repression of *steI2*, which encodes a key transcription factor for sexual development (11). We pointed out a possibility that the Ste11 protein autoregulates its expression, with its activity being down-regulated by phosphorylation by protein kinase A (11). Alternatively *steI2* may be regulated by another transcription factor that is a substrate of protein kinase A. Differentiation of these two possibilities will be required to identify the major substrate of protein kinase A in *S. pombe*.

Acknowledgments—We thank Drs. Yasushi Matsui, Kazuma Tanaka, and Akio Toh-e for *S. cerevisiae* strains, and the vector plasmid pKT10. We also thank Takako Iishiki and Yukako Iwahara for assistance in preparation of the manuscript. We are grateful to Dr. Charles Hoffman for critical reading of the manuscript.

References

1. Calleja, G. B., Johnson, B. F., and Yoo, B. Y. (1980) *Plant Cell Physiol.* 21, 613-624
2. Beach, D., Rodgers, L., and Gould, J. (1985) *Curr. Genet.* 10, 297-311
3. DeVito, J., Seydoux, G., Beach, D., and McLeod, M. (1991) *EMBO J.* 10, 3759-3766
4. Mochizuki, N., and Yamasato, M. (1992) *Mol. Gen. Genet.* 233, 17-24
5. Yamawaki-Katsuba, Y., Tamaoki, T., Choi, H.-R., Tanaka, H., and Katsuba, T. (1988) *Proc. Natl. Acad. Sci. U. S. A.* 85, 5695-5697
6. Young, D., Riggs, M., Field, J., Vogt, A., Brook, D., and Wigler, M. (1989) *Proc. Natl. Acad. Sci. U. S. A.* 86, 7989-7993
7. Mao, T., Mochizuki, N., and Yamamoto, M. (1990) *Proc. Natl. Acad. Sci. U. S. A.* 87, 7814-7818
8. Kawamura, K., Fugenschuh, M., and Young, D. (1991) *Cell Regul.* 2, 155-164
9. Robinson, G. A., Butcher, R., and Sutherland, E. W. (1971) *Cyclic AMP, Academic Press, New York*
10. Iishiki, T., Mochizuki, N., Maeda, T., and Yamamoto, M. (1992) *Genes & Dev.* 6, 2455-2462
11. Sugimoto, A., Iino, Y., Maeda, T., Watanabe, Y., and Yamamoto, M. (1991) *Genes & Dev.* 5, 1999-1999
12. Iino, Y., Sugimoto, A., and Yamamoto, M. (1990) *EMBO J.* 10, 221-226
13. Gutz, H., Hess, H., Leupold, U., and Loprieno, N. (1974) *Handbook of Genetics* (King, R. D., ed.), Vol. 1, pp. 395-446, Plenum Publishing Corp., New York
14. Sherman, F., Fink, G., and Hicks, J. (1986) *Methods in Yeast Genetics, Laboratory Course Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY*
15. Ito, H., Fukuda, Y., Murata, K., and Kimura, A. (1993) *J. Bacteriol.* 153, 163-165
16. Okazaki, K., Okazaki, N., Kume, K., Jinno, S., Tanaka, K., and Okayama, H. (1991) *Nucleic Acids Res.* 19, 6485-6489
17. Watanabe, Y., Iino, Y., Furuhata, K., Shimoda, C., and Yamamoto, M. (1988) *EMBO J.* 7, 761-767
18. Egel, R., and Egel-Mitani, M. (1974) *Exp. Cell Res.* 88, 127-134
19. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY*
20. Beach, D., Piper, M., and Nurse, P. (1982) *Mol. Gen. Genet.* 187, 326-329
21. Mulhis, R., and Fallica, A. (1987) *Methods Enzymol.* 155, 335-350
22. Southern, E. (1970) *Methods Enzymol.* 28, 351-359
23. Thomas, P. (1986) *Proc. Natl. Acad. Sci. U. S. A.* 77, 5201-5205
24. Sanger, F., Nicklen, S., and Coulson, A. (1977) *Proc. Natl. Acad. Sci. U. S. A.* 74, 5463-5467
25. Horikoff, S. (1984) *Genet. Anal.* 28, 351-359
26. Rathke, D. (1983) *Methods Enzymol.* 101, 202-211
27. Grimm, C., Kohli, J., Murray, J., and Maundrell, K. (1988) *Mol. Gen. Genet.* 215, 81-86
28. Tanaka, K., Nakashima, M., Tamaro, F., Kaziro, Y., Matsumoto, K., and Tob, E. A. (1990) *Mol. Cell. Biol.* 10, 4303-4313
29. Toda, T., Cameron, S., Sass, P., Zoller, M., and Wigler, M. (1987) *Cell* 50, 277-287
30. Toda, T., and Yamamoto, M. (1985) *Mol. Gen. Genet.* 198, 416-421
31. Iino, Y., and Yamamoto, M. (1985) *Proc. Natl. Acad. Sci. U. S. A.* 82, 2447-2451
32. Nurse, P. (1985) *Mol. Gen. Genet.* 198, 497-502
33. Matsumoto, K., Uno, I., and Ishikawa, T. (1985) *Proc. Natl. Acad. Sci. U. S. A.* 82, 497-502
34. Matsumoto, K., Uno, I., and Ishikawa, T. (1985) *Proc. Natl. Acad. Sci. U. S. A.* 82, 497-502
35. Camonis, J. H., Kalekin, M., Gendre, B., Garreau, H., Boy-Marceutte, R., and Jacquet, M. (1986) *EMBO J.* 5, 373-380
36. Broock, D., Toda, T., Michael, T., Levin, L., Brehme, C. Zoller, M., Powers, S., and Wigler, M. (1987) *Cell* 48, 788-799
37. Robinson, L. C., Gooch, J. B., Marshall, M. S., and Tatchell, K. (1987) *Science* 235, 1218-1221
38. Hoffman, C. S., and Winston, F. (1991) *Genes & Dev.* 5, 561-571
39. Hoffman, C. S., and Winston, F. (1990) *Genetics* 124, 867-876
40. Porter, L. L., Higgins, G. C., and Jackson, P. R. (1988) *J. Biol. Chem.* 263, 1676-1681
41. Kalderon, D., and Croizer, G. M. (1989) *Genes & Dev.* 2, 1539-1556
42. Shiekhattarian, L., scenic, N., Walsh, K. A., Fischer, H. E., and Titani, K. (1983) *Biochemistry* 22, 3762-3769