Cell, Vol. 48, 789–799, March 13, 1987, Copyright © 1987 by Cell Press

The S. cerevisiae *CDC25* Gene Product Regulates the *RAS*/Adenylate Cyclase Pathway

Daniel Broek, Takashi Toda, Tamar Michaeli, Lonny Levin, Carmen Birchmeier, Mark Zoller, Scott Powers, and Michael Wigler
Cold Spring Harbor Laboratory
Cold Spring Harbor, New York 11724

Summary

The gene corresponding to the S. cerevisiae cell division cycle mutant *cdc25* has been cloned and sequenced, revealing an open reading frame encoding a protein of 1589 amino acids that contains no significant homologies with other known proteins. Cells lacking *CDC25* have low levels of cyclic AMP and decreased levels of Mg^{2+}-dependent adenylate cyclase activity. The lethality resulting from disruption of the *CDC25* gene can be suppressed by the presence of the activated *RAS2*^{val19} gene, but not by high copy plasmids expressing a normal *RAS2* or *RAS1* gene. These results suggest that normal *RAS* is dependent on *CDC25* function. Furthermore, mutationally activated alleles of *CDC25* are capable of inducing a set of phenotypes similar to those observed in strains containing a genetically activated *RAS*/adenylate cyclase pathway, suggesting that *CDC25* encodes a regulatory protein. We propose that *CDC25* regulates adenylate cyclase by regulating the guanine nucleotide bound to *RAS* proteins.

Introduction

It is generally assumed that *RAS* proteins have a fundamental role in a wide variety of eukaryotic organisms, since they are so well conserved in evolution (Muller et al., 1982; Shilo and Weinberg, 1981; Defeo-Jones et al., 1983; Powers et al., 1984; Neuman-Silberberg et al., 1984). Many tumor cells contain mutationally activated *RAS* genes capable of tumorigenic transformation of established cell lines (for review see Bishop, 1985). In mammalian cells, neither the target of *RAS* proteins nor the regulation of *RAS* proteins is understood. The yeast Saccharomyces cerevisiae provides a good experimental system for understanding *RAS* function. First, the yeast *RAS* proteins are structurally related to their mammalian counterparts (Defeo-Jones et al., 1983; Powers et al., 1984), and like the mammalian *RAS* proteins they are capable of binding and hydrolyzing guanine nucleotides (Gibbs et al., 1984; Sweet et al., 1984; McGrath et al., 1984; Tamanoi et al., 1985). Second, yeast cells carrying the *RAS2*^{val19} mutation, a mutation analogous to the oncogenic human H-*ras*^{val12}, show a dominant phenotype including failure to arrest properly in G1 (Kataoka et al., 1984). Third, yeast cells lacking *RAS* function arrest in G1 (Kataoka et al., 1985a), a phenotype similar to that observed for mammalian cells after injection of a *ras*-specific monoclonal antibody (Mulcay et al., 1985). Fourth, the lethality that normally results from loss of yeast *RAS* function can be suppressed by the human H-*ras* gene (Kataoka et al., 1985a). Finally, the yeast *RAS* proteins are positive regulators of yeast adenylate cyclase (Toda et al., 1985; Broek et al., 1985), and although this function may not be conserved in evolution (Birchmeier et al., 1985), the human H-*ras* protein is also a potent activator of the yeast adenylate cyclase (Broek et al., 1985). Because of the structural, biochemical, and functional homology between the yeast and human *RAS* proteins, we are interested in identifying gene products in yeast that influence *RAS* function. Among these gene products will be proteins that are able to interact directly with the mammalian *RAS* proteins and that may have domains and functions which are conserved in evolution. In our attempt to identify such interacting proteins, we have investigated the temperature-sensitive cell division cycle (cdc) mutant *cdc25*, which causes G1 arrest at the nonpermissive temperature (Hartwell et al., 1973; Hartwell, 1974). In experiments presented here, we describe the cloning of the *CDC25* gene, present its DNA sequence, and demonstrate the involvement of *CDC25* in the control of the *RAS*/adenylate cyclase pathway.

Results

Cloning and Sequencing of *CDC25*

To isolate the *CDC25* gene, we screened libraries of yeast DNA for genes capable of restoring growth at the nonpermissive temperature to a *cdc25*-1 strain. We used strain 25-1 and TT25-6 for our screening (see Table 1 for descriptions of these and all other strains referred to below). Two libraries were used for this purpose. One library carried a *URA3* gene on a centromere-linked plasmid, and the other carried a *LEU2* gene on a high copy plasmid (see Experimental Procedures). After transformation, Ura^+ or Leu^+ transformants were selected at the permissive temperature and replica-plated at 35°C. Several transformants were able to grow at the higher temperature. Analysis of the plasmids from these transformants showed that two different yeast chromosomal loci were represented. The sequence of one of these genes, *TPK1*, indicated that it encodes a protein highly homologous to the catalytic subunit of the mammalian cAMP-dependent protein kinase (T. Toda unpublished data, and see below). The other gene was shown to be allelic to the *cdc25* locus by integrative mapping and complementation analysis (see Experimental Procedures). Restriction endonuclease cleavage maps of the yeast genomic inserts from the *CDC25* locus are shown in Figure 1.

A 2.0 kb BglII–SphI fragment of the *CDC25* clones that retained complementing activity was sequenced (Figure 1). Sequencing was continued until we had sequenced the entire open reading frame from this locus. The *CDC25* gene contains an open reading frame of 4767 nucleotides with the capacity to encode a protein of 1589 amino acids (Figure 2). An in-frame stop codon was found 13 codons upstream of the first methionine codon of the open read-

Table 1. Genotypes of Yeast Strains Used for These Studies

Strain	Genotype[a]
TT1A-1[b]	*MATα his3 leu2 ura3 trp1 ade8 cdc25::URA3* p*CDC25(TRP1)*-1.
TT1A-2	*MATα his3 leu2 ura3 trp1 ade8 cdc25::URA3* p*CDC25(LEU2)*-3.
TT1A-3	*MATα his3 leu2 ura3 trp1 ade8 cdc25::URA3* p*TPK1*.
TT1A-4	*MATα his3 leu2 ura3 trp1 ade8 cdc25::URA3* p*CDC25(LEU2)*-2.
TT1A-5	*MATα his3 leu2 ura3 trp1 ade8 cdc25::URA3* p*RAS2*^{val19}.
TMHS-20	*MATα his3 leu2 ura3 trp1 ade8 cdc25::URA3* p*CDC25*^{HS}(*LEU2*)-20.
SP1	*MATa his3 leu2 ura3 trp1 ade8 can1*.
SP12	*MATa his3 leu2 ura3 trp1 ade8 can1* p*TPK1*.
TTSD1	A diploid strain formed by mating SP1 and DC124.
DC124	*MATα leu2 trp1 ura3 ade8 his4*.
TT25-1	*MATa his3 leu2 trp1 can1 cdc25*-1.
TK161-R2V	*MATa his3 leu2 ura3 trp1 ade8 can1 RAS2*^{val19}.
TMRV-25	*MATa his3 leu2 ura3 trp1 ade8 can1 RAS2*^{val19} *cdc25::URA3*.
TT25-6[c]	*MATα leu2 ura3 trp1 can1 cdc25*-1.
TTS3801	*MATa/MATα his3/HIS3 his4/HIS4 leu2/leu2 ura3/ura3 trp1/trp1 ade8/ade8 can1/CAN1 cdc25::URA3/CDC25*.
25-1	*MATα leu2 ura1 ade2 cdc25*-1.
JR26-19B	*MATα leu2 his3 ura3 ade2 can1 lys1*.
TR56	A diploid strain formed by mating TT25-1 and JR26-19B.
TTS1201[d]	A *His3*$^+$ strain formed by transforming TR56 with the NcoI linearized p*CDC25(HIS3)*-1.
TTS1203[d]	An independent transformant similar to TTS1201.
TTS1204[d]	An independent transformant similar to TTS1201.
TTS1202[d]	An independent transformant similar to TTS1201.

[a] See Experimental Procedures for nomenclature used here and throughout the text. pXXX indicates that the given strain contain a particular extrachromosomal plasmid.
[b] TT1A-1 is a segregant of the strain TTS3801 after transformation with p*CDC25(TRP)*-1.
[c] TT25-6 is a segregant of a diploid that was formed by mating 25-1 (Hartwell et al., 1973) with SP1.
[d] See integrative mapping under Experimental Procedures.

ing frame. Northern blot analysis indicates that the transcript from this locus is 5.3 kb in length, suggesting that the entire open reading frame is used (data not shown). However, the BglII–SphI fragment of the *CDC25* gene (see p*CDC25(TRP1)*-1 in Figure 1), encoding amino acid positions 876 to 1552, retained complementing activity, indicating that only a portion of the *CDC25* gene is required for its essential function.

Suppression of *cdc25*$^-$ Growth Defects by Genes of the *RAS*/Adenylate Cyclase Pathway

We first tested whether, as expected, disruption of the *CDC25* gene caused growth arrest in haploid cells. For this purpose, we constructed a diploid strain, TTS3801, in which one *CDC25* allele was disrupted by the *URA3* auxotrophic marker (see Experimental Procedures). Tetrad analysis indicated that haploid spores lacking the *CDC25* gene were generally incapable of germination. In a few instances, such spores germinated and underwent a limited number of doublings. The diploid strain TTS3801, which is *trp1/trp1*, was then transformed with the plasmid p*CDC25-(TRP1)*-1, which contains the complementing BglII–SphI fragment of the *CDC25* gene, *ARS1*, and *TRP1* on a multicopy extrachromosomal replicating yeast plasmid (see Figure 1). One of the resulting Trp$^+$ transformants was sporulated and gave rise to haploid progeny strain TT1A-1, which lacks a chromosomal *CDC25* gene but carries an active BglII–SphI fragment of *CDC25* gene on the extrachromosomal plasmid. *ARS1*-containing plasmids of this type are normally unstable in the absence of selective pressure. However, p*CDC25(TRP1)*-1 was not lost from TT1A-1 even when this strain was grown for many generations in rich medium containing tryptophan. Experiments described below show the plasmid had not integrated into the yeast genome. These results confirm that a functional *CDC25* gene is required for vegetative growth.

To explore the relationship between the *CDC25* product and the genes encoding members of the *RAS*/adenylate cyclase pathway, we assayed the stability of the plasmid p*CDC25(TRP1)*-1 in strain TT1A-1 after transformation with high copy plasmids containing various genes. For this purpose we used the YEp213 plasmid, which contains only the *LEU2* marker, as well as p*RAS1*-2, p*RAS2*-1, p*RAS2*^{val19}, p*TPK1*, p*CYR1*-11, and p*CDC25(LEU2)*-2 (see Table 2 and Experimental Procedures for a description of these plasmids). After transformation of these plasmids into TT1A-1, Leu$^+$ transformants were selected. If cells contained a suppressor of *cdc25*$^-$, the Trp$^+$ phenotype would become unstable. To test this, the Leu$^+$ transformants were grown without selection in YPD (rich) medium for 48 hr and plated onto YPD. The resulting colonies were replica plated onto YPD and onto tryptophan prototrophic medium (see Table 2). The p*CDC25(TRP1)*-1 plasmid was not lost from any of the Leu$^+$ transformants carrying YEp213, p*RAS1*-2, or p*RAS2*-1. Thus, high copy plasmids carrying the *RAS1* or *RAS2* genes do not suppress *cdc25*$^-$. In contrast p*CDC25(TRP1)*-1 was readily lost from the transformants carrying p*CDC25(LEU)*-2, p*RAS2*^{val19}, or p*TPK1*. The p*CDC25(TRP1)*-1 plasmid could also be lost, although at a lower frequency, from transformants carrying p*CYR1*-11. These demonstrate that the growth defect of cells lacking *CDC25* can be suppressed by high copy plasmids encoding *RAS2*^{val19} protein, the catalytic subunit of cAMP-dependent protein kinase

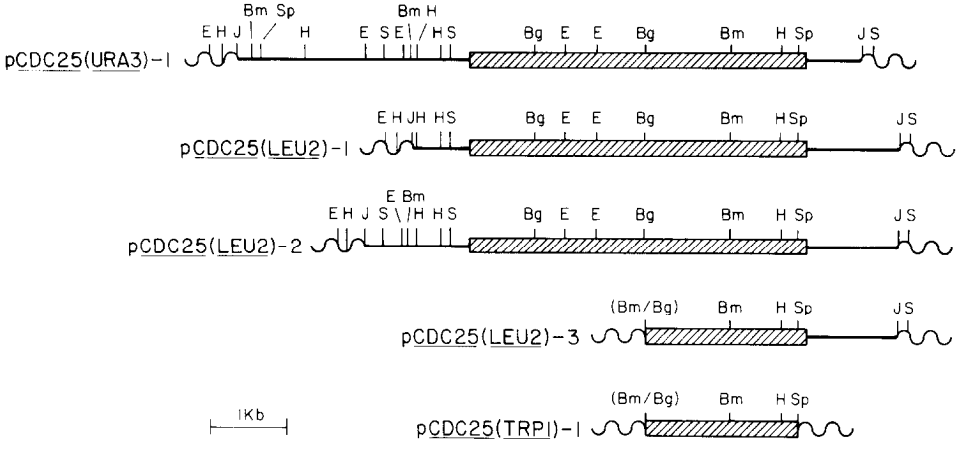

Figure 1. Structure of the *CDC25* Gene and Plasmids Used for These Studies

Restriction maps of the inserts and flanking regions of vectors are shown for the *CDC25* containing plasmids used for these studies. Coding sequences for the *CDC25* gene are indicated by hatched bars. J represents a junction between an insert DNA and the vector. Wavy lines represent flanking vector sequences. p*CDC25(URA3)*-1 was isolated from a YCp50 genomic library as a suppressor of the temperature-sensitive phenotype of yeast strain TT25-6. p*CDC25(LEU2)*-1 and p*CDC25(LEU2)*-2 were isolated from a YEp13 genomic library as a suppressor of the temperature-sensitive phenotype of strain 25-1. The construction of p*CDC25(TRP1)*-1 and p*CDC25(LEU2)*-3 is described in Experimental Procedures. Abbreviations used are as follows: Bm, BamHI; Bg, BglII; E, EcoRI; H, HindIII; S, SalI; Sp, SphI.

(*TPK1*), or adenylate cyclase (*CYR1*). These results suggest that *CDC25* may participate in the *RAS*/adenylate cyclase pathway.

Altered Adenylate Cyclase Activity in Cells Lacking *CDC25*

We investigated the possible interaction of *CDC25* with the adenylate cyclase pathway at the biochemical level. For this purpose, we first measured cAMP levels in yeast strains lacking chromosomal *CDC25* but carrying high copy suppressor plasmids. Strain TT1A-3 carried p*TPK1*, which expresses the catalytic subunit of the cAMP-dependent protein kinase (cAPK), and strain TT1A-4 carried p*CDC25(LEU2)*-2, which contains the full-length *CDC25* gene. As shown in Table 3, strain TT1A-3, which lacks *CDC25*, has only 15%–30% of the cAMP level of strain TT1A-4, which contains a functional *CDC25* gene. To rule out the possibility that the high copy *TPK1* plasmid resulted in reduction of cAMP levels by negative feedback, we compared the levels of cAMP present in a wild-type strain, SP1, to the levels in the same strain harboring the high copy *TPK1* plasmid. No differences in cAMP levels were observed (Table 3). Thus, we conclude that cells lacking *CDC25* function cannot maintain normal levels of cAMP.

We next tested whether the disruption of the *CDC25* gene affected adenylate cyclase as assayed in vitro. Membranes were prepared from various yeast strains, and adenylate cyclase activity was measured in the presence of Mn^{2+}, which measures the amount of adenylate cyclase catalytic subunit independent of *RAS* and guanine nucleotides (Casperson et al., 1983; Toda et al., 1985; Broek et al., 1985). Strains lacking *CDC25* and strains containing *CDC25* did not show dramatic differences in the levels of adenylate cyclase activity when assayed in the presence of Mn^{2+} (see Table 4). Thus, *CDC25* does not appear to directly affect the amount of adenylate cyclase. However, strains lacking *CDC25* showed less than 10% of the Mg^{2+}-dependent adenylate cyclase activity observed in strains containing *CDC25*.

We have previously shown that in the presence of Mg^{2+}, yeast adenylate cyclase activity is *RAS*-dependent. *RAS* proteins must bind guanine triphosphates (GTP or Gpp(NH)p, a nonhydrolyzable analogue of GTP) to effectively stimulate adenylate cyclase (Toda et al., 1985; Broek et al., 1985; J. Field, unpublished data). This probably accounts for the stimulation of adenylate cyclase activity by Gpp(NH)p in wild-type strain backgrounds (Casperson et al., 1983; Toda et al., 1985). The addition of Gpp(NH)p to membranes from the strain lacking *CDC25* stimulated the Mg^{2+}-dependent adenylate cyclase activity to a level similar to that seen in strains with wild-type backgrounds (see Table 4 and Figure 3). Indeed, the guanine nucleotide concentration required to stimulate adenylate cyclase in strains without *CDC25* function is very similar to the concentration required in wild-type strains (Figure 3). Thus, the *RAS* proteins in membranes lacking *CDC25* are capable of function. However, the lower levels of Mg^{2+}-dependent adenylate cyclase activity (in the absence of Gpp(NH)p) seen in membranes from the strain lacking *CDC25*, TT1A-3, could indicate that basal levels of *RAS* function may be impaired.

Effects of *CDC25* Disruption in Strains Containing Single Copy *RAS2*^{val19}

We previously showed that high copy plasmids expressing *RAS2*^{val19}, but not wild-type *RAS2*, can suppress the lethality that otherwise results from disruption of *CDC25* (Table 2). This is true even for cells containing a single copy of *RAS2*^{val19}. The strain TK161-R2V (see Table 1) was

```
-312                                                                                                  GTCGACGTCAGTCACCTCCAGGTTATTATGT
-281  AATTCGCTAAAACGACTTTTAAACATCGCGGGAGACGGATCTTTGACAGAGACCTGGAGGAAAAAATAAAATAGAAAAATAAGCTCAAAAGTTACAGAGAAAACTGCATCACCAACACG
-162  GCGGAGACAGTTATAGTGACTTGATCACCGTGAAGCAATTCCTTTAGTTAGATAGACTGTTTCTTTTCGGAAAGAAATTGAATAAAAAGAGCCACATTTTTCTTCGAATAAATACTAA

   1        ***                                          MET SER ASP THR ASN THR SER ILE PRO ASN THR SER SER ALA ARG GLU
 -43  AAAATAA AAC GAA AAA GCA AGG TGG ATA TTG GAT AGT TGT ATC ATG TCC GAT ACT AAC ACG TCT ATT CCC AAT ACA AGT TCT GCA AGG GAG

  17  ALA GLY ASN ALA SER GLN THR PRO SER ILE SER SER SER SER ASN THR SER THR THR THR ASN THR GLU SER SER SER ALA SER LEU SER
  49  GCA GGC AAT GCT TCA CAA ACT CCA TCG ATC AGC TCT TCA TCT AAC ACT TCC ACT ACC ACT AAC ACA GAA TCA TCC TCA GCT TCT CTT TCT

  47  SER SER PRO SER THR SER GLU LEU THR SER ILE ARG PRO ILE GLY ILE VAL VAL ALA ALA TYR ASP PHE ASN TYR PRO ILE LYS LYS ASP
 139  TCT TCC CCC TCG ACA AGT GAG TTG ACC AGC ATT CGT CCA ATT GGA ATA GTA GTC GCT GCT TAT GAC TTT AAT TAT CCC ATT AAA AAA GAC

  77  SER SER SER GLN LEU LEU SER VAL GLN GLN GLY GLU THR ILE TYR ILE LEU ASN LYS ASN SER SER GLY TRP TRP ASP GLY LEU VAL ILE
 229  AGT TCT TCG CAA CTT TTG TCT GTA CAA CAA GGG GAA ACC ATT TAT ATA CTT AAC AAA AAC TCA TCT GGG TGG TGG GAT GGA TTA GTT ATT

 107  ASP ASP SER ASN GLY LYS VAL ASN ARG GLY TRP PHE PRO GLN ASN PHE GLY ARG PRO LEU ARG ASP SER HIS LEU ARG LYS HIS SER HIS
 319  GAC GAC AGT AAT GGG AAA GTT AAC AGA GGC TGG TTT CCT CAA AAC TTC GGT AGA CCT TTA AGA GAC AGT CAT CTC AGA AAG CAC AGT CAT

 137  PRO MET LYS LYS TYR SER SER SER LYS SER SER ARG ARG SER SER LEU ASN SER LEU GLY ASN SER ALA TYR LEU HIS VAL PRO ARG ASN
 409  CCG ATG AAA AAA TAT AGT TCC AGT AAG AGC TCA AGG CGC AGC AGC TTA AAT AGC TTG GGC AAT AGT GCA TAT TTA CAT GTG CCT AGA AAT

 167  PRO SER LYS SER ARG ARG GLY SER SER THR LEU SER ALA SER LEU SER ASN ALA HIS ASN ALA GLU THR SER SER GLY HIS ASN ASN THR
 499  CCG AGC AAG AGC AGG AGG GGG AGT TCT ACT TTA TCA GCG TCT TTA TCA AAT GCC CAC AAT GCA GAA ACA AGT TCC GGC CAC AAT AAC ACC

 197  VAL SER MET ASN ASN SER PRO PHE SER ALA PRO ASN ASP ALA SER HIS ILE THR PRO GLN SER SER ASN PHE ASN SER ASN ALA SER LEU
 589  GTA TCG ATG AAT AAT TCT CCC TTT TCA GCG CCA AAC GAT GCT TCC CAC ATA ACC CCT CAA TCT TCG AAC TTT AAT TCC AAT GCT AGT CTA

 227  SER GLN ASP MET THR LYS SER ALA ASP GLY SER SER GLU MET ASN THR ASN ALA ILE MET ASN ASN ASN GLU THR ASN LEU GLN THR SER
 679  TCC CAG GAT ATG ACA AAG AGT GCA GAT GGC TCA TCT GAG ATG AAT ACA AAC GCA ATT ATG AAT AAC AAT GAA ACA AAT TTA CAA ACT TCT

 257  GLY GLU LYS ALA GLY PRO PRO LEU VAL ALA GLU GLU THR ILE LYS ILE LEU PRO LEU GLU GLU ILE GLU MET ILE ILE ASN GLY ILE ARG
 769  GGT GAG AAA GCA GGT CCC CCA CTA GTA GCA GAA GAA ACA ATT AAG ATA TTA CCG TTG GAA GAG ATA GAA ATG ATT ATT AAT GGT ATA CGT

 287  SER ASN ILE ALA SER THR TRP SER PRO ILE PRO LEU ILE THR LYS THR SER ASP TYR LYS LEU VAL TYR TYR ASN LYS ASP LEU ASP ILE
 859  TCG AAC ATT GCT TCG ACT TGG TCC CCC ATA CCA CTG ATA ACG AAA ACA TCC GAT TAC AAG TTG GTA TAC TAT AAC AAA GAC CTT GAT ATA

 317  TYR CYS SER GLU LEU PRO LEU ILE SER ASN SER ILE MET GLU SER ASP ASP ILE CYS ASP SER GLU PRO LYS PHE PRO PRO ASN ASP HIS
 949  TAC TGT TCA GAA TTA CCC TTG ATT TCT AAC TCA ATT ATG GAA TCC GAT GAC ATT TGT GAC AGC GAA CCA AAA TTC CCG CCC AAT GAT CAT

 347  LEU VAL ASN LEU TYR THR ARG ASP LEU ARG LYS ASN ALA ASN ILE GLU ASP SER SER THR ARG SER LYS GLN SER GLU SER GLU GLN ASN
1039  CTT GTT AAC CTA TAT ACT AGA GAT CTG AGG AAA AAT GCG AAT ATT GAG GAC AGT TCT ACG AGA TCG AAG CAA TCG GAA AGT GAA CAA AAT

 377  ARG SER SER LEU LEU MET GLU LYS GLN ASP SER LYS GLU THR ASP GLY ASN ASN ASN SER ILE ASN ASP ASP ASP ASN ASN ASN GLU ASN
1129  AGA TCA AGC CTT CTA ATG GAA AAA CAG GAT TCA AAA GAA ACT GAT GGA AAT AAT AAC AGT ATT AAT GAT GAT GAT AAT AAT AAC GAA AAT

 407  ASN LYS ASN GLU PHE ASN GLU ALA GLY PRO SER SER LEU ASN SER LEU SER ALA PRO ASP LEU THR GLN ASN ILE GLN SER ARG VAL VAL
1219  AAC AAA AAC GAA TTC AAT GAG GCT GGT CCT TCA TCA TTA AAT TCT TTA TCT GCT CCA GAT TTA ACG CAG AAT ATT CAA TCA AGG GTA GTT

 437  ALA PRO SER ARG SER SER ILE LEU ALA LYS SER ASP ILE PHE TYR HIS TYR SER ARG ASP ILE LYS LEU TRP THR GLU LEU GLN ASP LEU
1309  GCC CCA AGT CGC TCT TCT ATA CTG GCC AAG AGT GAC ATC TTT TAT CAC TAT TCA AGA GAT ATA AAA TTG TGG ACA GAA TTA CAA GAC CTA

 467  THR VAL TYR TYR THR LYS THR ALA HIS LYS MET PHE LEU LYS GLU ASN ARG LEU ASN PHE THR LYS TYR PHE ASP LEU ILE SER ASP SER
1399  ACA GTT TAT TAT ACT AAA ACG GCT CAC AAG ATG TTC CTT AAA GAG AAT CGT CTC AAT TTC ACG AAA TAC TTT GAT TTG ATA TCA GAT TCA

 497  ILE VAL PHE THR GLN LEU GLY CYS ARG LEU MET GLN HIS GLU ILE LYS ALA LYS SER CYS SER LYS GLU ILE LYS LYS ILE PHE LYS GLY
1489  ATA GTC TTC ACA CAG TTA GGC TGC AGG CTA ATG CAA CAT GAA ATT AAA GCC AAA AGT TGC AGC AAG GAG ATT AAG AAG ATT TTC AAA GGT

 527  LEU ILE SER SER LEU SER ARG ILE SER ILE ASN SER HIS LEU TYR PHE ASP SER ALA PHE HIS ARG LYS LYS MET ASP THR MET ASN ASP
1579  CTA ATC TCT TCA TTG TCA AGG ATA AGT ATC AAT TCT CAT TTA TAT TTC GAT TCA GCT TTT CAC AGA AAA AAA ATG GAT ACT ATG AAT GAC

 557  LYS ASP ASN ASP ASN GLN GLU ASN ASN CYS SER ARG THR GLU GLY ASP ASP GLY LYS ILE GLU VAL ASP SER VAL HIS ASP LEU VAL SER
1669  AAG GAT AAC GAT AAT CAG GAA AAT AAT TGT TCT AGG ACG GAA GGG GAT GAT GGT AAA ATT GAA GTA GAT AGT GTA CAT GAT CTA GTT TCA

 587  VAL PRO LEU SER GLY LYS ARG ASN VAL SER THR SER THR THR ASP THR LEU THR PRO MET ARG SER SER PHE SER THR VAL ASN GLU ASN
1759  GTT CCA TTG TCC GGT AAA CGT AAT GTA AGT ACC AGT ACA ACG GAT ACA TTG ACT CCA ATG AGA TCA TCA TTC AGT ACA GTC AAT GAG AAC

 617  ASP MET GLU ASN PHE SER VAL LEU GLY PRO ARG ASN SER VAL ASN SER VAL VAL THR PRO ARG THR SER ILE GLN ASN SER THR LEU GLU
1849  GAT ATG GAA AAT TTC TCA GTC TTA GGT CCA AGA AAT AGT GTT AAT TCT GTC GTA ACA CCA AGG ACT TCA ATA CAA AAT TCT ACT TTG GAA

 647  ASP PHE SER PRO SER ASN LYS ASN PHE LYS SER ALA LYS SER ILE TYR GLU MET VAL ASP VAL GLU PHE SER LYS PHE LEU ARG HIS VAL
1939  GAT TTT TCA CCG TCC AAC AAA AAT TTT AAG TCA GCT AAA TCG ATT TAC GAA ATG GTT GAT GTG GAA TTC TCG AAA TTT TTA AGG CAT GTT

 677  GLN LEU LEU TYR PHE VAL LEU GLN SER SER VAL PHE SER ASP ASP ASN THR LEU PRO GLN LEU LEU PRO ARG PHE PHE LYS GLY SER PHE
2029  CAG TTA CTT TAT TTT GTG TTA CAG AGC TCA GTC TTC TCA GAT GAT AAT ACT TTA CCA CAG TTG CTC CCA AGA TTT TTT AAA GGT TCA TTT

 707  SER GLY GLY SER TRP THR ASN PRO PHE SER THR PHE ILE THR ASP GLU PHE GLY ASN ALA THR LYS ASN LYS ALA VAL THR SER ASN GLU
2119  AGC GGT GGT TCT TGG ACA AAT CCA TTT TCG ACT TTT ATT ACG GAT GAA TTT GGT AAT GCG ACA AAG AAC AAA GCT GTC ACA TCT AAT GAA

 737  VAL THR ALA SER SER SER LYS ASN SER SER ILE SER ARG ILE PRO PRO LYS MET ALA ASP ALA ILE ALA SER ALA SER GLY TYR SER ALA
2209  GTG ACC GCT TCG TCG TCC AAA AAT TCC TCA ATA TCA AGG ATT CCA CCA AAG ATG GCA GAT GCT ATT GCC TCT GCG TCA GGA TAT AGC GCT

 767  ASN SER GLU THR ASN SER GLN ILE ASP LEU LYS ALA SER SER ALA ALA SER GLY SER VAL PHE THR PRO PHE ASN ARG PRO SER HIS ASN
2299  AAT TCA GAA ACA AAT TCC CAA ATT GAT TTA AAA GCA AGC AGT GCC GCG TCT GGT TCA GTT TTT ACA CCT TTC AAC CGT CCT TCT CAT AAC

 797  ARG THR PHE SER ARG ALA ARG VAL SER LYS ARG LYS LYS LYS TYR PRO LEU THR VAL ASP THR LEU ASN THR MET LYS LYS LYS SER SER
2389  AGA ACC TTT TCA AGA GCA AGA GTT TCA AAA AGG AAG AAA AAA TAT CCA TTA ACT GTA GAC ACT TTG AAT ACA ATG AAG AAG AAA TCC TCG

 827  GLN ILE PHE GLU LYS LEU ASN ASN ALA THR GLY GLU HIS LEU LYS ILE ILE SER LYS PRO LYS SER ARG ILE ARG ASN LEU GLU ILE ASN
2479  CAA ATT TTT GAA AAA TTA AAT AAT GCT ACA GGT GAA CAC TTA AAA ATT ATA AGT AAA CCC AAA AGC AGA ATT AGG AAT TTG GAA ATA AAT

 857  SER SER THR TYR GLU GLN ILE ASN GLN ASN VAL LEU LEU LEU GLU ILE LEU GLU ASN LEU ASP LEU SER ILE PHE ILE ASN LEU LYS ASN
2569  TCA AGC ACA TAC GAA CAA ATA AAT CAG AAT GTT TTA CTA TTG GAG ATA CTG GAG AAT TTA GAT CTG TCA ATT TTC ATC AAT TTG AAA AAC

 887  LEU ILE LYS THR PRO SER ILE LEU LEU ASP LEU GLU SER GLU GLU PHE LEU VAL HIS ALA MET SER SER VAL SER SER VAL LEU THR GLU
2659  CTG ATT AAG ACA CCC AGT ATT TTG TTG GAT TTG GAA AGC GAG GAA TTT TTA GTT CAC GCC ATG TCT TCG GTC TCC TCA GTA CTA ACA GAG

 917  PHE PHE ASP ILE LYS GLN ALA PHE HIS ASP ILE VAL ILE ARG LEU ILE MET THR THR GLN GLN THR THR LEU ASP ASP PRO TYR LEU PHE
2749  TTT TTT GAT ATA AAG CAG GCT TTT CAT GAC ATC GTC ATC AGA TTA ATA ATG ACA ACG CAA CAA ACG ACC TTA GAC GAC CCG TAT TTG TTT

 947  SER SER MET ARG SER ASN PHE PRO VAL GLY HIS HIS GLU PRO PHE LYS ASN ILE SER ASN THR PRO LEU VAL LYS GLY PRO PHE HIS LYS
2839  TCC TCA ATG AGG TCC AAT TTC CCT GTC GGA CAT CAT GAA CCT TTC AAG AAT ATC TCT AAT ACA CCT TTG GTC AAG GGC CCC TTC CAT AAA

 977  LYS ASN GLU GLN LEU ALA LEU SER LEU PHE HIS VAL LEU VAL SER GLN ASP VAL GLU PHE ASN ASN LEU GLU PHE LEU ASN ASN SER ASP
2929  AAA AAT GAA CAA TTG GCA CTC TCC TTA TTT CAC GTA TTG GTG AGT CAA GAT GTG GAG TTC AAT AAC CTT GAA TTT TTA AAC AAC TCC GAC
```

```
1007  ASP PHE LYS ASP ALA CYS GLU LYS TYR VAL GLU ILE SER ASN LEU ALA CYS ILE ILE VAL ASP GLN LEU ILE GLU GLU ARG GLU ASN LEU
3019  GAT TTT AAA GAT GCT TGT GAA AAG TAT GTC GAG ATT TCT AAT CTT GCG TGT ATT ATT GTT GAT CAA TTG ATT GAA GAA AGA GAA AAT TTG

1037  LEU ASN TYR ALA ALA ARG MET MET LYS ASN ASN LEU THR ALA GLU LEU LEU LYS GLY GLU GLN GLU LYS TRP PHE ASP ILE TYR SER GLU
3109  CTG AAC TAC GCA GCA AGA ATG ATG AAG AAT AAT TTG ACT GCA GAA CTA TTG AAA GGT GAG CAA GAA AAA TGG TTT GAT ATT TAT TCC GAG

1067  ASP TYR SER ASP ASP ASP SER GLU ASN ASP GLU ALA ILE ILE ASP ASP GLU LEU GLY SER GLU ASP TYR ILE GLU ARG LYS ALA ALA ASN
3199  GAC TAT AGT GAT GAC GAT TCA GAA AAT GAT GAA GCT ATC ATC GAT GAC GAA TTA GGA TCT GAG GAC TAT ATT GAA CGC AAA GCT GCG AAC

1097  ILE GLU LYS ASN LEU PRO TRP PHE LEU THR SER ASP TYR GLU THR SER LEU VAL TYR ASP SER ARG GLY LYS ILE ARG GLY GLY THR LYS
3289  ATA GAG AAA AAC CTT CCA TGG TTT TTA ACT TCA GAT TAT GAA ACT AGT CTT GTC TAT GAC TCA AGA GGA AAA ATT CGT GGC GGG ACA AAA

1127  GLU ALA LEU ILE GLU HIS LEU THR SER HIS GLU LEU VAL ASP ALA ALA PHE ASN VAL THR MET LEU ILE THR PHE ARG SER ILE LEU THR
3379  GAG GCA CTG ATT GAA CAT TTA ACC AGT CAT GAA CTT GTT GAT GCG GCT TTC AAT GTT ACA ATG TTA ATA ACT TTC AGA AGT ATA TTA ACC

1157  THR ARG GLU PHE PHE TYR ALA LEU ILE TYR ARG TYR ASN LEU TYR PRO PRO GLU GLY LEU SER TYR ASP ASP TYR ASN ILE TRP ILE GLU
3469  ACA AGA GAG TTT TTT TAT GCC CTG ATT TAC AGG TAC AAC TTG TAT CCT CCT GAA GGG CTG AGT TAC GAT GAT TAC AAT ATT TGG ATA GAA

1187  LYS LYS SER ASN PRO ILE LYS CYS ARG VAL VAL ASN ILE MET ARG THR PHE LEU THR GLN TYR TRP THR ARG ASN TYR TYR GLU PRO GLY
3559  AAA AAG TCA AAC CCG ATT AAA TGC CGT GTG GTC AAC ATT ATG AGA ACA TTT TTG ACG CAG TAT TGG ACA AGA AAT TAT TAT GAA CCT GGC

1217  ILE PRO LEU ILE LEU ASN PHE ALA LYS MET VAL VAL SER GLU LYS ILE PRO GLY ALA GLU ASP LEU LEU GLN LYS ILE ASN GLU LYS LEU
3649  ATA CCA CTG ATT CTA AAT TTT GCC AAG ATG GTT GTA TCG GAG AAA ATA CCG GGG GCA GAG GAT CTT TTG CAA AAG ATA AAT GAA AAA CTG

1247  ILE ASN GLU ASN GLU LYS GLU PRO VAL ASP PRO LYS GLN GLN ASP SER VAL SER ALA VAL VAL GLN THR THR LYS ARG ASP ASN LYS SER
3739  ATA AAT GAG AAT GAG AAA GAA CCA GTG GAT CCT AAG CAA CAA GAT TCG GTA TCG GCA GTC GTA CAG ACA ACT AAA CGT GAC AAT AAA TCA

1277  PRO ILE HIS MET SER SER SER SER LEU PRO SER SER ALA SER SER ALA PHE PHE ARG LEU LYS LYS LEU LYS LEU LEU ASP ILE ASP PRO
3829  CCG ATA CAC ATG TCT TCG TCT TCT TTA CCA TCT TCT GCT TCT TCA GCG TTT TTT AGA TTG AAG AAA TTG AAG CTC TTG GAT ATT GAC CCA

1307  TYR THR TYR ALA THR GLN LEU THR VAL LEU GLU HIS ASP LEU TYR LEU ARG ILE THR MET PHE GLU CYS LEU ASP ARG ALA TRP GLY THR
3919  TAC ACA TAT GCC ACA CAA TTG ACT GTA CTT GAA CAT GAC TTA TAC CTC AGG ATC ACT ATG TTT GAA TGC TTG GAT AGG GCA TGG GGT ACC

1337  LYS TYR CYS ASN MET GLY GLY SER PRO ASN ILE THR LYS PHE ILE ALA ASN ALA ASN THR LEU THR ASN PHE VAL SER HIS THR ILE VAL
4009  AAG TAT TGT AAT ATG GGT GGT TCT CCG AAC ATT ACG AAA TTT ATA GCT AAT GCT AAT ACG CTA ACT AAT TTT GTT TCT CAT ACC ATT GTA

1367  LYS GLN ALA ASP VAL LYS THR ARG SER LYS LEU THR GLN TYR PHE VAL THR VAL ALA GLN HIS CYS LYS GLU LEU ASN ASN PHE SER SER
4099  AAA CAG GCA GAT GTC AAG ACA CGT TCA AAA TTA ACG CAA TAT TTT GTT ACC GTT GCC CAG CAT TGT AAA GAG TTG AAT AAT TTT TCT TCA

1397  MET THR ALA ILE VAL SER ALA LEU TYR SER SER PRO ILE TYR ARG LEU LYS LYS THR TRP ASP LEU VAL SER THR GLU SER LYS ASP LEU
4189  ATG ACT GCC ATA GTG TCC GCT TTG TAT TCC TCC CCA ATC TAC CGA CTG AAA AAG ACA TGG GAT TTA GTT TCC ACT GAG TCG AAG GAC CTT

1427  LEU LYS ASN LEU ASN ASN LEU MET ASP SER LYS ARG ASN PHE VAL LYS TYR ARG GLU LEU LEU ARG SER VAL THR ASP VAL ALA CYS VAL
4279  CTG AAG AAC CTA AAC AAC CTT ATG GAT TCC AAG AGA AAT TTT GTG AAG TAT AGA GAG CTG TTG CGA TCC GTG ACG GAC GTT GCA TGT GTT

1457  PRO PHE PHE GLY VAL TYR LEU SER ASP LEU THR PHE THR PHE VAL GLY ASN PRO ASP PHE LEU HIS ASN SER THR ASN ILE ILE ASN PHE
4369  CCC TTT TTT GGT GTA TAC CTA TCT GAT TTA ACA TTT ACG TTT GTC GGA AAC CCA GAT TTT CTT CAC AAT TCA ACC AAC ATA ATA AAC TTC

1487  SER LYS ARG THR LYS ILE ALA ASN ILE VAL GLU GLU ILE ILE SER PHE LYS ARG PHE HIS TYR LYS LEU LYS ARG LEU ASP ASP ILE GLN
4459  AGC AAG AGG ACT AAA ATC GCA AAT ATA GTG GAG GAA ATT ATA AGC TTT AAA AGA TTC CAT TAC AAG CTG AAA CGA TTG GAT GAT ATT CAG

1517  THR VAL ILE GLU ALA SER LEU GLU ASN VAL PRO HIS ILE GLU LYS GLN TYR GLN LEU SER LEU GLN VAL GLU PRO ARG SER GLY ASN THR
4549  ACC GTT ATA GAA GCG TCT TTG GAA AAT GTC CCC CAC ATT GAA AAG CAG TAT CAA TTA TCA CTG CAA GTG GAA CCG AGA TCA GGA AAC ACC

1547  LYS GLY SER THR HIS ALA SER SER ALA SER GLY THR LYS THR ALA LYS PHE LEU SER GLU PHE THR ASP ASP LYS ASN GLY ASN PHE LEU
4639  AAA GGC AGT ACG CAT GCT TCT TCT GCT AGC GGT ACA AAA ACT GCA AAA TTC CTA AGT GAA TTT ACA GAT GAT AAA AAT GGC AAT TTT TTG

1577  LYS LEU GLY LYS LYS LYS PRO PRO SER ARG LEU PHE ARG •••
4729  AAG CTA GGT AAG AAA AAA CCT CCT TCT AGG TTA TTT CGA TAAAAGTTTATACAATTTGCTAATCAAGAAGAACCTTAGCTTTATGTTTGATTGCTACACTCTATTA

4836  TTTAAGATGGCTGCTTTTACTTAATATTCTTCGTGATAATACTGTACTGGTGGAGTGTTTTTCGTTTTCGAGGATTTGAGAGTACGCTTCATTTGCAGTTCTTCTTGATAAAGTTCGTT
4955  TTATATATATATATCTATTTTATATCTTTATATATTTTATTACACCCAGTTAAGTTATCGATCCAAGATTTTAAATGCCCGATTAGAGGAAACTTATTACCTGAAAAAATATCAATTAG
5074  TGATTCTATGAAAA
```

Figure 2. Nucleotide Sequence and Deduced Amino Acid Sequence of the *CDC25* Gene

The nucleotide sequence and the deduced amino acid sequence of the one long open reading frame of *CDC25* are presented. In-frame stop codons, upstream and downstream from the putative first methionine, are indicated by the asterisks. Coordinates in the left margin indicate nucleotide and amino acid positions and are used throughout this paper. The boxed region around nucleotide position 2635 represents the BglII site used for the construction of p*CDC25(LEU2)*-3 and p*CDC25(TRP1)*-1. The oval indicates the position of the first methionine following the BglII site and thus is the putative first methionine of the *CDC25* protein expressed by p*CDC25(LEU2)*-3 and p*CDC25(TRP1)*-1. The boxed region around nucleotide position 4652 indicates the SphI site used for the construction of p*CDC25(LEU2)*-3.

readily transformed with the 3.2 kb SalI fragment of p*CDC25::URA3* (see Experimental Procedures) with a resulting disruption of the *CDC25* locus (Southern blotting data not shown). These transformants display the *RAS2*^{val19} phenotypes of heat-shock and starvation sensitivity (see Figures 4 and 5). There is a minor attenuation of the *RAS2*^{val19} phenotype in *cdc25*$^{-}$ strains (see Figure 4B). These results prove that the effects of *RAS2*^{val19} do not require *CDC25*, although the *RAS2*^{val19} gene product may show a minor dependence on the *CDC25* product. It was therefore of interest to examine the biochemical properties of adenylate cyclase in strains lacking *CDC25* but containing *RAS2*^{val19}.

We have previously shown that membranes prepared for yeast strains carrying the activated *RAS2*^{val19} mutation have elevated levels of Mg^{2+}-dependent adenylate cyclase activity relative to wild-type strains and that the Mg^{2+}-dependent activity of *RAS2*^{val19} strains cannot be stimulated by addition of Gpp(NH)p (Toda et al., 1985). This is illustrated in Table 4, Experiment IV, for the *RAS2*^{val19} strain, TK161-R2V. In the strain TT1A-5, which contains *RAS2*^{val19} but lacks a functional *CDC25* gene, the profile of adenylate cyclase activity is slightly changed. Basal levels are high, although they are modestly stimulated by Gpp(NH)p. This result suggests that while adenylate cyclase is under the control of the *CDC25* product in wild-type *RAS2* strains, it largely escapes this control in *RAS2*^{val19} strains. This is consistent with the notion that the *RAS2*^{val19} product does not have a strong dependency on *CDC25* while the *RAS2* product does. We do note that we repeatedly see a 2-fold stimulation of adenylate cyclase by GTP in the *RAS2*^{val19} *cdc25*$^{-}$ strain, although we

Table 2. Suppressors of *cdc25*$^-$ Growth Defects

LEU2[a] Transforming Plasmid	Protein Encoded	Experiment Number	Number of Trp$^+$ Colonies / Total Number of Colonies Trp$^+$/YPD[b]	Suppressor Activity[c]
YEp213	None	1	127/127	None
		2	155/155	None
p*CDC25(LEU2)*-2	*CDC25*	1	30/399	Strong
		2	5/313	Strong
p*RAS1*-2	*RAS1*	1	124/124	None
p*RAS2*-1	*RAS2*	1	81/81	None
		2	42/42	None
p*RAS2*^{val19}	*RAS2*^{val19}	1	1/166	Strong
		2	0/190	Strong
p*TPK1*	cAMP-dependent protein kinase catalytic subunit	1	4/376	Strong
		2	10/299	Strong
p*CYR1*-11	Adenylate cyclase	1	205/232	Weak
		2	239/257	Weak

[a] Strain TT1A-1, which contains a disrupted chromosomal *CDC25* but carries the *TRP1* plasmid, p*CDC25(TRP1)*-1, was transformed with *LEU2* plasmids expressing the indicated gene product. After growing the Leu$^+$ Trp$^+$ transformants without selection, cells were plated onto YPD medium, which served as a master plate. The presence of the Trp$^+$ marker in the colonies grown up on YPD was determined by replica-plating onto synthetic medium lacking tryptophan as described in Experimental Procedures.

[b] The stabilities of the Trp$^+$ marker expressed as the ratio of the number of Trp$^+$ colonies to the number of colonies on the YPD master plate are indicated.

[c] The ability of the indicated plasmid to destabilize p*CDC25(TRP-1)*-1, and hence suppress loss of *CDC25*, is indicated.

Table 3. Intracellular cAMP Levels of *CDC25* Mutants

Strain	Genotype[a]	Cyclic AMP Level[b] (pmol cAMP per mg Protein) Exp. 1	Exp. 2	Exp. 3
TT1A-4	*cdc25*$^-$ p*CDC25(LEU2)*-2	3.2	4.0	7.9
TT1A-3	*cdc25*$^-$ p*TPK1*	0.5	1.5	2.6
SP12	*CDC25* p*TPK1*	3.7	4.3	ND
SP1	*CDC25*	3.9	4.8	8.2

[a] Full descritions of the genotypes of the indicated strains are given in Table 1.

[b] Intracellular cAMP levels of the indicated strains were determined as described in Experimental Procedures. For experiment 1 and 2, yeast strain SP1 was grown in synthetic media containing leucine and the other strains were grown in synthetic medium lacking leucine. For experiment 3, the yeast strains were grown in (rich) YPD medium.

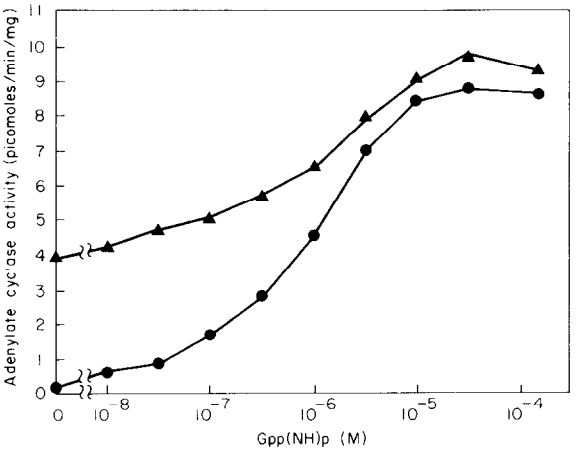

Figure 3. Gpp(NH)p Dependence of Adenylate Cyclase Activity in a *cdc25*$^-$ Strain

Adenylate cyclase activities of the wild-type strain, SP1 (triangles), and the strain lacking *CDC25*, TT1A-3 (circles), are plotted as a function of the Gpp(NH)p concentration. $MgCl_2$ (2.5 mM) was used as the divalent cation. Adenylate cyclase activity was determined as described in Experimental Procedures except that ATP, [α-^{32}P]ATP, creatine phosphokinase, and creatine phosphate were added after a 10 min preincubation at room temperature. See Table 1 for a description of these strains.

do not see this stimulation in *RAS2*^{val19} *CDC25* strains. Thus the *RAS2*^{val19} product may have a minor dependency on the *CDC25* product. This would explain the weak attenuation of the *RAS2*^{val19} phenotype seen in *cdc25*$^-$ strains (see Figure 4).

Induction of a Dominant Phenotype by a Mutationally Activated *CDC25* Gene

Activating mutations in genes encoding regulatory components of the *RAS*/adenylate cyclase pathway give rise to a cluster of phenotypes. *RAS2*^{val19} (Kataoka et al., 1984), *CYR1::HIS3*, a gene that overexpresses the catalytic domain of adenylate cyclase (Kataoka et al., 1985b), and *bcy1*, a gene that encodes a nonfunctional regulatory subunit of the cAMP-dependent protein kinase (Uno et al., 1982; Sass et al., 1986; Toda et al., 1987), all activate the *RAS*/adenylate cyclase pathway. Yeast carrying these ac-

Table 4. Adenylate Cyclase Activity in Yeast Membranes with Disrupted *CDC25*

Strain	Genotype[a]	Adenylate Cyclase Activity[b] (pmol cAMP per min per mg)		
		Mn^{2+}	Mg^{2+}	Mg^{2+} plus Gpp(NH)p
Experiment I				
SP1	Wild type	49	2.4	10.0
TT1A-4	*cdc25*⁻p*CDC25(LEU2)-2*	56	4.1	10.4
TT1A-3	*cdc25*⁻p*TPK1*	29	0.1	5.6
Experiment II				
SP1	Wild type	51	3.1	11.3
TT1A-4	*cdc25*⁻p*CDC25(LEU2)-2*	40	3.3	9.6
TT1A-3	*cdc25*⁻p*TPK1*	37	0.3	9.5
Experiment III				
SP1	Wild type	58	4.0	10.5
TT1A-4	*cdc25*⁻p*CDC25(LEU2)-2*	42	4.4	9.7
TT1A-3	*cdc25*⁻p*TPK1*	39	0.2	9.4
Experiment IV				
SP1	Wild type	68	2.1	8.0
TK161-R2V	$RAS2^{val19}$	79	11.7	12.6
TT1A-5	*cdc25*⁻p$RAS2^{val19}$	71	4.0	8.4

[a] Membranes were prepared from the indicated strains as described in Experimental Procedures. Membranes were assayed in the presence of either 2.5 mM $MnCl_2$, 2.5 mM $MgCl_2$, or 2.5 mM $MgCl_2$ and 10 μM Gpp(NH)p. Experiments I, II, III, and IV represent four independent experiments carried out using different membrane preparations. Adenylate cyclase activity is expressed in units, where 1 unit is the generation of 1 pmol of cAMP per min per mg of membrane protein. Data shown are the average of duplicate data points, which did not differ from each other by more than 10%.
[b] The full genotypes of the indicated strains are given in Table 1.

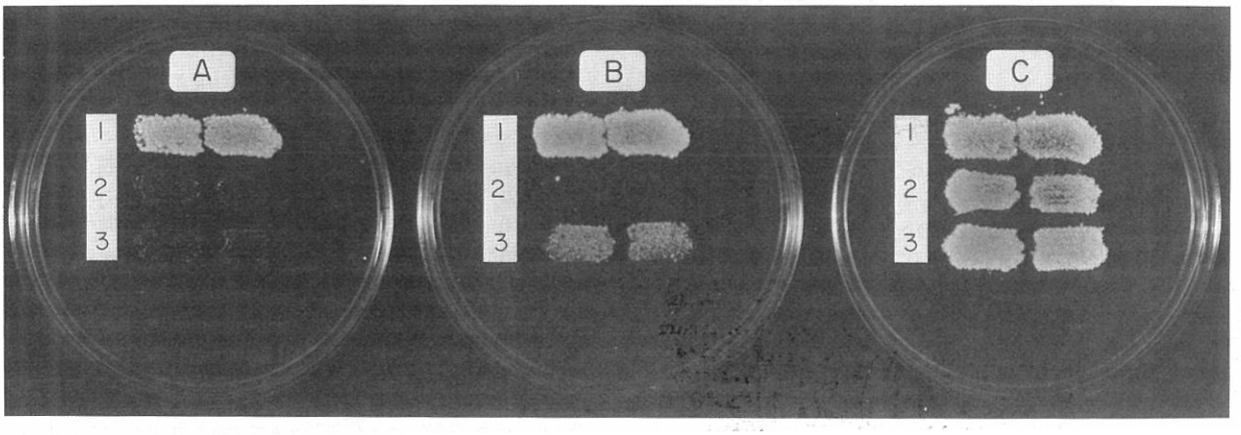

Figure 4. Heat-Shock Sensitivity of Strains Lacking *CDC25* and Containing $RAS2^{val19}$

Yeast strains were patched in duplicate onto YPD plates, incubated at 30°C for 3 days, replica-plated onto YPD plates, and heat-shocked (at 55°C) for (A) 30 min, (B) 15 min, or (C) 0 min. After heat-shock treatment the plates were incubated at 30°C for 1 day. The strains used for this experiment are (1) the wild-type strain, SP1, (2) the $RAS2^{val19}$-containing strain, TK161-R2V, and (3) the $RAS2^{val19}$-containing strain lacking *CDC25*, TMRV-25. See Table 1 for a description of these strains.

tivating mutations are heat-shock-sensitive and lose viability rapidly upon starvation. We tested whether similar activating mutations could be found in the *CDC25* gene. For this purpose, we passaged the plasmid p*CDC25-(LEU2)-2* in a mutator strain of E. coli, LE30 (Silhavy et al., 1984). Pools of mutagenized plasmids were then transformed into a wild-type yeast strain, and the transformants were screened for heat-shock sensitivity. Several Leu^+ transformants were found to be heat-shock-sensitive. When isolated, plasmids from these strains could reproducibly induce heat-shock sensitivity when introduced into wild-type strains (Figure 6). Thus, activating mutants of *CDC25* can cause at least some of the phenotypic abnormalities seen in activating mutants of other genes of the *RAS*/adenylate cyclase pathway. This result suggests that *CDC25* encodes a regulatory component of the pathway.

Discussion

We have cloned and sequenced *CDC25*, the wild-type allele of a temperature-sensitive mutant causing a G1 cell cycle arrest at the nonpermissive temperature. The sequence of S. cerevisiae *CDC25* gene has also recently been reported by others (Camonis et al., 1986). Our se-

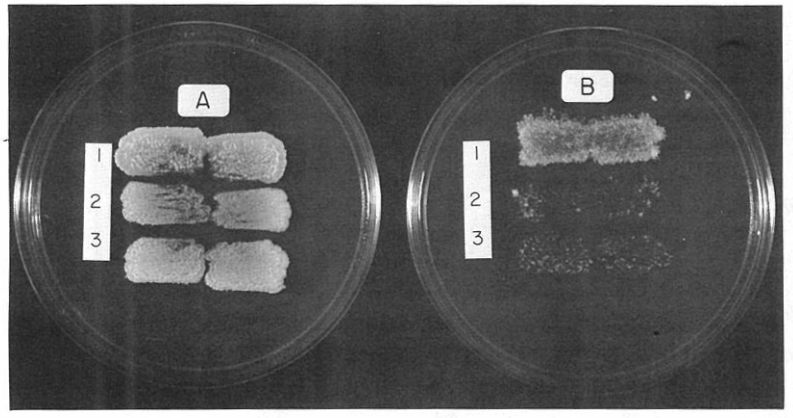

Figure 5. Starvation Sensitivity of Strains Lacking *CDC25* and Containing *RAS2*^{val19}

Yeast strains were patched in duplicate onto a YPD (rich medium) plate. After 2 days at 30°C, the patches were replica-plated onto a YNB-N plate that lacked a source of nitrogen and onto a YPD plate (A) (0 time of nitrogen starvation) that was incubated at 30°C for 2 days. After 9 days at 30°C, the YNB-N plate was replica-plated onto a YPD plate (B), which was incubated at 30°C for 2 days. The strains used for this experiment are (1) the wild-type strain, SP1, (2) the *RAS2*^{val19} strain, TK161-R2V, and (3) the *RAS2*^{val19} strain lacking *CDC25*, TMRV-25. See Table 1 for a description of these strains.

quence agrees substantially with that sequence, but differs in that it is 3 bp longer within the *CDC25* coding region. The cytosine at position 2861 and cytosine–thymine at positions 2894–2895 of our sequence are not found in the sequence reported previously. Consequently, we predict an amino acid sequence at positions 952 to 966, NFPVGHHEPFKNISN, whereas Camonis and co workers (Camonis et al., 1986) predict NFLSVIMNLSRISN at position 952–965. These differences lie outside the minimum subgenic fragment required for *CDC25* function (see Figure 1 and Camonis et al., 1986) and may thus reflect genetic variation between our strains. Computer analysis of the predicted *CDC25* amino acid sequence (Figure 2) revealed that the *CDC25* protein is not closely related to any of the proteins contained in two protein sequence data banks (see Experimental Procedures). The *CDC25* sequence does not contain the consensus sequence for a protein kinase, nor is it homologous to adenylate cyclase, *RAS* proteins or other known proteins; in addition, there are no consensus nucleotide binding sequences (Robinshaw et al., 1986; Knopf et al., 1986). We were unable to find any hydrophobic domain of sufficient length to span the plasma membrane.

Others have reported (Camonis et al., 1986) that *cdc25*^{ts} strains have decreased levels of cAMP at the nonpermissive temperature, suggesting that the normal function of *CDC25* might be to directly or indirectly influence cAMP levels. Our own data confirm this (see Table 3). Moreover, disruptions of *CDC25* are lethal, but lethality is suppressed in cells carrying extra copies of the genes encoding adenylate cyclase or cAMP-dependent protein kinase catalytic subunits. Thus, if *CDC25* has other essential functions besides regulating cAMP levels, these functions are redundant with functions of the cAMP effector pathway. Most importantly, we have shown that the adenylate cyclase activity in membranes prepared from strains lacking *CDC25* is abnormally low when assayed in the presence of magnesium (see Table 4 and Figure 3). Thus the function of the *CDC25* gene product is required for the normal function of adenylate cyclase.

CDC25 appears to have a regulatory function. Not all mutations capable of causing G1 arrest are mutations in regulatory genes. For example *cdc19* encodes a temperature-sensitive pyruvate kinase (Frankel, 1982). Temperature-sensitive mutations in *RAM* cause G1 arrest, but *RAM* encodes a protein needed for the maturation of *RAS* and other proteins (Powers et al., 1986). If *CDC25* does encode a regulatory component of the adenylate cyclase pathway, we reasoned that it should be possible to isolate mutations in *CDC25* that give the characteristic phenotypes of mutations which activate adenylate cyclase. Mutations such as in *RAS2*^{val19} and *CYR1::HIS3* (Toda et al., 1985; Kataoka et al., 1985b) lead to defects in resistance to starvation and heat shock (Sass et al., 1986). Indeed we have found dominantly acting mutant alleles of *CDC25* that cause sensitivity to heat shock (see Figure 6). This result suggests that *CDC25* encodes a regulatory component of the signal transduction pathway controlling adenylate cyclase.

The relationship between *CDC25* and *RAS* genes is not completely resolved by our experiments. Both *CDC25* and *RAS* appear to regulate adenylate cyclase catalytic activity. Three possibilities can be envisioned: first, *RAS* acts through *CDC25*; second, *CDC25* acts through *RAS*; and third, both *RAS* and *CDC25* act coordinately to control adenylate cyclase. We can clearly dismiss the first possibility, since the activated *RAS2*^{val19} gene suppresses the lethality resulting from loss of *CDC25*. The distinctive phenotype of *RAS2*^{val19} penetrates even in the absence of *CDC25* function. Moreover, biochemical analysis shows that the adenylate cyclase in *RAS2*^{val19} *cdc25*$^{-}$ strains behaves much like the adenylate cyclase of *RAS2 CDC25* strains. Thus, *CDC25* cannot mediate the effector function of *RAS2*.

The two remaining possibilities are hard to resolve without additional biochemical data, although we lean strongly toward the hypothesis that *CDC25* controls *RAS* function. It seems simplest to us that, in the absence of *CDC25* function, *RAS* proteins are in a "ground," or inactive, state, perhaps bound to GDP or without bound guanine nucleotides. Thus the addition of a nonhydrolyzable GTP analogue to broken membrane preparations fully restores adenylate cyclase activity (see Table 3 and Figure 3). In this model, *RAS2*^{val19} protein is less dependent on

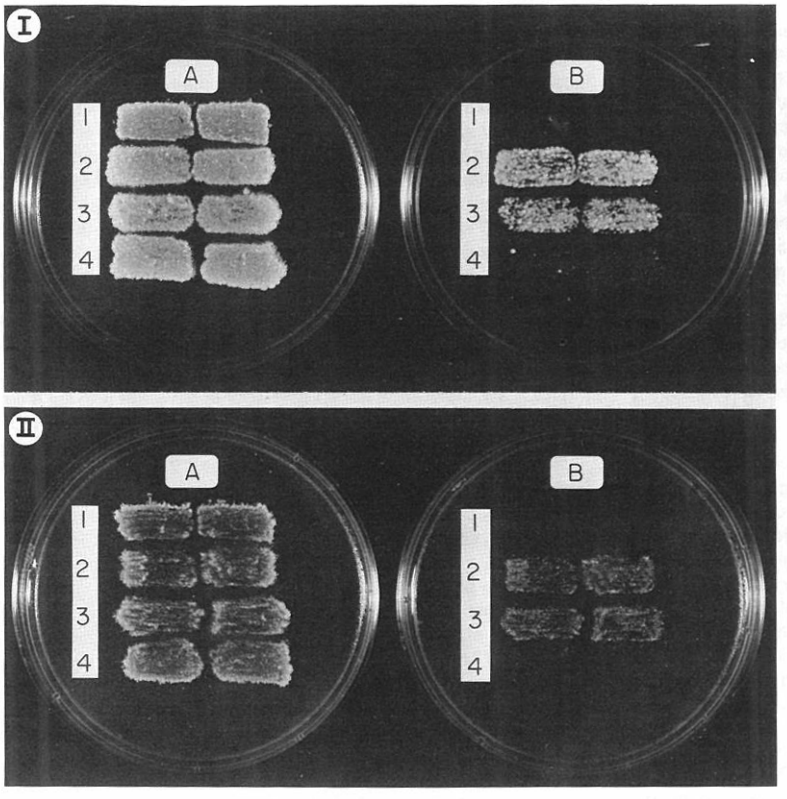

Figure 6. Heat-Shock-Sensitive Phenotype Induced by Mutationally Activated *CDC25* Alleles

Yeast strains were patched in duplicate onto (I) YPD or (II) SC-leu plates, incubated at 30°C for 4 days, replica-plated onto (I) YPD or (II) SC-leu plates, and heat-shocked (at 55°C) for (A) 0 min, or (B) 30 min. Following heat-shock treatment, the plates were incubated at 30°C for 1 day. The strains used in (I) are (1) a heat-shock-sensitive *RAS2*^{val19} strain, TK161-R2V, (2) a wild-type strain, SP1, (3) strain TT1A-4, containing a disrupted chromosomal *CDC25* and carrying the p*CDC25(LEU2)*-2 plasmid, and (4) strain TMHS-20, containing a disrupted chromosomal *CDC25* and carrying the mutationally activated p*CDC25*^{HS}*(LEU2)*-20. The strains used in (II) are (1) a heat-shock-sensitive *RAS2*^{val19} strain, TK161-R2V, carrying an extrachromosomal *LEU2* plasmid (YEp13), (2) a wild-type strain SP1 carrying a *LEU2* plasmid (YEp13), (3) strain SP1 carrying the unmutated p*CDC25(LEU2)*-2 plasmid, and (4) strain SP1 carrying the mutationally activated p*CDC25*^{HS}-*(LEU2)*-20 plasmid. See Table 1 for a description of these strains.

CDC25 function because, due to its decreased GTPase activity, it remains longer in its GTP bound or "activated" state. It is possible that the *CDC25* protein may function analogously to receptor proteins of animal cells that regulate G proteins by promoting GDP/GTP exchange (Gilman, 1984).

Recently, we have obtained evidence for a direct interaction between *CDC25* and *RAS* gene products. One of us (S. P.) has isolated a mutant *RAS2* gene that has a temperature-sensitive (ts) lethal phenotype even in the presence of wild-type *RAS* genes. Like *cdc25*$^{-}$ strains, this phenotype can be suppressed by high copy plasmids expressing *RAS2*^{val19}, but not by high copy plasmids expressing *RAS2*. The ts phenotype can also be suppressed by high copy plasmids expressing *CDC25*, but only in the presence of a wild-type *RAS1* or *RAS2* gene. The likely explanation for these observations is that the mutant *RAS2* protein forms an ineffective and irreversible complex with *CDC25* protein, consuming the supply available from a single copy of the gene. This defect can be overcome by supplying additional *CDC25* product, but only in the presence of a wild-type *RAS* product.

Experimental Procedures

Strains, Media, Yeast Genetics, and Yeast Genetic Nomenclature

S. cerevisiae strains used are listed in Table 1. E. coli HB101 was used for plasmid propagation and isolation. Media used were as follows: 1% Bacto-yeast extract, 2% Bacto peptone, and 2% Detrose (YPD); 0.67% yeast nitrogen base without amino acids (Difco) and 2% Dextrose and all auxotrophic requirements (80 μg/ml) (SC) (Sherman et al., 1979); and 0.17% yeast nitrogen base without amino acids and without ammonium sulfate (Difco) and 2% dextrose (YNB-N). Plates contained 2% agar. Standard yeast genetic methods were followed as described previously (Sherman et al., 1979). Standard yeast genetics nomenclature is used throughout. Wild-type and dominant mutations are denoted by capital italicized letters, recessive mutations by lowercase italicized letters, and gene disruptions by lowercase letters representing the disrupted gene followed by two colons and the wild-type gene marker. For example *ras1::HIS3* indicates the *RAS1* gene disrupted by the *HIS3* gene marker. In the text of this paper, gene disruptions are abbreviated by lowercase italicized letters representing the gene followed by a superscript minus sign, such as *ras1*$^{-}$.

DNA

Plasmid DNA was isolated from E. coli by the alkali lysis method (Maniatis et al., 1982). Yeast DNA was prepared essentially as described (Nasmyth and Tatchell, 1980). Digests with restriction endonucleases and ligations with T4 DNA ligase were performed under conditions recommended by suppliers (New England Biolabs or Bethesda Research Labs).

Plasmids

Several of the plasmids used for these studies have been described elsewhere: p*RAS1*-2 and p*RAS2*-1, by Powers et al. (1984); p*CYR1*-11, by Kataoka et al. (1985b). p*RAS2*^{val19} was constructed by inserting a 2.3 kb HindIII–EcoRV DNA fragment containing the entire *RAS2*^{val19} coding region into the *LEU2*-containing YEp213 (Sherman et al., 1982) vector that had been cut by HindIII and PvuII. P*TPK1* is a YEp13 vector (Sherman et al., 1982) containing the *TPK1* gene (T. Toda, unpublished data). Other plasmids used for this study are described in Figure 1. The vector used for the construction of p*CDC25(TRP)*-1 is TTp7. TTp7 was constructed by inserting the 1.4 kb EcoRI *TRP1–ARS1* fragment into the EcoRI site of PUC8 (Messing and Vieira, 1982). p*CDC25(LEU2)*-3 was constructed by deleting the 3.1 kb BamHI–BglII fragment from p*CDC25(LEU2)*-2 (see Figure 1). p*cdc25::URA3* was constructed by deleting the 3.9 kb ClaI–HindIII fragment from the *CDC25* coding region of p*CDC25*(LEU2)-2, treating the resulting vector with Klenow, and inserting a 1.1 kb, blunt-ended HindIII–HindIII fragment containing the complete *URA3* gene.

Cloning of *CDC25*

The *CDC25* gene was isolated from two different yeast genomic libraries. Yeast strain TT25-6 (see Table 1 for its genotype) was transformed with a yeast genomic YCp50 (Rose and Fink, unpublished data) library by the lithium acetate method (Ito et al., 1983). Transformants were incubated at 35°C directly on synthetic plates containing all auxotrophs except uracil (SC-ura). Two independent transformants were obtained and two different plasmids were isolated from each two transformants. One of these, p*CDC25(URA3)*-1, is shown in Figure 1.

Yeast strain 25-1 (generously supplied by Dr. Johnston) was transformed with the yeast genomic library constructed in the YEp13 (Nasmyth and Tatchell, 1980) library by the spheroplast method (Hinnen et al., 1978). Leu^+ transformants grown at 23°C were pooled, plated on YPD, and incubated at 37°C. Colonies grown at 37°C were replica-plated onto SC-leu plates. Eight transformants that could grow at 37°C were isolated. Seven out of eight transformants contained the same plasmid, p*CDC25(LEU2)*-1, and the other one contained overlapping plasmid, p*CDC25(LEU2)*-2 (see Figure 1).

DNA Sequencing

The complete nucleotide sequence of the coding region of *CDC25* was determined by dideoxy sequencing of both strands of DNA (Sanger et al., 1977). Restriction fragments of about 500 nucleotides were subcloned into the M13 vectors mp18 and mp19 (Yannisch-Perron et al., 1985). Each subclone was sequenced in its entirety using a combination of the universal M13 sequencing primer and synthetic oligonucleotide primers complementary to the M13 subclone. Unidirectional deletions were constructed at the 5′ end of the gene in order to complete the sequencing of the second strand without the aid of additional synthetic primers (Henikoff, 1984).

Protein Sequence Homology Searches

Homologies to the *CDC25* sequence were searched for among the protein sequences listed in GENEBANK and PIR database using the homology search program previously described (Goad and Kanehisa et al., 1982).

Disruption of *CDC25*

A 3.2 kb SalI fragment of p*cdc25::URA3* was transformed into diploid yeast cells TTSD1, and transformants were selected by uracil prototrophy (see, for example, TTS3801, Table 1). Tetrad analyses of TTS3801 showed a 2:2 (viable:nonviable) pattern and the two viable haploids were always uracil-requiring phenotypes.

Integration Mapping

The 2.1 kb Klenow-treated BglII–SphI fragment of *CDC25* was inserted into pUC8 that had been cut by SalI and treated with Klenow. The 1.7 kb BamHI fragment of *HIS3* was inserted into the BamHI site of the resulting plasmid to generate p*CDC25(HIS1)*-1. p*CDC25(HIS1)*-1 was linearized by cutting at the NcoI site located in the *CDC25* coding region and transformed to diploid cells, TR56. Tetrad analyses showed two types of segregation pattern. In type I, all 4 spores in tetrads segregated 4:0 for temperature sensitivity and 2:2 for histidine auxotrophy (in TTS1201, TTS1203, and TTS1204). In type II, both temperature sensitivity and histidine auxotrophy cosegregated 2:2 (in TTS1202). These results suggest that the sequence we had cloned integrated into the *CDC25* locus. In type I, the plasmid sequence integrated at the *cdc25-1*^{ts} locus, and in type II, integration occurred in the wild-type *CDC25* locus.

CDC25 Suppression Experiment

TTS3801 was transformed with p*CDC25(TRP1)*-1 (Figure 1), a tryptophan prototroph was selected, and tetrad analysis was performed. Ura^+ (and therefore *cdc25*$^-$) haploids were always stably Trp^+, showing that cells with disrupted *cdc25* were viable only because they carried p*CDC25(TRP1)*-1. One of the Ura^+ Trp^+ segregants (TT1A-1) was used for suppression experiments. TT1A-1 was transformed with various *LEU2* gene-containing multicopy plasmids and plated onto SC-leu plates. Individual Leu^+ transformants were grown in 2 ml of YPD at room temperature for 2 days. Cells were plated on YPD after appropriate dilution and incubated at 30°C for 2 days. Colonies were then replica-plated onto SC-leu, SC-trp, and YPD. Maintenance or loss of plasmids was checked by leucine and tryptophan auxotrophy.

Heat-Shock Experiments

p*CDC25(LEU2)*-2 was transformed into a mutator strain of E. coli, LE30, and grown for 36 hr in rich medium as described (Fowler et al., 1974). The mutagenized plasmid was rescued from the E. coli and transformed into the wild-type SP1 strain. Transformants were selected on SC-leu, grown at 30°C for 4 days, replica-plated again onto SC-leu, and placed at 55°C for 0, 15, 30, or 45 min. Colonies that exhibited heat-shock sensitivity (compared with SP1 carrying a unmutated p*CDC25-(LEU2)*-2) were retested for heat-shock sensitivity after loss of the mutated p*CDC25(LEU2)*-2. The plasmid p*CDC25*^{HS}(LEU2)-20 was isolated from one of the heat-shock-sensitive transformants.

cAMP Assays

The yeast strains were grown in synthetic media to a cell density of 0.5 × 10^7 cells per ml, or in rich media to a cell density of 1 × 10^7 cell per ml. After pelleting and washing, the cells were extracted with 1 M $HClO_3$ and the extracts were prepared for cAMP assay with the Amersham cAMP determination kit. For experiment 2 in Table 3 the following modification of the published procedures was introduced. The crude $HClO_3$ extracts were applied to a 1 ml DOWEX AG 50W-X4 column, eluted with H_2O, and lyophilized. Samples were resuspended in 100 mM Tris–HCl and neutralized with 1 M NaOH before cAMP determination using the Amersham cAMP assay kit.

Adenylate Cyclase Assays

Yeast membrane fractions were prepared as previously described (Broek et al., 1985). Protein determinations were performed by the method of Lowry (Lowry et al., 1951). Adenylate cyclase activity was assayed as previously described (Casperson et al., 1983; Broek et al., 1985), and [^{32}P]cAMP produced was determined as described (Solomon et al., 1973).

Acknowledgments

We are appreciative to P. Bird for preparation of this manuscript. This work was supported by grants from the National Institutes of Health, the American Business Foundation for Cancer Research, American Cancer Society, and Pfizer Biomedical Research Award. D. B. is a postdoctoral fellow of the Damon Runyon–Walter Winchell Cancer Fund. C. B. is supported by the Swiss National Science Foundation. T. M. is supported by an NIH postdoctoral fellowship. L. L. is a graduate student from the Department of Genetics at the State University of New York at Stony Brook. M. W. is an American Cancer Society Research Professor.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received September 29, 1986; revised December 22, 1986.

References

Bishop, J. M. (1985). Viral Oncogenes. Cell *42*, 23–38.

Birchmeier, C., Broek, D., and Wigler, M. (1985). *RAS* proteins can induce meiosis in Xenopus oocytes. Cell *43*, 615–621.

Broek, D., Samiy, N., Fasano, O., Fujiyama, A., Tamanoi, F., Northup, J., and Wigler, M. (1985). Differential activation of yeast adenylate cyclase by wild-type and mutant *RAS* proteins. Cell *41*, 763–769.

Camonis, J. H., Kalekine, M., Bernard, G., Garreau, H., Boy-Marcotte, E., and Jacquet, M. (1986). Characterization, cloning and sequence analysis of the CDC25 gene which controls the cyclic AMP level of Saccharomyces cerevisiae. EMBO J. *5*, 375–380.

Casperson, G., Walker, N., Brasier, A., and Bourne, H. (1983). A guanine nucleotide-sensitive adenylate cyclase in the yeast Saccharomyces cerevisiae. J. Biol. Chem. *258*, 7911–7914.

Defeo-Jones, D., Scolnick E., Koller, R., and Dhar, R. (1983). *ras*-related gene sequences identified and isolated from Saccharomyces cerevisiae. Nature *306* , 707–709.

Fowler, R. G., Degnon, G. E., and Cox, E. C. (1974). Mutational specificity of a conditional Escherichia coli mutator, mutD5. Mol. Gen. Genet. *133*, 179–185.

Frankel, D. G. (1982). Carbohydrate metabolism. In The Molecular Biology of the Yeast Saccharomyces: Metabolism and Gene Expression (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory), pp. 1–37.

Gibbs, J., Sigal, I., Poe, M., and Scolnick, E. (1984). Intrinsic GTPase activity distinguishes normal and oncogenic *ras* p21 molecules. Proc. Natl. Acad. Sci. USA *81*, 5704–5708.

Gilman, A. G. (1984). G proteins and dual control of adenylate cyclase. Cell *36*, 577–579.

Goad, W. B., and Kanehisa, M. I. (1982). Pattern recognition in nucleic acid sequences. I. A general method for finding local homologies and symmetries. Nucl. Acids Res. *10*, 247–263.

Hartwell, L. H. (1974). Saccharomyces cerevisiae cell cycle. Bacteriol. Rev. *38*, 164–198.

Hartwell, L. H., Mortiner, R. K., Culotti, J., and Culotti, M. (1973). Genetic control of the cell division cycle in yeast: genetic analysis of *cdc* mutants. Genetics *74*, 267–286.

Henikoff, S. (1984). Unidirectional digestion with Exonuclease III creates targeted breakpoints for DNA sequencing. Gene *28*, 351–359.

Hinnen, A., Hicks, J. B., and Fink, G. R. (1978). Transformation in yeast. Proc. Natl. Acad. Sci. USA *75*, 1929–1933.

Ito, H., Fukuda, Y., Murata, K., and Kimura, A. (1983). Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. *153*, 163–168.

Kataoka, T., Powers, S., McGill, C., Fasano, O., Strathern, J., Broach, J., and Wigler, M. (1984). Genetic analysis of yeast *RAS1* and *RAS2* genes. Cell *37*, 437–445.

Kataoka, T., Powers, S., Cameron, S., Fasano, O., Goldfarb, M., Broach, J., and Wigler, M. (1985a). Functional homology of mammalian and yeast *RAS* genes. Cell *40*, 19–26.

Kataoka, T., Broek, D., and Wigler, M. (1985b). DNA sequence and characterization of the S. cerevisiae gene encoding adenylate cyclase. Cell *43*, 493–505.

Knopf, J. L., Lee, M. H., Sultzman, L. A., Kriz, R. W., Loomis, C. R., Hewick, R. M., and Bell, R. M. (1986). Cloning and expression of multiple protein kinase C cDNAs. Cell *46*, 491–502.

Lowry, O., Rosebrough, N., Farr, A., and Randall, R. (1951). Protein measurement with a phenol reagent. J. Biol. Chem. *193*, 265–275.

Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982). Molecular Cloning: A Laboratory Manual (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory).

Matsumoto, K., Uno, I., Oshima, Y., and Ishikawa, T. (1982). Isolation and characterization of yeast mutants deficient in adenylate cyclase and cyclic AMP dependent protein kinase. Proc. Natl. Acad. Sci. USA *79*, 2355–2359.

McGrath, J., Capon, D., Goeddel, D., and Levinson, A. (1984). Comparative biochemical properties of normal and activated human *ras* p21 protein. Nature *310*, 644–655.

Messing, J., and Vieira, J. (1982). A new pair of M13 vectors used for the selection of either DNA strand of double digested restriction fragments. Gene *19*, 269–276.

Mulcay, L., Smith, M., and Stacey, D. (1985). Requirements of *ras* proto-oncogene function during serum stimulated growth of NIH3T3 cells. Nature *313*, 241–243.

Muller, R., Slamon, D., Tremblay, J., Kline, M., and Verma, I. (1982). Differential expression of cellular oncogenes during pre- and postnatal development of the mouse. Nature *299*, 640–644.

Nasmyth, K. A., and Tatchell, K. (1980). The structure of transposable yeast mating type loci. Cell *19*, 753–764.

Neuman-Silberberg, F. S., Schejter, E., Hoffmann, F. M., and Shilo, B.-Z. (1984). The Drosophila *ras* oncogenes: structure and nucleotide sequence. Cell *37*, 1027–1033.

Powers, S., Kataoka, T., Fasano, O., Goldfarb, M., Strathern, J., Broach, J., and Wigler, M. (1984). Genes in S. cerevisiae encoding proteins with domains homologous to the mammalian *ras* proteins. Cell *36*, 607–612.

Powers, S., Michaelis, S., Broek, D., Anna-A., S. S., Field, J., Herskowitz, I., and Wigler, M. (1986). *RAM*, a gene of yeast required for a functional modification of *RAS* proteins and for production of mating pheromone a-factor. Cell *47*, 413–422.

Reed, S. I. (1980). The selection of S. cerevisiae mutants defective in the start event of cell division. Genetics *95*, 561–577.

Robinshaw, J. D., Russell, D. W., Harris, B. A., Smigel, M. D., and Gilman, A. D. (1986). Deduced primary structure of the α subunit of the GTP-binding stimulatory protein of adenylate cyclase. Proc. Natl. Acad. Sci. USA *83*, 1251–1255.

Sanger, F., Nicklen, S., and Coulson, A. (1977). DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA *74*, 5463–5467.

Sass, P., Field, J., Nikawa, J., Toda, T., and Wigler, M. (1986). Cloning and characterization of the high affinity cAMP phosphodiesterase of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA *83*, 9303–9307.

Sherman, F., Fink, G. R., and Lawrence, C. W. (1979). Methods in Yeast Genetics (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory).

Sherman, F., Fink, G. R., and Hicks, J. B. (1982). Methods in Yeast Genetics (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory).

Shilo, B., and Weinberg, R. (1981). DNA sequences homologous to vertebrate oncogenes are conserved in Drosophila melanogaster. Proc. Natl. Acad. Sci. USA *78*, 6789–6792.

Silhavy, T. J., Berman, M. L., and Enquist, L. W. (1984). Experiments with Gene Fusion (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory), pp. 75–77.

Solomon, Y., Landos, C., and Rodbell, M. (1973). A highly sensitive adenylate cyclase assay. Anal. Biochem. *58*, 541–548.

Sweet, R., Yokoyama, S., Kamata, T., Feramisco, J., Rosenberg, M., and Gross, M. (1984). The product of *ras* is a GTPase and the T24 oncogenic mutant is deficient in this activity. Nature *311*, 273–275.

Tamanoi, F., Samiy, N., Rao, M., and Walsh, M. (1985). Enzymatic properties of yeast *RAS2* protein. In Cancer Cells III: Growth Factors and Transformation, J. Feramisco, B. Ozanne, and L. Stiles, eds. (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory), pp. 251–256.

Tatchell, K., Chaleff, D., Defeo-Jones, D., and Scolnick, E. (1984). Requirement of either of a pair of *ras* related genes of Saccharomyces cerevisiae for spore viability. Nature *309*, 523–527.

Toda, T., Uno, I., Ishikawa, T., Powers, S., Kataoka, T., Broek, D., Cameron, S., Broach, J., Matsumoto, K., and Wigler, M. (1985). In yeast, *RAS* proteins are controlling elements of adenylate cyclase. Cell *40*, 27–36.

Toda, T., Cameron, S., Sass, P., Zoller, M., Scott, J. D., McMullen, B., Hurwitz, M., Krebs, E. G., and Wigler, M. (1987). Cloning and characterization of *BCY1*, a locus encoding a regulatory subunit of the cAMP dependent protein kinase in yeast. Mol. Cell. Biol., in press.

Uno, I., Matsumoto, K., and Ishikawa, T. (1982). Characterization of cyclic AMP-requiring yeast mutants altered in the regulatory subunit of protein kinase. J. Biol. Chem. *257*, 14110–14115.

Yannisch-Perron, C., Vieira, J., and Messing, J. (1985). Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene *33*, 109–119.