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SCH9, a gene of *Saccharomyces cerevisiae* that encodes a protein distinct from, but functionally and structurally related to, cAMP-dependent protein kinase catalytic subunits

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A new gene, *SCH9*, was isolated from *Saccharomyces cerevisiae* by its ability to complement a *cdc25*^{ts} mutation. Sequence analysis indicates that it encodes a 90,000-dalton protein with a carboxy-terminal domain homologous to yeast and mammalian cAMP-dependent protein kinase catalytic subunits. In addition to suppressing loss of *CDC25* function, multicopy plasmids containing *SCH9* suppress the growth defects of strains lacking the *RAS* genes, the *CYR1* gene, which encodes adenylyl cyclase, and the *TPK* genes, which encode the cAMP-dependent protein kinase catalytic subunits. Cells lacking *SCH9* grow slowly and have a prolonged G_1 phase of the cell cycle. This defect is suppressed by activation of the cAMP effector pathway. We propose that *SCH9* encodes a protein kinase that is part of a growth control pathway which is at least partially redundant with the cAMP pathway.

[*Key Words*: cAMP; growth control; *Saccharomyces cerevisiae*]

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In the yeast *Saccharomyces cerevisiae* the cAMP effector pathway may play an important role in growth regulation. Two of the previously identified temperature-sensitive cell-division-cycle 'start' mutants, *cdc25*^{ts} and *cdc35*^{ts}, which cause G_1 arrest at the nonpermissive temperature, block production of cAMP (Matsumoto et al. 1984; Camonis et al. 1986; Martegani et al. 1986b). *CDC35* is the same as *CYR1*, the gene encoding adenylyl cyclase (Boutelet et al. 1985). *CDC25* encodes a protein that regulates adenylyl cyclase activity, probably through control of the *RAS* proteins (Broek et al. 1987; Robinson et al. 1987). Similarly, mutations of the *RAS* genes, which control the adenylyl cyclase activity of yeast, produce abnormalities of growth control (Kataoka et al. 1985b; Toda et al. 1985; Marshall et al. 1987). We and others have cloned *CDC25* by complementation screening (Camonis et al. 1986; Martegani et al. 1986b; Broek et al. 1987; Robinson et al. 1987), and in the process we also isolated the *TPK* genes, which encode the cAMP-dependent protein kinase (cAPK) catalytic subunits (Toda et al. 1987a). We report here another gene, provisionally called *SCH9*, that is also capable of suppressing the growth arrest of *cdc25*^{ts} when present on multicopy plasmids. *SCH9* encodes a protein with a domain most homologous to the catalytic subunits of the cAPK, the cGMP-dependent protein kinase (cGPK), and protein kinase C. Like the latter two protein kinases, the *SCH9* protein has a large amino-terminal domain. We also describe genetic experiments that examine the interaction of the *SCH9* product with the members of cAMP pathway. Overexpression of *SCH9* suppresses the growth defects that result from loss of *CDC25*, both *RAS* genes, *CYR1*, or all three *TPK* genes. Like cells overexpressing components of the cAMP pathway, cells overexpressing *SCH9* are sensitive to heat shock. *SCH9* is not itself an essential gene, but *sch9*$^-$ cells grow slowly. This phenotype is suppressed by activation of the cAMP pathway. Based on these results, we propose that *SCH9* encodes an effector kinase for a growth regulatory pathway, which is, to a large extent, redundant with the cAMP pathway.

Results

Isolation of the SCH9 *gene*

We transformed a temperature-sensitive *cdc25* strain, TT25-6 (see Table 1), with pooled DNA from a *S. cerevisiae* genomic library that had been constructed in YEp213 (Sherman et al. 1986; Toda et al. 1987a). Trans-

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Table 1. *Strain description*

Strain	Genotype and derivation	
TT25-6	*Matα leu2 ura3 trp1 can1 cdc25*-1	Broek et al. (1987)
SP1	*Mata his3 leu2 ura3 trp1 ade8 can1*	Cold Spring Harbor Laboratory collection
DC124	*Matα his4 leu2 ura3 trp1 ade8 can1*	Cold Spring Harbor Laboratory collection
TTSD 1	A diploid strain formed by mating SP1 and DC124	Broek et al. (1987)
TT1A-1	*Matα his3 leu2 ura3 trp1 ade8 cdc25::URA3*, containing p*CDC25(TRP1)*-1	Broek et al. (1987)
KPPK-1	*Mata/Matα his3/his3 leu2/leu2 ura3/ura3 trp1/trp1 ade8/+ can1/+ ras1::HIS3/+ ras2::URA3/+*	Toda et al. (1985)
KPPK-1T	A transformant of KPPK-1 with p*TPK1-TRP1*	
SPK-3T	*Matα his3 leu2 ura3 trp1 ade8 can1 ras1::HIS3 ras2::URA3*, containing p*TPK1-TRP1*	A segregant of tetrads from KPPK-1T
T158	*Mata/Matα his3/+ his4/+ leu2/leu2 ura3/ura3 trp1/trp1 ade8/ade8 can1/+ cyr1::URA3/+*	Transformant of TTSD 1 with 2.3-kb *Bgl*II fragment of p*cyr1::URA3*[a]
T158-T	A transformant of T158 with p*TPK1-TRP1*	
T158-5AT	*Mata his3 leu2 ura3 trp1 ade8 cyr1::URA3* containing p*TPK1-TRP1*	A segregant of tetrads from T158-T
T168	*MATa/Matα his3/his3 leu2/leu2 ura3/ura3 trp1/trp1 ade8/ade8 tpk1::URA3/+ tpk2::HIS3/+ tpk3::TRP1/+*	Toda et al. (1987a)
T168-T	A transformant of T168 with YRp*TPK1-ADE8*	
T168-6BT	*Matα his3 leu2 ura3 trp1 ade8 tpk1::URA3 tpk2::HIS3 tpk3::TRP1* containing p*TPK1-ADE8*	A segregant from tetrads of T168-T
T198	*Mata/Matα his3/+ his4/+ leu2/leu2 ura3/ura3 trp1/trp1 ade8/ade8 can1/+ sch9::ADE8/+*	A transformant of TTSD 1 with the *Pvu*II fragment of p*sch9::ADE8*
T198-8B	*Mata his3 leu2 ura3 trp1 ade8 sch9::ADE8*	A segregant from tetrads of T198
TT152	*Mata his3 leu2 ura3 trp1 ade8*	A segregant from tetrads of TTSD 1
T213	*MATa/α his3/his3 leu2/leu2 ura3/ura3 trp1/trp1 ade8/ade8 sch9::ADE8/+*	A diploid strain formed by crossing T198-8B and TT152
T213-4A	*Mata his3 leu2 ura3 trp1 ade8 sch9::ADE8*	A segregant from tetrads of T213
S13-3A	*MATα his3 leu2 ura3 trp1 ade8 tpk2::HIS3 tpk3::TRP1 byc1::LEU2*	S. Cameron et al. (in prep.)
S25	*MATa/MATα his3/his3 leu2/leu2 ura3/ura3 trp1/trp1 ade8/ade8 tpk2::HIS3/+ tpk3::TRP1/+ bcy1::LEU2/+ sch9::ADE8/+*	Diploid strain formed by crossing T198-8B and S13-3A
S25-31C	*MATa his3 leu2 ura3 trp1 ade8 tpk2::HIS3 tpk3::TRP1 bcy1::LEU2 sch9::ADE8*	Segregant from diploid strain S25

formants growing at 35°C were picked, and their plasmids were transferred to *Escherichia coli* and mapped for restriction endonuclease sites. Nine different plasmids were isolated. Five of these nine contained known genes: Three plasmids contained *TPK1*, which is one of the three genes that encode the catalytic subunits of the cAPK (Toda et al. 1987a); one contained *CDC25* itself (Camonis et al. 1986; Martegani et al. 1986b; Broek et al. 1987; Robinson et al. 1987); and the other contained *CYR1*, which encodes adenylyl cyclase (Kataoka et al. 1985a). From restriction mapping and Southern hybridization data, the remaining four plasmids did not contain *CDC25*, *RAS1*, *RAS2*, *CYR1*, *TPK1*, *TPK2*, or *TPK3*, all genes capable of suppressing *cdc25*^{ts} when they are carried on multicopy yeast plasmids. Two of the four remaining plasmids contained overlapping restriction fragments and represented a common locus (Fig. 1). We designated the gene of this locus as *SCH9* and describe its characterization here. The other two plasmids contain two new genes, which we have provisionally named *SCH1* and *SCH2*. These are currently under study in our lab and are not described here.

The SCH9*-gene product has homology to protein kinases*

To locate the coding region of *SCH9*, various restriction fragments were isolated and subcloned into the yeast shuttle vector, YEp213 (Sherman et al. 1986). These plasmids were transformed into the *cdc25*^{ts} strain TT25-6 (see Table 1) and examined for complementing activity (Fig. 1). The 6.5-kb *Hin*dIII fragment was shown to be capable of suppressing *cdc25*^{ts}, but the 3.0-kb *Pvu*II fragment was not (Fig. 1). This information provided us with two restriction endonuclease sites from which we began nucleotide sequencing. One long open reading frame was found in this region (Fig. 2). If the first methionine in the open reading frame is used, the *SCH9* gene would encode a protein of 824 amino acid residues. An in-frame stop codon appears 48 nucleotides upstream from that ATG. Disruption of this open reading frame destroys the activity of the *SCH9* gene (data not presented).

The deduced amino acid sequence of the *SCH9* gene contains consensus sequences found in all of the known protein kinases (Hunter and Cooper 1986). The sequence Gly X Gly X X Gly (where X is any amino acid), followed

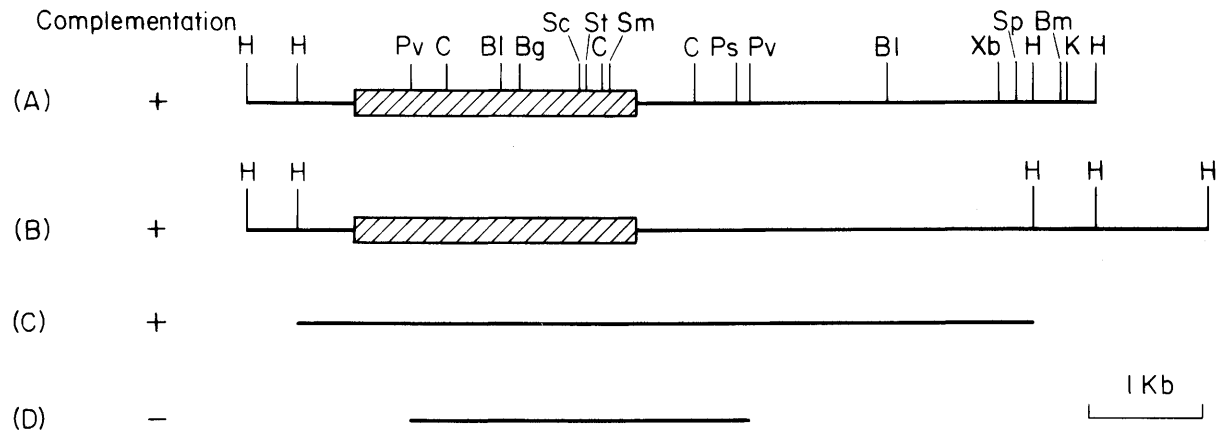

Figure 1. Restriction map and subcloning analysis of the *SCH9* locus. Structure and subcloning results of the *SCH9* locus are shown. (*A* and *B*) Two original suppressor plasmids of *cdc25*^{ts}. The 6.5-kb *Hin*dIII fragment (*C*) or the 3.0-kb *Pvu*II fragment (*D*) was inserted in the corresponding site of YEp213 (Sherman et al. 1986). The resultant subclones were transformed into the *cdc25*^{ts} strain TT25-6 (see Table 1), and the suppression of temperature sensitivity was examined. Coding sequences for the *SCH9* gene are indicated by hatching. The direction of transcription is from the left to the right. Abbreviations used are as follows: (Bg) *Bgl*II; (Bl) *Bal*I; (Bm) *Bam*HI; (C) *Cla*I; (H) *Hin*dIII; (K) *Kpn*I; (Ps) *Pst*I; (Pv) *Pvu*II; (Sc) *Sac*I; (Sm) *Sma*I; (Sp) *Sph*I; (St) *Stu*I; (Xb) *Xba*I.

7–16 residues later by Lys, is thought to be part of the ATP-binding site (Zoller et al. 1981; Kamps et al. 1984; Hannink and Donoghue 1985). It is shown with inverted triangles in Figure 2. Downstream of the ATP-binding site, two other protein kinase consensus sequences are found: Asp Phe Gly, shown with closed circles in Figure 2; and Ala Pro Glu, shown with open circles in Figure 2. The sequence of the *SCH9*-gene product was used in a computer search of GenBank and the NBRF (PIR) data base. As expected, significant homology was found between *SCH9* and the entire family of protein kinases. The *SCH9* protein was most homologous to the catalytic subunits of the cAMP-dependent protein kinases from both yeast and mammals (see Table 2; Fig. 3). In fact, the putative catalytic domain of *SCH9* is closer to the cAPK catalytic domains than any other protein kinase we have encountered. Homologies with other protein kinases were also apparent. The *SCH9* protein has lower but very significant homology to the catalytic domains of protein kinase C and cGPK. The *SCH9* protein shows less homology to various other protein kinases. An alignment of the putative catalytic domain of *SCH9* with that of the bovine and yeast cAPKs is seen in Figure 3.

Based on these results, we presume that *SCH9* encodes a protein kinase. Like protein kinase C and cGPK, the *SCH9* protein has a large amino-terminal domain. We presume that this domain is regulatory, but we can find no homology between it and the regulatory domains of other protein kinases.

Disruptions of the cAMP pathway are suppressed by SCH9 *carried on a multicopy plasmid*

A multicopy plasmid carrying the *SCH9* gene can suppress a temperature-sensitive *cdc25* allele, as can other multicopy plasmids expressing components of the cAMP pathway. To better understand the relationship of the *SCH9*-gene product to the cAMP pathway, we tested the ability of a multicopy plasmid carrying *SCH9* to suppress other mutations of the cAMP pathway. For this purpose, we used the plasmid exchange method (Broek et al. 1987). Strains were constructed that lacked *CDC25* (TT1A-1), both *RAS1* and *RAS2* genes (SPK-3T), the *CYR1* gene (T158-5AT), or all three *TPK* genes (T168-6BT). The strains were viable because they contained suppressor genes on a multicopy plasmid: *CDC25* as the suppressor gene for strain TT1A-1 and *TPK1* as the suppressor gene for strains SPK-3T, T158-5AT, and T168-6BT. The construction of these strains is described in Materials and methods. We then tested the ability of a multicopy plasmid containing the *SCH9* gene to replace, or 'exchange,' for the resident suppressor plasmid. The results are shown in Table 3. The multicopy plasmid containing the *SCH9* gene could exchange for the resident suppressor plasmid in all cases. Overexpression of the *SCH9*-gene product therefore appears to compensate for disruption of all the components of the cAMP effector pathway we tested.

Cells that overexpress SCH9 *are heat shock sensitive*

Cells with mutations that activate the cAMP pathway are abnormally sensitive to a heat shock (Sass et al. 1986; Broek et al. 1987; Nikawa et al. 1987b; Toda et al. 1987a). The above experiments suggest that the *SCH9*-gene product has functions that overlap those of the yeast cAMP pathway. We therefore asked whether overexpression of *SCH9* results in a heat-shock-sensitive phenotype. For this purpose, we constructed a plasmid, YEp*ADH-SCH9*, in which the *SCH9*-coding sequences were under the control of the strong alcohol dehydrogenase I gene promoter (for details, see Materials and methods). This plasmid also contains the *LEU2* gene. Leu2$^-$ cells were transformed with *YEpADH-SCH9*, and independent transformants were tested for their ability

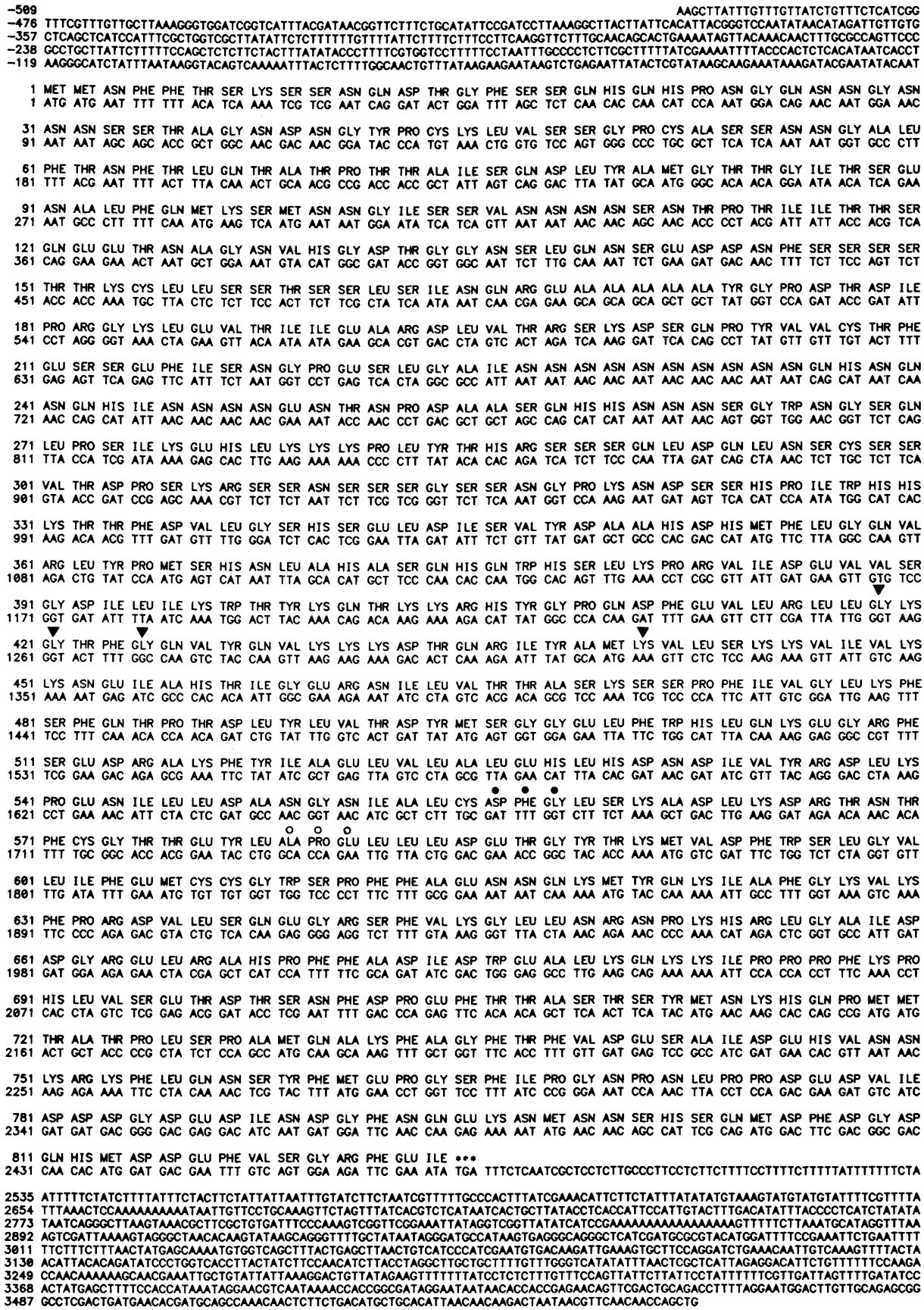

Figure 2. {*See facing page for legend.*}

Table 2. *Protein kinases homologous to* SCH9

Protein kinase	Score	Source
Yeast cAPK (*TPK1*)	41	Lisziewicz et al. (1987)
Bovine cAPK	39	Shoji et al. (1981)
Bovine cGPK	34	Takio et al. (1984)
Rat protein kinase C-type I	32	Knopf et al. (1986)
Rabbit muscle phosphorylase b kinase	30	Reimann et al. (1984)
Yeast *STE7*	18	Teague et al. (1986)
Yeast *CDC28*	14	Lorincz et al. (1983)

Alignment scores (see Materials and methods for details) for eight protein kinases are presented. Higher scores indicate greater similarity to *SCH9*. The average alignment score when *SCH9* was compared with 31 different protein kinases was 13 with a S.D. of 12.

to withstand a heat shock treatment. The results (Fig. 4) clearly indicate that overexpression of the *SCH9* gene leads to heat shock sensitivity.

Cells with disrupted SCH9 *grow slowly*

To examine the effect on cells of perturbing the *SCH9* gene, one-step gene disruption (Rothstein 1983) was carried out. An *SCH9* disruption plasmid was constructed by inserting the 3.4-kb *Bam*HI/*Bgl*II *ADE8* marker (White et al. 1985) into the *Bgl*II site, which is located in the middle of the *SCH9*-coding sequence (see Fig. 1; Materials and methods). Insertion of *ADE8* into the *SCH9* at this coding sequence site causes an interruption at the 487th amino acid. This separates the consensus ATP-binding sequence, Gly X Gly X X Gly, from the consensus sequence Asp Phe Gly. Both of these regions are thought to be important for kinase activity, and, therefore, we would predict that a disruption in that region would inactivate the *SCH9* protein. An *sch9 :: ADE8* fragment was used to transform the diploid TTSD 1 (Table 1). One resultant transformant, T198, was sporulated, and tetrad dissection carried out. As shown in Figure 5, two normal-sized colonies and two small colonies were obtained in nearly all the tetrads. A test for auxotrophic markers showed that the normal-sized colonies were always Ade$^-$ and the small colonies were Ade$^+$. Genomic Southern hybridization of the haploid segregants derived from T198 showed that the *SCH9* gene was indeed disrupted in Ade$^+$ cells (data not shown). This result indicates that disruption of the *SCH9* gene is not lethal but that cells without *SCH9* have a growth disadvantage. The doubling time of the *sch9*$^-$ cells was about two times longer than that of wild type-cells (Table 4). The proportion of unbudded cells was measured during exponential growth, and from this we calculated (Rivin and Fangmann 1980) that the increase in the doubling time was due entirely to an increase in the duration of the G_1 phase of the cell cycle.

Small colony size of sch9$^-$ *is suppressed by activation of the cAMP pathway*

Next, we determined whether activation of the cAMP pathway could compensate for the growth defects observed in *sch9*$^-$ cells. The regulatory subunit of the cAPK, encoded by the *BCY1* gene, is responsible for controlling the activity of the cAPK (Matsumoto et al. 1982;

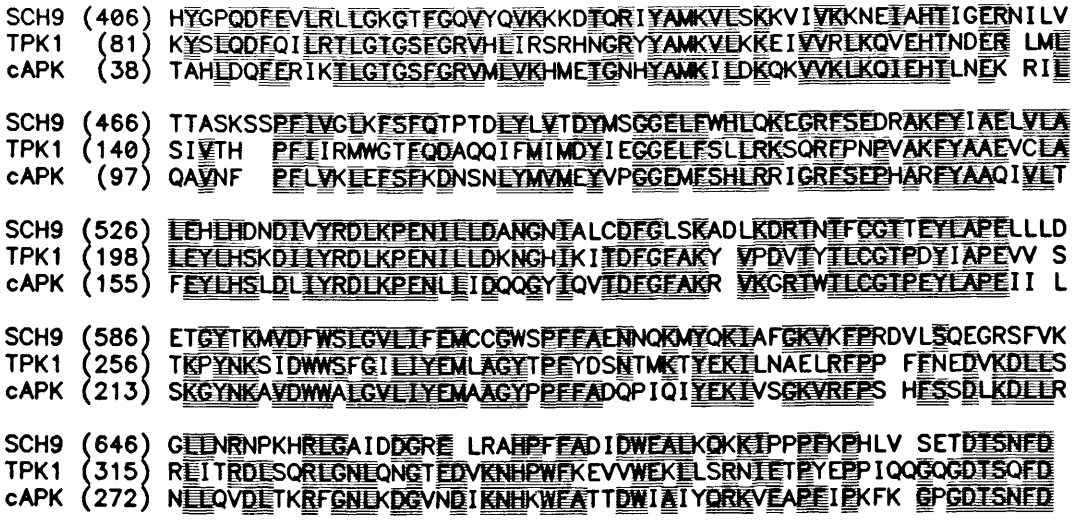

Figure 3. Amino acid sequence comparison between *SCH9* and cAPK catalytic subunits. A portion of the amino acid sequence of *SCH9* is compared to that of *TPK1*, one of three yeast genes that encode the catalytic subunits of the cAPK (Toda et al. 1987a), and the bovine Cα catalytic subunit (Shoji et al. 1981; Showers and Mauver 1986). Identical amino acids are shadowed.

Figure 2. Nucleotide sequence and deduced amino acid sequence of *SCH9*. The sequence starts at the *Hin*dIII site at −509 bp and continues through the second *Pvu*II site (see Fig. 1). In addition to the deduced amino acid sequence, 4084 nucleotides of *SCH9* are shown. (▼) The consensus sequence for amino acids at the ATP-binding site. Invariant sequences among known protein kinases are indicated: (●) Asp Phe Gly; (○) Ala Pro Glu. (*) The 3′ termination codon.

Table 3. *The suppression profile of the* SCH9 *gene carried on a multicopy plasmid*

Viability of cells carrying extrachromosomal high copy gene[a]	Viability of cells with chromosomal genotype			
	cdc25$^-$	*ras1*$^-$ *ras2*$^-$	*cyr1*$^-$	*tpk1*$^-$ *tpk2*$^-$ *tpk3*$^-$
ϕ	−	−	−	−
CDC25	+	−	−	NT
RAS2	−	+	−	NT
RAS2^{Val19}	+	+	−	NT
CYR1	+	+	+	NT
TPK1	+	+	+	+
SCH9	+	+	+	+

Yeast strains were constructed that contain disruptions of chromosomal genes but were viable because they contained known suppressor genes on multicopy plasmids (see Materials and methods). Genes being tested (left-hand column) for suppression of the indicated chromosomal mutations were introduced into these strains on multicopy plasmids, and the ability of one plasmid to replace, or "exchange," for the resident suppressor plasmid was determined (Broek et al. 1987). (+) The exchange could occur, and the specified gene, when present on multicopy plasmid, can suppress the indicated chromosomal mutations. (−) The specified multicopy plasmid could not exchange for the resident suppressor plasmid. (NT) Not tested. TT1A-1, SPK-3T, T158-5AT, and T168-6BT were used for *cdc25*$^-$, *ras1*$^-$ *ras2*$^-$, *cyr1*$^-$, and *tpk1*$^-$ *tpk2*$^-$ *tpk3*$^-$, respectively (Table 1).

[a] p*CDC25* (*LEU2*)-2 (Broek et al. 1987), YEp*RAS2*-1 (Powers et al. 1984), p*RAS2*^{Val19} (Broek et al. 1987), YEp13-*CYR1-11* (Kataoka et al. 1985a), YEp*TPK1* (Toda et al. 1987a), and YEp*SCH9* (Fig. 1A) were multicopy plasmids containing the indicated genes. The negative control, ϕ, was the multicopy plasmid YEp13 (Sherman et al. 1986).

Johnson et al. 1987; Toda et al. 1987b). Disruption of the *BCY1* gene activates the cAPK (Toda et al. 1987b). Therefore, we asked whether disruption of the *BCY1* gene can suppress the *sch9*$^-$ growth defect. To this end, a yeast strain, S25-31C, was constructed in which both the *SCH9* and *BCY1* genes were disrupted (for details, see Materials and methods). This strain was then transformed with a multicopy plasmid carrying the *BCY1* gene. Plasmid segregation analysis (Fig. 6) reveals that loss of the *BCY1* gene, with resulting activation of the cAMP pathway, suppresses the small colony size of *sch9*$^-$ cells. Similar experiments with *RAS2*^{val19}, a mutant *RAS2* gene that activates the cAMP pathway (Toda et al. 1985), lead to similar conclusions (data not presented).

Discussion

We have isolated and characterized *SCH9*, a new gene of *S. cerevisiae*. Judging by primary sequence analysis, *SCH9* encodes a protein kinase with a catalytic domain that closely resembles the cAPK catalytic domain of bovine or yeast origin. Indeed, the *SCH9* kinase is as related to the bovine cAPK in that domain as is the yeast cAPK. Like protein kinase C and the cGPK, but unlike the authentic cAPK catalytic subunits, the *SCH9*-encoded protein has a large amino-terminal domain. Although this domain does not resemble that of the C kinases or the cGPK, we nevertheless presume that it has a regulatory function.

The similarity between *SCH9* and the *TPK* genes,

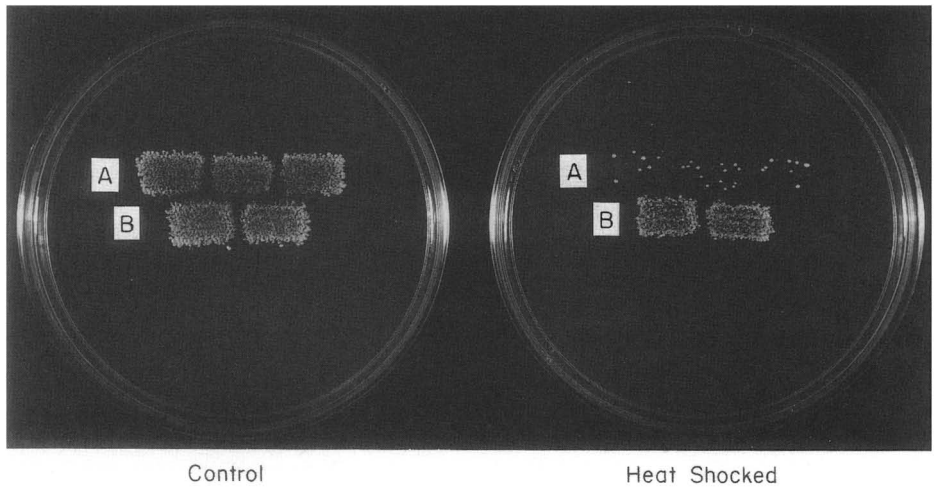

Figure 4. Heat shock sensitivity of cells overexpressing *SCH9*. The wild-type yeast strain SP1 was transformed with a multicopy plasmid carrying *SCH9* under the control of the alcohol dehydrogenase I gene promoter (*A*) or a control plasmid lacking the *SCH9* gene (*B*). Independent transformants were picked, grown on an SC-Leu plate for 2 days, replica plated onto a preheated SC-Leu plate, and incubated at 55°C for 45 min. Patches of cells on heat-shocked plates were incubated at 30°C for 2 days before being photographed.

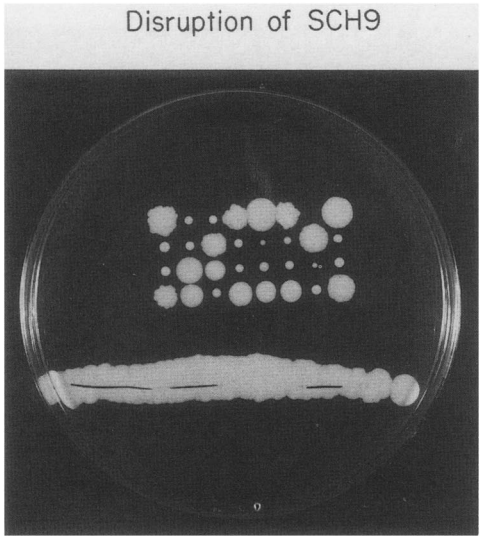

Figure 5. Phenotype of *sch9*$^-$ cells. A diploid heterozygous for a disruption of the *SCH9* gene, T198 (Table 1), was sporulated and asci dissected. The picture was taken after 5 days of incubation at 30°C. Vertical rows are spores from single asci.

which encode the yeast cAPK catalytic subunits, extends beyond the primary sequence of their encoded proteins. *SCH9* was isolated as a suppressor of temperature-sensitive alleles of *CDC25*, a gene required for the maintenance of cAMP levels in yeast. In fact, multicopy plasmids containing *SCH9* can suppress the defects resulting from loss of several of the components of the cAMP pathway. Moreover, overexpression of the *SCH9* gene leads to a phenotype, heat shock sensitivity, seen in cells with an activated cAMP pathway. Conversely, the slow-growth phenotype of cells bearing a disrupted *SCH9* gene can be reversed by the activation of the cAMP pathway. These results indicate that the functions of the cAPK and the *SCH9* protein kinase could be largely redundant.

We have not formally excluded the unlikely possibility that the *SCH9* gene encodes a highly divergent form of cAPK. We can test this possibility directly once we are able to assay the kinase activity of the *SCH9* protein. It seems more likely that in yeast, as in higher eukaryotes (Rasmussen 1986), physiologic events are controlled by multiple signaling pathways. Recent experiments by our lab strongly support this idea. We have isolated mutant *TPK* genes, called *tpk*^{w} alleles, which suppress all of the defects observed in strains lacking the *BCY1* gene (Cameron et al. 1988). *bcy1*$^-$ strains containing the *tpk*^{w} alleles (*bcy1*$^-$·*tpk*^{w} strains) respond appropriately to changing nutrient conditions (Cameron et al. 1988). Intracellular cAMP levels can be varied over a 10,000-fold range in the *bcy1*$^-$ *tpk*^{w} strains without noticeable phenotypic effects (Nikawa et al. 1987a; Cameron et al. 1988). These results would tend to exclude the possibility that *SCH9* encodes a cAPK. Rather, the experiments suggest that many of the nutrient responses thought to be dependent upon the cAMP effector system may also be under the parallel or redundant control of other, cAMP-independent signaling systems. The *SCH9* protein kinase is certainly a candidate component of such a system.

Table 4. *Growth characteristics of* sch9$^-$ *cells*

Strain	Genotype[a]	Doubling time (D)[b]	Fraction unbudded (F)[b]	G_1[b]	D − G_1[b]
SP1	*SCH9*	114	0.43	40	74
T198-8B	*sch9*$^-$	207	0.67	122	85
T213-4A	*sch9*$^-$	252	0.77	176	76

[a] The full genotypes of these strains are described in Table 1.
[b] The doubling time (D) of cells incubated in rich YPD medium was measured during exponential growth at 30°C. The fraction (F) of unbudded cells (cells in G_1) during exponential growth was determined by the microscopic examination of 200 cells. The duration of G_1 was determined using the formula $G_1 = D[1 - \log(2 - F)/\log 2]$ (Rivin and Fangmann 1980). The difference (D − G_1) represents the duration of the cell cycle, excluding G_1. Time values are in minutes.

Materials and methods

Strains, media, genetic procedures, and nomenclature

Yeast strains used in this study are listed in Table 1. *E. coli* HB101 was used for plasmid construction and purification. Yeast media have been described (Toda et al. 1985). Standard yeast genetic procedures were followed throughout (Sherman et al. 1986). The lithium acetate method was used for transformation of yeast cells (Ito et al. 1983). Heat shock experiments were performed as described (Sass et al. 1986). Gene disruptions are denoted by lowercase letters, followed by two colons, followed by the wild-type prototrophic marker used for disruption. In the text, gene disruptions are often abbreviated by lowercase letters, followed by a superscript minus sign, such as *sch9*$^-$. A strain that has a temperature-sensitive recessive mutation is shown by lowercase letters, followed by a superscript 'ts,' such as *cdc25*^{ts}.

DNA preparation and manipulation

Plasmid DNA was isolated from *E. coli* using the alkali–lysis method (Maniatis et al. 1982). Yeast DNA was prepared essentially as described (Nasmyth and Reed 1980). Restriction endonucleases, T4 DNA ligase, and the large fragment of DNA polymerase I were used as recommended by their suppliers (New England Biolabs, Inc., Bethesda Research Labs, Inc., or Boehringer Mannheim Biochemicals). Standard molecular cloning techniques were as described (Maniatis et al. 1982).

Nucleotide sequence determination

The dideoxy sequence method (Sanger et al. 1977) using [α-^{35}S] dATP as a substrate (Biggin et al. 1983) was carried out in combination with the unidirectional progressive deletion method (Henikoff 1984) using double-stranded plasmid DNA as template (Hattori and Sakaki 1986). A 3.0-kb *Pvu*II fragment (see Fig. 1) was subcloned into the *Sma*I site of pUC18 (Yanisch-Perron et al. 1985) in both orientations. *Bam*HI and *Sph*I were used subsequently to linearize these plasmids before deletion by *Exo*III and *Exo*VII. A 6.5-kb *Hin*dIII fragment (see Fig. 1) was subcloned into the *Hin*dIII site of pUC19 (Yanisch-Perron et al. 1985) in both orientations. *Sal*I and *Kpn*I were used for linear-

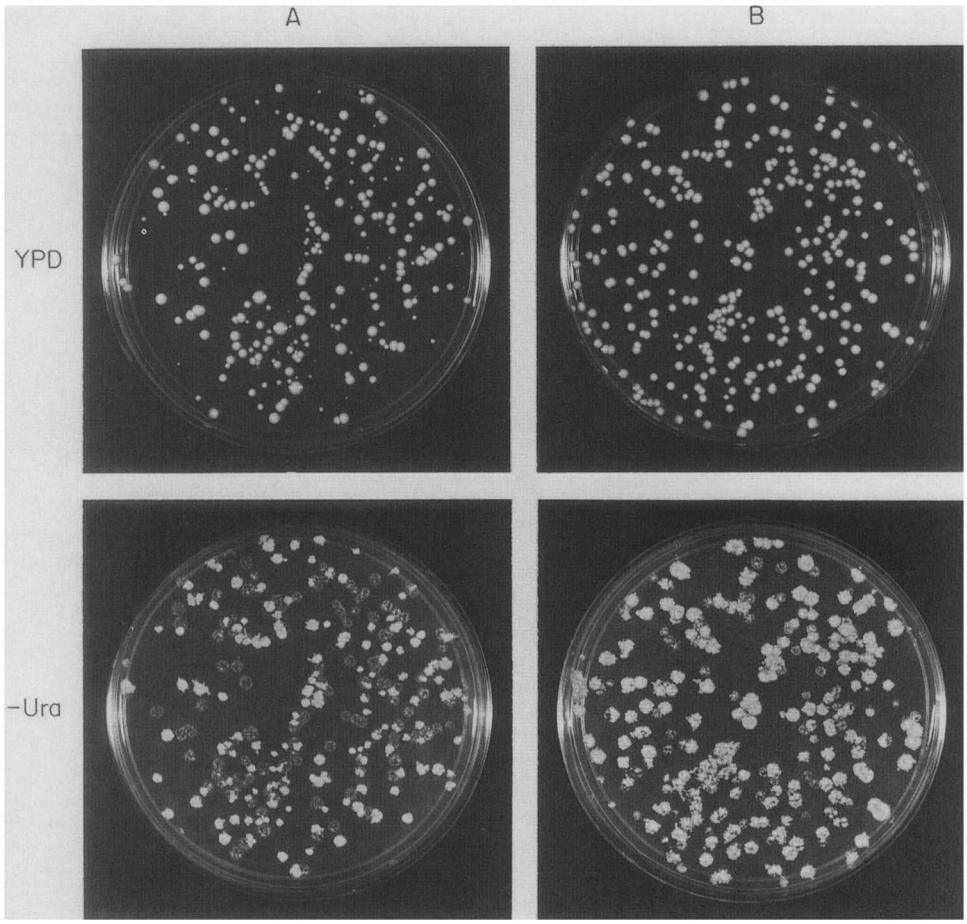

Figure 6. Suppression of the *sch9*⁻ growth defect by *bcy1*⁻. The strain S25-31C (see Table 1), a *ura3*⁻ strain that lacks both the *SCH9* gene and the gene for the regulatory subunit of the cAPK (*BCY1*), was transformed by multicopy plasmids carrying the *URA3* gene (*B*) or both the *URA3* and the *BCY1* genes (*A*). A transformant was grown overnight in YPD medium, diluted, plated onto YPD plates, and grown at 30°C for 3 days before being photographed. In the absence of Ura selection, the *BCY1* plasmids were unstable, and some colonies on each YPD plate were formed from cells that had lost their respective plasmid. After being photographed, the YPD plates were replica plated onto SC-Ura plates (which select for Ura⁺ cells) to reveal which colonies were formed from plasmid-containing cells. Colony sizes are distorted by replica plating, but notice that colony sizes in *A* are very heterogeneous on the master plate. Small colonies on the YPD plate are Ura⁺ (i.e., contain *BCY1*), and large colonies on the YPD plate are Ura⁻ (i.e., are *bcy1*⁻). On the other hand colony sizes in *B* are more uniform on the master plate and are generally large. These cells are all *sch9*⁻ *bcy1*⁻. No consistent size difference is noted in the segregants from the transformant carrying the control plasmid. Multicopy plasmids containing the *BCY1* gene do not affect the growth rate of wild-type strains (data not presented).

izing these plasmids. A total of 4084 nucleotides, from *Hin*dIII to the second *Pvu*II site, were sequenced on both strands.

Analysis of protein sequence similarities

The IFIND program from IntelliGenetics was used to identify and optimally align the *SCH9* protein with similar proteins in the NBRF (PIR) protein data base and with translations of nucleic acid sequences in GenBank. The alignment algorithm used by the IFIND program is based on the work of Dumas and Ninio (1982), Needleman and Wunsch (1970), and Wilbur and Lipman (1983). The parameter settings used were window size = 20, word length = 1, gap penalty = 2, fast = yes, and density = less.

Isolation of genes that can suppress a temperature-sensitive cdc25

The temperature-sensitive *cdc25*ᵗˢ strain, TT25-6 (see Table 1), was transformed with a yeast genomic library that was constructed by inserting yeast DNA partially digested with *Hin*dIII into YEp213, which contains the *LEU2* marker (Sherman et al. 1986; Toda et al. 1987a). Transformants were selected for temperature-sensitive suppression, either by incubating directly at 35°C on synthetic plates lacking leucine or by first incubating at room-temperature on plates lacking leucine and then replica-plating onto YPD plates followed by incubation at 35°C. Colonies that could grow at 35°C were picked, and plasmid segregation analysis was performed. Transformants whose growth at 35°C was plasmid dependent were chosen, and their plasmids recovered by transforming *E. coli*. Each plasmid was transformed back into TT25-6 to confirm its ability to suppress the temperature-sensitive phenotype.

Yeast strain constructions

To test whether the *SCH9* gene carried on a multicopy plasmid could suppress loss of genes involved in the *RAS*/cAMP

pathway, several mutant haploid strains were made. (1) The haploid strain, SPK-3T (see Table 1), is a tetrad segregant from the diploid KPPK-1T. KPPK-1T was derived from the diploid KPPK-1 (Toda et al. 1985) by transformation with p*TPK1-TRP1*. KPPK-1 is a diploid heterozygous at both of the *RAS* genes (Toda et al. 1985). p*TPK1-TRP1* contains the 2.4-kb *Hin*dIII/*Sph*I fragment of *TPK1* (Toda et al. 1987a) and the 1.4-kb *Eco*RI fragment of *ARS1-TRP1* (Tschumper and Carbon 1980) in the pBR322 vector. SPK-3T lacks any *RAS* genes but is viable because it has the *TPK1* gene on a multicopy plasmid (Toda et al. 1987a). (2) T158-5AT (see Table 1) is a tetrad segregant from a diploid T158-T derived from the diploid T158 by transformation with p*TPK1-TRP1*. T158 is a diploid heterozygous at the *CYR1* locus, which encodes adenylyl cyclase (Kataoka et al. 1985a). The *CYR1* gene was disrupted by using p*cyr1* :: *URA3*, which was constructed as follows. The entire coding sequence of the *CYR1* gene (Kataoka et al. 1985a) was removed by digesting p*CYR1*-2 (Kataoka et al. 1985a) with *Pvu*II and *Bal*I. These enzymes cleave in the 5′- and 3′-flanking regions of *CYR1*, respectively. The *Pvu*II to *Bal*I region was replaced with the 1.0-kb *Hin*dIII/*Sma*I fragment of *URA3*. A 2.3-kb *Bgl*II fragment that contained the *URA3* gene and flanking sequences from the *CYR1* locus was used for disruption of the *CYR1* gene. T158-5AT does not contain a functional *CYR1* gene but is viable because it has the *TPK1* gene on a multicopy plasmid. (3) T168-6BT (see Table 1) was a segregant of tetrads from a diploid T168-T that was transformed with the plasmid p*TPK1-ADE8*. T168 is a diploid heterozygous at all of the three *TPK* genes, which encode the catalytic subunits of the cAMP-dependent protein kinase (Toda et al. 1987a). p*TPK1-ADE8* was constructed by inserting the 3.4-kb *Bam*HI/*Bgl*II fragment of *ADE8* (White et al. 1985) into the *Bam*HI site of p*TPK1-TRP1*. T168-6BT lacks any functional chromosomal *TPK* genes but is viable because it has the *TPK1* gene on a multicopy plasmid. (4) TT1A-1 has been described (see Table 1; Broek et al. 1987). TT1A-1 lacks a functional chromosomal *CDC25* gene but is viable because it has the *CDC25* gene on a multicopy plasmid.

To test whether activation of the cAMP pathway can suppress the growth defects of *sch9*$^-$ cells, we constructed a strain, S25-31C, in which the chromosomal *SCH9* and *BCY1* genes are disrupted. We then introduced the *BCY1* gene on a multicopy plasmid and performed plasmid segregation analysis. This allowed us to assess the growth properties of *sch9*$^-$ strains in the presence or absence of *BCY1* and, thus, with either normal or increased activity of the cAMP pathway, respectively. Yeast strains containing three functional *TPK* genes and lacking the *BCY1* gene are phenotypically abnormal (Cannon and Tatchell 1987; Toda et al. 1987b), and cannot be transformed (S. Cameron, unpubl.). The *TPK* genes are required for these effects; disruption of two of the *TPK* genes in a strain lessens the severity of the *bcy1*$^-$ phenotype (Cameron et al. 1988) and renders them transformable (S. Cameron, unpubl.). Because we needed to introduce multicopy plasmids by transformation, S25-31C lacks the *TPK2* and *TPK3* genes (partial genotype: *sch9*$^-$ *bcy1*$^-$ *TPK1 tpk2*$^-$ *tpk3*$^-$) and was constructed by tetrad analysis of a diploid strain made by crossing T198-8B with S13-3A. Activation of any one of the *TPK* genes through disruption of the *BCY1* gene will suppress the growth defect of an *sch9*$^-$ strain (S. Cameron, unpubl.).

Disruption of the SCH9 *gene*

To disrupt the *SCH9* gene, we constructed the plasmid p*sch9* :: *ADE8*, as follows. The 6.5-kb *Hin*dIII fragment of *SCH9* (see Fig. 1) was subcloned into the corresponding site of pUC8 (Viera and Messing 1982). This plasmid was linearized at the single *Bgl*II site located in the middle of the coding region of *SCH9* (see Fig. 1), and the 3.4-kb *Bam*HI/*Bgl*II fragment of *ADE8* (White et al. 1985) was inserted, creating p*sch9* :: *ADE8*. The 6.4-kb *Pvu*II fragment of p*sch9* :: *ADE8*, which contains *ADE8* flanked by sequences of *SCH9*, was used for gene disruption by transforming a diploid TTSD 1.

Construction of the SCH9 *overexpressor plasmid*

In characterizing *SCH9* plasmids made by unidirectional deletion (Henikoff 1984), a plasmid, pUC(*SCH9*A9), was isolated that contains the *Eco*RI site of pUC19 (Yanisch-Perron et al. 1985), precisely 14 nucleotides 5′ of the initiating ATG of the *SCH9* gene. This modified *SCH9* gene was used to construct the plasmid YEp*ADH-SCH9*. A 6.4-kb *Eco*RI/*Hin*dIII fragment containing the complete coding sequence of *SCH9* was removed from pUC(*SCH9*A9) and treated with the large fragment of DNA polymerase. This fragment was then ligated into a *Hin*dIII cut, vector, pAD-1, which had been treated with the large fragment of DNA polymerase I. pAD-1 is a *LEU2*-2μ-based vector constructed in this laboratory that contains the alcohol dehydrogenase I promoter adjacent to a polylinker (J. Field et al., in prep.). The resulting plasmid, YEp*ADH-SCH9*, contains the *SCH9* gene under the control of the *ADH1*-gene promoter.

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References

Biggin, M.D., T.J. Gibson, and G.F. Hong. 1983. Buffer gradient gels and ^{35}S label as an aid to rapid DNA sequence determination. *Proc. Natl. Acad. Sci.* **80:** 3963–3965.

Boutelet, F., A. Petitjean, and F. Hilger. 1985. Yeast *CDC35* mutants are defective in adenylate cyclase and are allelic with *CYR1* mutants while *CAS1*, a new gene, is involved in the regulation of adenylate cyclase. *EMBO J.* **4:** 2635–2642.

Broek, D., T. Toda, T. Michaeli, L. Levin, C. Birchmeier, M. Zoller, S. Powers, and M. Wigler. 1987. The *S. cerevisiae CDC25* gene product regulates the *RAS*/adenylate cyclase pathway. *Cell* **48:** 789–799.

Cameron, S., L. Levin, M. Zoller, and M. Wigler. 1988. cAMP-independent control of sporulation, glycogen metabolism and heat shock resistance in *S. cerevisiae*. *Cell* (in press).

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

Cannon, J. and K. Tatchell. 1987. Characterization of *Saccharomyces cerevisiae* genes encoding subunits of cyclic AMP-dependent protein kinase. *Mol. Cell. Biol.* **7:** 2653–2663.

Dumas, J.P. and J. Ninio. 1982. Fast algorithms to deal with nucleic acid sequences. *Nucleic Acids Res.* **10:** 197–206.

Hannink, M. and D.J. Donoghue. 1985. Lysine residue 121 in the proposed ATP-binding site of the v-mos protein is required for transformation. *Proc. Natl. Acad. Sci.* **82:** 7894–7898.

Hattori, M. and Y. Sakaki. 1986. Dideoxy sequencing method using denatured plasmid templates. *Anal. Biochem.* **152:** 232–238.

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

Hunter, T. and J.A. Cooper. 1986. Viral oncogenes and tyrosine phosphorylation. *Annu. Rev. Biochem.* **54:** 897–930.

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

Johnson. K.E., S. Cameron, T. Toda, M. Wigler, and M. Zoller. 1987. Expression in *Escherichia coli* of *BCY1*, the regulatory subunit of the cAMP-dependent protein kinase from *Saccharomyces cerevisiae*. *J. Biol. Chem.* **262:** 8636–8642.

Kamps, M.P., S.S. Taylor, and B.M. Sefton. 1984. Direct evidence that oncogenic tyrosine protein kinases and cAMP-dependent protein kinase have homologous ATP-binding sites. *Nature* **310:** 589–592.

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

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

Knopf, J.L., M.-H. Lee, L.A. Sultzmann, R.W. Kriz, C.R. Loomis, R.M. Hewick, and R.M. Bell. 1986. Cloning and expression of multiple protein kinase C cDNA's. *Cell* **46:** 491–502.

Lisziewicz, J.A., H. Godany, H. Foerster, and H. Kuentzel. 1987. Isolation and nucleotide sequence of a *Saccharomyces cerevisiae* protein kinase gene suppressing the cell cycle start mutation *cdc25*. *J. Biol. Chem.* **262:** 2549–2553.

Lorincz, A.T. and S.I. Reed. 1983. Primary structure homology between the product of yeast cell division control gene *CDC28* and vertebrate oncogenes. *Nature* **307:** 183–185.

Maniatis, T., E.F. Fritsch, and J. Sambrook. 1982. *Molecular cloning: A laboratory manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Marshall, M.S., J.B. Gibbs, E.M. Scolnick, and I.S. Sigal. 1987. Regulatory function of the *Saccharomyces cerevisiae RAS* C-terminus. *Mol. Cell. Biol.* **7:** 2309–2315.

Martegani, E., M.D. Baroni, and M. Wanoni. 1986a. Interaction of cAMP with the *CDC25*-mediated step in the cell cycle of *Saccharomyces cerevisiae*. *EMBO J.* **5:** 375–380.

Martegani, E., M.D. Baroni, G. Frascotti, and L. Alberghina. 1986b. Molecular cloning and transcriptional analysis of the start gene *CDC25* of *Saccharomyces cerevisiae*. *EMBO J.* **5:** 2363–2369.

Matsumoto, K., I. Uno, and T. Ishikawa. 1984. Identification of the structural gene and nonsense alleles for adenylate cyclase in *Saccharomyces cerevisiae*. *J. Bacteriol.* **157:** 277–287.

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

Nasmyth, K.A. and S.I. Reed. 1980. Isolation of genes by complementation in yeast: Molecular cloning of a cell cycle gene. *Proc. Natl. Acad. Sci.* **77:** 2119–2123.

Needleman, S.B. and C.D. Wunsch. 1970. A general method applicable to the search for similarities in the amino acid sequences of two proteins. *J. Mol. Biol.* **48:** 443–453.

Nikawa, J., P. Sass, and M. Wigler. 1987a. Cloning and characterization of the low-affinity cyclic AMP phosphodiesterase gene of *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **7:** 3629–3636.

Nikawa, J., S. Cameron, T. Toda, K.M. Ferguson, and M. Wigler. 1987b. Rigorous feedback control of cAMP levels in *Saccharomyces cerevisiae*. *Genes Dev.* **1:** 931–937.

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

Rasmussen, H. 1986. The calcium messenger system. *N. Engl. J. Med.* **314:** 1094–1101, 1164–1170.

Reimann, E.M., K. Titani, L.H. Ericsson, R.D. Wade, E.H. Fischer, and K.A. Walsh. 1984. Homology of the gamma-subunit of phosphorylase-b kinase with cAMP-dependent protein kinase. *Biochemistry* **23:** 4185–4192.

Rivin, C.J. and W.L. Fangmann. 1980. Cell-cycle phase expansion in nitrogen-limited cultures of *Saccharomyces cerevisiae*. *J. Cell. Biol.* **85:** 96–107.

Robinson, L.C., J.B. Gibbs, M.S. Marshall, I.S. Sigal, and K. Tatchell. 1987. *CDC25*: A component of the RAS-adenylate cyclase pathway in *Saccharomyces cerevisiae*. *Science* **235:** 1218–1221.

Rothstein, R.J. 1983. One-step gene disruption in yeast. *Methods Enzymol.* **101:** 202–211.

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

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

Sherman, F., G.R. Fink, and J.B. Hicks. 1986. In *Laboratory course manual for methods in yeast genetics.* (ed. F. Sherman, G.R. Fink, and J.B. Hicks) Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Shoji, S., D. Parmelee, R. Wade, S. Kumar, L. Ericsson, K. Walsh, H. Neurath, G. Long, J. Demaille, E. Fischer, and K. Titani. 1981. Complete amino acid sequence of the catalytic subunit of bovine cardiac muscle cyclic AMP-dependent protein kinase. *Proc. Natl. Acad. Sci.* **78:** 848–851.

Showers, M.O. and R.A. Mauver. 1986. A cloned bovine cDNA encodes an alternate form of the catalytic subunit of cAMP-dependent protein kinase. *J. Biol. Chem.* **261:** 16288–16291.

Takio, K., R.D. Wade, S.B. Smith, E.G. Krebs, K.A. Walsh, and K. Titani. 1984. Guanosine cyclic 3',5'-phosphate dependent protein kinase, a dimeric protein homologous with 2 separate protein families. *Biochemistry* **23:** 4207–4218.

Teague, M.A., D.T. Chaleff, and B. Errede. 1986. Nucleotide sequence of the yeast regulatory gene *STE7* predicts a protein homologous to protein kinsaes. *Proc. Natl. Acad. Sci.* **83:** 7371–7375.

Toda, T., S. Cameron, P. Sass, M. Zoller, and M. Wigler. 1987a. Three different genes in *S. cerevisiae* encode the catalytic subunits of the cAMP-dependent protein kinase. *Cell* **50:** 277–287.

Toda, T., S. Cameron, P. Sass, M. Zoller, J.D. Scott, B. McMullen, M. Hurwitz, E.G. Krebs, and M. Wigler. 1987b. Cloning and characterization of *BCY1*, a locus encoding a regulatory subunit of the cAMP dependent protein kinase in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **7:** 1371–1377.

Toda, T., I. Uno, T. Ishikawa, S. Powers, T. Kataoka, D. Broek, S. Cameron, J.R. Broach, K. Matsumoto, and M. Wigler.

1985. In yeast, *RAS* proteins are controlling elements of adenylate cyclase. *Cell* **40:** 27–36.

Tschumper, G. and J. Carbon. 1980. Sequence of a yeast DNA fragment containing a chromosomal replicator and the TRP1 gene. *Gene* **10:** 157–166.

Viera, J. and J. Messing. 1982. The pUC plasmids, an M13up7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. *Gene* **19:** 259–268.

White, J.H., K. Lusnak, and S. Fogel. 1985. Mismatch-specific post-meiotic segregation frequency in yeast suggests a heteroduplex recombination intermediate. *Nature* **315:** 350–352.

Wilbur, W.J. and D.J. Lipman. 1983. Rapid similarity searches of nucleic acid and protein data banks. *Proc. Natl. Acad. Sci.* **80:** 726–730.

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

Zoller, M.J., N.C. Nelson, and S.S. Taylor. 1981. Affinity labeling of cAMP-dependent protein kinase with p-fluorosulfonyl benzoyl adenosine. Covalent modification of lysine 71. *J. Biol. Chem.* **256:** 10837–10842.

Zoller, M.J., J. Kuret, S. Cameron, L. Levin, and K. Johnson. 1988. Purification of C1, the catalytic subunit of *Saccharomyces cerevisiae* cAMP-dependent protein kinase encoded by *TPK1*. *J. Biol. Chem.* (in press).