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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*]

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	*Mata his3 leu2 ura3 trp1 ade8 cdc25::URA3*, containing pCDC25(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 pTPK1-TRP1	
SPK-3T	*Matα his3 leu2 ura3 trp1 ade8 can1 ras1::HIS3 ras2::URA3*, containing pTPK1-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 BglII fragment of pcyr1::URA3[a]
T158-T	A transformant of T158 with pTPK1-TRP1	
T158-5AT	*Mata his3 leu2 ura3 trp1 ade8 cyr1::URA3* containing pTPK1-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 YRpTPK1-ADE8	
T168-6BT	*Mata his3 leu2 ura3 trp1 ade8 tpk1::URA3 tpk2::HIS3 tpk3::TRP1* containing pTPK1-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 PvuII fragment of psch9::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 bcy1::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 *Hind*III 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
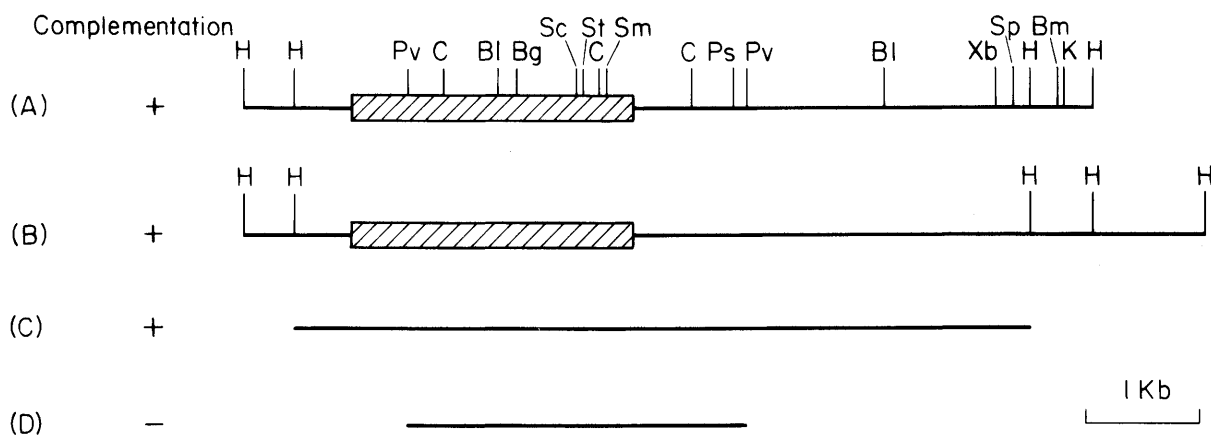
Figure 1. Restriction map and subcloning analysis of the *SCH9* locus. Structure and subcloning results of the *SCH9* locus are shown. (*A* and *B*) Two original suppressor plasmids of *cdc25*^ts. The 6.5-kb *Hind*III fragment (*C*) or the 3.0-kb *Pvu*II fragment (*D*) was inserted in the corresponding 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) *Hind*III; (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 YEp*ADH-SCH9*, and independent transformants were tested for their ability

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-509                                                                          AAGCTTATTTGTTTGTTATCTGTTTCTCATCGG
-478 TTTCGTTTGTTGCTTAAAGGGTGGATCGGTCATTTACGATAACGGTTCTTTCTGCATATTCCGATCCTTAAAGGCTTACTTATTCACATTACGGGTCCAATATAACATAGATTGTTGTG
-357 CTCAGCTCATCCATTTCGCTGGTCGCTTATATTCTCTTTTTTGTTTTATTCTTTTCTTTCCTTCAAGGTTCTTTGCAACAGCACTGAAAATAGTTACAAACAACTTTGCGCCAGTTCCC
-238 GCCTGCTTATTCTTTTTCCAGCTCTCTTCTACTTTATATACCCTTTTCGTGGTCCTTTTTCCTAATTTGCCCCTCTTCGCTTTTTATCGAAAATTTTACCCACTCTCACATAATCACCT
-119 AAGGGCATCTATTTAATAAGGTACAGTCAAAAATTTACTCTTTTGGCAACTGTTTATAAGAAGAATAAGTCTGAGAATTATACTCGTATAAGCAAGAAATAAAGATACGAATATACAAT

   1 MET MET ASN PHE PHE THR SER LYS SER SER ASN GLN ASP THR GLY PHE SER SER GLN HIS GLN HIS PRO ASN GLY GLN ASN ASN GLY ASN
   1 ATG ATG AAT TTT TTT ACA TCA AAA TCG TCG AAT CAG GAT ACT GGA TTT AGC TCT CAA CAC CAA CAT CCA AAT GGA CAG AAC AAT GGA AAC

  31 ASN ASN SER SER THR ALA GLY ASN ASP ASN GLY TYR PRO CYS LYS LEU VAL SER SER GLY PRO CYS ALA SER SER ASN ASN GLY ALA LEU
  91 AAT AAT AGC AGC ACC GCT GGC AAC GAC AAC GGA TAC CCA TGT AAA CTG GTG TCC AGT GGG CCC TGC GCT TCA TCA AAT AAT GGT GCC CTT

  61 PHE THR ASN PHE THR LEU GLN THR ALA THR PRO THR THR ALA ILE SER GLN ASP LEU TYR ALA MET GLY THR THR GLY ILE THR SER GLU
 181 TTT ACG AAT TTT ACT TTA CAA ACT GCA ACG CCG ACC ACC GCT ATT AGT CAG GAC TTA TAT GCA ATG GGC ACA ACA GGA ATA ACA TCA GAA

  91 ASN ALA LEU PHE GLN MET LYS SER MET ASN ASN GLY ILE SER SER VAL ASN ASN ASN ASN SER ASN THR PRO THR ILE ILE THR THR SER
 271 AAT GCC CTT TTT CAA ATG AAG TCA ATG AAT AAT GGA ATA TCA TCA GTT AAT AAT AAC AAC AGC AAC ACC CCT ACG ATT ATT ACC ACG TCA

 121 GLN GLU GLU THR ASN ALA GLY ASN VAL HIS GLY ASP THR GLY GLY ASN SER LEU GLN ASN SER GLU ASP ASP ASN PHE SER SER SER SER
 361 CAG GAA GAA ACT AAT GCT GGA AAT GTA CAT GGC GAT ACC GGT GGC AAT TCT TTG CAA AAT TCT GAA GAT GAC AAC TTT TCT TCC AGT TCT

 151 THR THR LYS CYS LEU LEU SER SER THR SER SER LEU SER ILE ASN GLN ARG GLU ALA ALA ALA ALA ALA TYR GLY PRO ASP THR ASP ILE
 451 ACC ACC AAA TGC TTA CTC TCT TCC ACT TCT TCG CTA TCA ATA AAT CAA CGA GAA GCA GCA GCA GCT GCT TAT GGT CCA GAT ACC GAT ATT

 181 PRO ARG GLY LYS LEU GLU VAL THR ILE ILE GLU ALA ARG ASP LEU VAL THR ARG SER LYS ASP SER GLN PRO TYR VAL VAL CYS THR PHE
 541 CCT AGG GGT AAA CTA GAA GTT ACA ATA ATA GAA GCA CGT GAC CTA GTC ACT AGA TCA AAG GAT TCA CAG CCT TAT GTT GTT TGT ACT TTT

 211 GLU SER SER GLU PHE ILE SER ASN GLY PRO GLU SER LEU GLY ALA ILE ASN ASN ASN ASN ASN ASN ASN ASN ASN ASN GLN HIS ASN GLN
 631 GAG AGT TCA GAG TTC ATT TCT AAT GGT CCT GAG TCA CTA GGC GCC ATT AAT AAT AAC AAC AAT AAC AAC AAC AAT AAT CAG CAT AAT CAA

 241 ASN GLN HIS ILE ASN ASN ASN ASN GLU ASN THR ASN PRO ASP ALA ALA SER GLN HIS HIS ASN ASN ASN SER GLY TRP ASN GLY SER GLN
 721 AAC CAG CAT ATT AAC AAC AAC AAC GAA AAT ACC AAC CCT GAC GCT GCT GCT AGC CAG CAT CAT AAT AAT AAC AGT GGT TGG AAC GGT TCT CAG

 271 LEU PRO SER ILE LYS GLU HIS LEU LYS LYS LYS SER PRO LEU TYR THR HIS ARG SER SER SER GLN LEU ASP GLN LEU ASN SER CYS SER SER
 811 TTA CCA TCG ATA AAA GAG CAC TTG AAG AAA AAA CCC CTT TAT ACA CAC AGA TCA TCT TCC CAA TTA GAT CAG CTA AAC TCT TGC TCT TCA

 301 VAL THR ASP PRO SER LYS ARG SER SER ASN SER SER SER GLY SER SER ASN GLY PRO LYS ASN ASP SER SER HIS PRO ILE TRP HIS HIS
 901 GTA ACC GAT CCG AGC AAA CGT TCT TCT AAT TCT TCG TCG GGT TCT TCA AAT GGT CCA AAG AAT GAT AGT TCA CAT CCA ATA TGG CAT CAC

 331 LYS THR THR PHE ASP VAL LEU GLY SER HIS SER GLU LEU ASP ILE SER VAL TYR ASP ALA ALA HIS ASP HIS MET PHE LEU GLY GLN VAL
 991 AAG ACA ACG TTT GAT GTT TTG GGA TCT CAC TCG GAA TTA GAT ATT TCT GTT TAT GAT GCT GCC CAC GAC CAT ATG TTC TTA GGC CAA GTT

 361 ARG LEU TYR PRO MET SER HIS ASN LEU ALA HIS ALA SER GLN HIS GLN TRP HIS SER LEU LYS PRO ARG VAL ILE ASP GLU VAL VAL SER
1081 AGA CTG TAT CCA ATG AGT CAT AAT TTA GCA CAT GCT TCC CAA CAC CAA TGG CAC AGT TTG AAA CCT CGC GTT ATT GAT GAA GTT GTG TCC
                                                                                                                       ▼
 391 GLY ASP ILE LEU ILE LYS TRP THR TYR LYS GLN THR LYS LYS ARG HIS TYR GLY PRO GLN ASP PHE GLU VAL LEU ARG LEU LEU GLY LYS
1171 GGT GAT ATT TTA ATC AAA TGG ACT TAC AAA CAG ACA AAG AAA AGA CAT TAT GGC CCA CAA GAT TTT GAA GTT CTT CGA TTA TTG GGT AAG
         ▼                ▼                                                                              ▼
 421 GLY THR PHE GLY GLN VAL TYR GLN VAL LYS LYS LYS ASP THR GLN ARG ILE TYR ALA MET LYS VAL LEU SER LYS LYS VAL ILE VAL LYS
1261 GGT ACT TTT GGC CAA GTC TAC CAA GTT AAG AAG AAA GAC ACT CAA AGA ATT TAT GCA ATG AAA GTT CTC TCC AAG AAA GTT ATT GTC AAG

 451 LYS ASN GLU ILE ALA HIS THR ILE GLY GLU ARG ASN ILE LEU VAL THR THR ALA SER LYS SER SER PRO PHE ILE VAL GLY LEU LYS PHE
1351 AAA AAT GAG ATC GCC CAC ACA ATT GGC GAA AGA AAT ATC CTA GTC ACA ACG TCC AAA TCG TCC CCA TTC ATT GTC GGA TTG AAG TTT

 481 SER PHE GLN THR PRO THR ASP LEU TYR LEU VAL THR ASP TYR MET SER GLY GLY GLU LEU PHE TRP HIS LEU GLN LYS GLU GLY ARG PHE
1441 TCC TTT CAA ACA CCA ACA GAT CTG TAT TTG GTC ACT GAT TAT ATG AGT GGT GGA GAA TTA TTC TGG CAT TTA CAA AAG GAA GGC CGT TTT

 511 SER GLU ASP ARG ALA LYS PHE TYR ILE ALA GLU LEU VAL LEU ALA LEU GLU HIS LEU HIS ASP ASN ASP ILE VAL TYR ARG ASP LEU LYS
1531 TCG GAA GAC AGA GCG AAA TTC TAT ATC GCT GAG TTA GTC CTA GCG TTA GAA CAT TTA CAC GAT AAC GAT ATC GTT TAC AGG GAC CTA AAG
                                                                                               •    •    •
 541 PRO GLU ASN ILE LEU LEU ASP ALA ASN GLY ASN ILE ALA LEU CYS ASP PHE GLY LEU SER LYS ALA ASP LEU LYS ASP ARG THR ASN THR
1621 CCT GAA AAC ATT CTA CTC GAT GCC AAC GGT AAC ATC GCT CTT TGC GAT TTT GGT CTT TCT AAA GCT GAC TTG AAG GAT AGA ACA AAC ACA
                                                             o    o    o
 571 PHE CYS GLY THR THR GLU TYR LEU ALA PRO GLU LEU LEU LEU ASP GLU THR GLY TYR THR LYS MET VAL ASP PHE TRP SER LEU GLY VAL
1711 TTT TGC GGC ACC ACG GAA TAC CTG GCA CCA GAA TTG TTA CTG GAC GAA ACC GGC TAC ACC AAA ATG GTC GAT TTC TGG TCT CTA GGT GTT

 601 LEU ILE PHE GLU MET CYS CYS GLY TRP SER PRO PHE PHE ALA GLU ASN ASN GLN LYS MET TYR GLN LYS ILE ALA PHE GLY LYS VAL LYS
1801 TTG ATA TTT GAA ATG TGT TGT GGT TGG TCC CCT TTC TTT GCG GAA AAT AAT CAA AAA ATG TAC CAA AAA ATT GCC TTT GGT AAA GTC AAA

 631 PHE PRO ARG ASP VAL LEU SER GLN GLU GLY ARG SER PHE VAL LYS GLY LEU LEU ASN ARG ASN PRO LYS HIS ARG LEU GLY ALA ILE ASP
1891 TTC CCC AGA GAC GTA CTG TCA CAA GAG GGG AGG TCT TTT GTA AAG GGT TTA CTA AAC AGA AAC CCC AAA CAT AGA CTC GGT GCC ATT GAT

 661 ASP GLY ARG GLU LEU ARG ALA HIS PRO PHE PHE ALA ASP ILE ASP TRP GLU ALA LEU LYS GLN LYS LYS ILE PRO PRO PRO PHE LYS PRO
1981 GAT GGA AGA GAA CTA CGA GCT CAT CCA TTT TTC GCA GAT ATC GAC TGG GAG GCC TTG AAG CAG AAA AAA ATT CCA CCA CCT TTC AAA CCT

 691 HIS LEU VAL SER GLU THR ASP THR SER ASN PHE ASP PRO GLU PHE THR THR ALA SER THR SER TYR MET ASN LYS HIS GLN PRO MET MET
2071 CAC CTA GTC TCG GAG ACG GAT ACC TCG AAT TTT GAC CCA GAG TTC ACA ACA GCT TCA ACT TCA TAC ATG AAC AAG CAC CAG CCG ATG ATG

 721 THR ALA THR PRO LEU SER PRO ALA MET GLN ALA LYS PHE ALA GLY PHE THR PHE VAL ASP GLU SER ALA ILE ASP GLU HIS VAL ASN ASN
2161 ACT GCT ACC CCG CTA TCT CCA GCC ATG CAA GCA AAG TTT GCT GGT TTC ACC TTT GTT GAT GAG TCC GCC ATC GAT GAA CAC GTT AAT AAC

 751 LYS ARG LYS PHE LEU GLN ASN SER TYR PHE MET GLU PRO GLY SER PHE ILE PRO GLY ASN PRO ASN LEU PRO PRO ASP GLU ASP VAL ILE
2251 AAG AGA AAA TTC CTA CAA AAC TCG TAC TTT ATG GAA CCT GGT TCC TTT ATC CCG GGA AAT CCA AAC TTA CCT CCA GAC GAA GAT GTC ATC

 781 ASP ASP ASP GLY ASP GLU ASP ILE ASN ASP GLY PHE ASN GLN GLU LYS ASN MET ASN ASN SER HIS SER GLN MET ASP PHE ASP GLY ASP
2341 GAT GAT GAC GGG GAC GAG GAC ATC AAT GAT GGA TTC AAC CAA GAG AAA AAT ATG AAC AAC AGC CAT TCG CAG ATG GAC TTC GAC GGC GAC

 811 GLN HIS MET ASP ASP GLU PHE VAL SER GLY ARG PHE GLU ILE •••
2431 CAA CAC ATG GAT GAC GAA TTT GTC AGT GGA AGA TTC GAA ATA TGA TTTCTCAATCGCTCCTCTTGCCCTTCCTCTTCTTTTCCTTTTCTTTTTATTTTTTCTA

2535 ATTTTTCTATCTTTTATTTCTACTTCTATTATTAATTTGTATCTTCTAATCGTTTTTGCCCACTTTATCGAAACATTCTTCTATTTATATATGTAAAGTATGTATGTATTTTCGTTTTA
2654 TTTAAACTCCAAAAAAAAATAATTGTTCCTGCAAAGTTCTAGTTTATCACGTCTCATAATCACTGCTTATACCTCCACCATTCCATTGTACTTTGACATATTTACCCCTCATCTATATA
2773 TAATCAGGGCTTAAGTAAACGCTTCGCTGTGATTTCCCAAAGTCGGTTCGGAAATTATAGGTCGGTTATATCATCCGAAAAAAAAAAAAAAAAAGTTTTTCTTAAATGCATAGGTTTAA
2892 AGTCGATTAAAAGTAGGGCTAACACAAGTATAAGCAGGGTTTTGCTATAATAGGGATGCCATAAGTGAGGGCAGGGCTCATCGATGCGCGTACATGGATTTTCCGAAATTCTGAATTTT
3011 TTCTTTCTTTAACTATGAGCAAAATGTGGTCAGCTTTACTGAGCTTAACTGTCATCCCATCGAATGTGACAAGATTGAAAGTGCTTCCAGGATCTGAAACAATTGTCAAAGTTTTACTA
3130 ACATTACACAGATATCCCTGGTCACCTTACTATCTTCCAACATCTTACCTAGGCTTGCTGCTTTTGTTTGGGTCATATATTTAACTCGCTCATTAGAGGACATTCTGTTTTTTCCAAGA
3249 CCAACAAAAAAGCAACGAAATTGCTGTATTATTAAAGGACTGTTATAGAAGTTTTTTATCCTCTCTTTGTTTCCAGTTATTCTTATTCCTATTTTTTCGTTGATTAGTTTTGATATCC
3368 ACTATGAGCTTTTCCACCATAAATAGGAACGTCAATAAAACCACCGGCGATAGGAATAATAACACCACCGAGAACAGTTCGACTGCAGACCTTTTAGGAATGGACTTGTTGCAGAGCGG
3487 GCCCTGACTGATGAACACGATGCAGCCAAACAACTCTTCTGACATGCTGCACATTAACAACAAGACTAATAACGTTCAACAACCAGCTG
```

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 487[th] 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;

```
SCH9 (406)  HYGPQDFEVLRLLGKGTFGQVYQVKKKDTQRIYAMKVLSKKVIVKKNEIAHTIGERNILV
TPK1 ( 81)  KYSLQDFQILRTLGTGSFGRVHLIRSRHNGRYYAMKVLKKEIVVRLKQVEHTNDER LML
cAPK ( 38)  TAHLDQFFERIKTLGTGSFGRVMLVKHMETGNHYAMKILDKQKVVKLKQIEHTLNEK RIL

SCH9 (466)  TTASKSSPFLVGLKFSFQTPTDLYLVTDYMSGGELFWHLQKEGRFSEDRAKFYIAELVLA
TPK1 (140)  SIVTH  PFLIRMWGTFQDAQQIFMIMDYIEGGELFSLLRKSQRFPNPVAKFYAAEVCLA
cAPK ( 97)  QAVNF  PFLVKLEFSFKDNSNLYMVMEYVPGGEMFSHLRRIGRFSEPHARFYAAQIVLT

SCH9 (526)  LEHLHDNDIVYRDLKPENILLDANGNIALCDFGLSKADLKDRTNTFCGTTEYLAPELLLD
TPK1 (198)  LEYLHSKDILYRDLKPENILLDKNGHIKITDFGFAKY VPDVTYTLCGTPDYIAPEVV S
cAPK (155)  FEYLHSLDLIYRDLKPENLEIDQQGYIQVTDFGFAKR VKGRTWTLCGTPEYLAPEII L

SCH9 (586)  ETGYTKMVDFWSLGVLIFEMCCGWSPFFAENNQKMYQKIAFGKVKFPRDVLSQEGRSFVK
TPK1 (256)  TKPYNKSIDWWSFGILIYEMLAGYTPFYDSNTMKTYEKILNAELRFPP FFNEDVKDLLS
cAPK (213)  SKGYNKAVDWWALGVLIYEMAAGYPPFFADQPIQIYEKIVSGKVRFPS HFSSDLKDLLR

SCH9 (646)  GLLNRNPKHRLGAIDDGRE LRAHPFFADIDWEALKQKKIPPPFKPHLV SETDTSNFD
TPK1 (315)  RLITRDLSQRLGNLQNGTEDVKNHPWFKEVVWEKLLSRNIETPYEPPIQQGQGDTSQFD
cAPK (272)  NLLQVDLTKRFGNLKDGVNDIKNHKWFATTDWIAIYQRKVEAPFIPKFK GPGDTSNFD
```

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 *Hind*III 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] pCDC25 (*LEU2*)-2 (Broek et al. 1987), YEpRAS2-1 (Powers et al. 1984), pRAS2^{Val19} (Broek et al. 1987), YEp13-CYR1-11 (Kataoka et al. 1985a), YEpTPK1 (Toda et al. 1987a), and YEpSCH9 (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,

Control   Heat Shocked
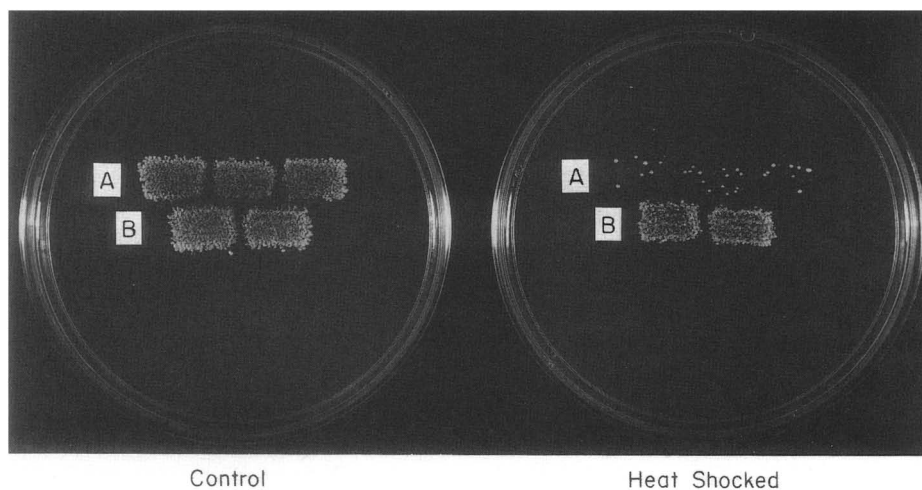
Figure 4. Heat shock sensitivity of cells overexpressing *SCH9*. The wild-type yeast strain SP1 was transformed with a multicopy plasmid carrying *SCH9* under the control of the alcohol dehydrogenase I gene promoter (*A*) or a control plasmid lacking the *SCH9* gene (*B*). Independent transformants were 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.
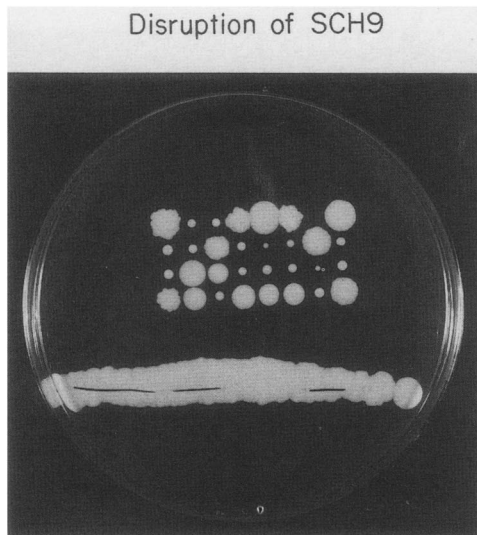
Disruption of SCH9

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.

Table 4. *Growth characteristics of* sch9⁻ *cells*

Strain	Geno-type[a]	Doubling time (D)[b]	Fraction unbudded (F)[b]	G_I[b]	$D - G_I$[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_I) during exponential growth was determined by the microscopic examination of 200 cells. The duration of G_I was determined using the formula $G_I = D[1 - \log(2 - F)/\log 2]$ (Rivin and Fangmann 1980). The difference $(D - G_I)$ represents the duration of the cell cycle, excluding G_I. Time values are in minutes.

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 *tpkʷ* alleles, which suppress all of the defects observed in strains lacking the *BCY1* gene (Cameron et al. 1988). *bcy1⁻* strains containing the *tpkʷ* alleles (*bcy1⁻·tpkʷ* 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⁻ tpkʷ* 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.

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 *cdc25ᵗˢ*.

### 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-³⁵S] 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 *PvuII* fragment (see Fig. 1) was subcloned into the *SmaI* site of pUC18 (Yanisch-Perron et al. 1985) in both orientations. *BamHI* and *SphI* were used subsequently to linearize these plasmids before deletion by *ExoIII* and *ExoVII*. A 6.5-kb *HindIII* fragment (see Fig. 1) was subcloned into the *HindIII* site of pUC19 (Yanisch-Perron et al. 1985) in both orientations. *SalI* and *KpnI* were used for linear-
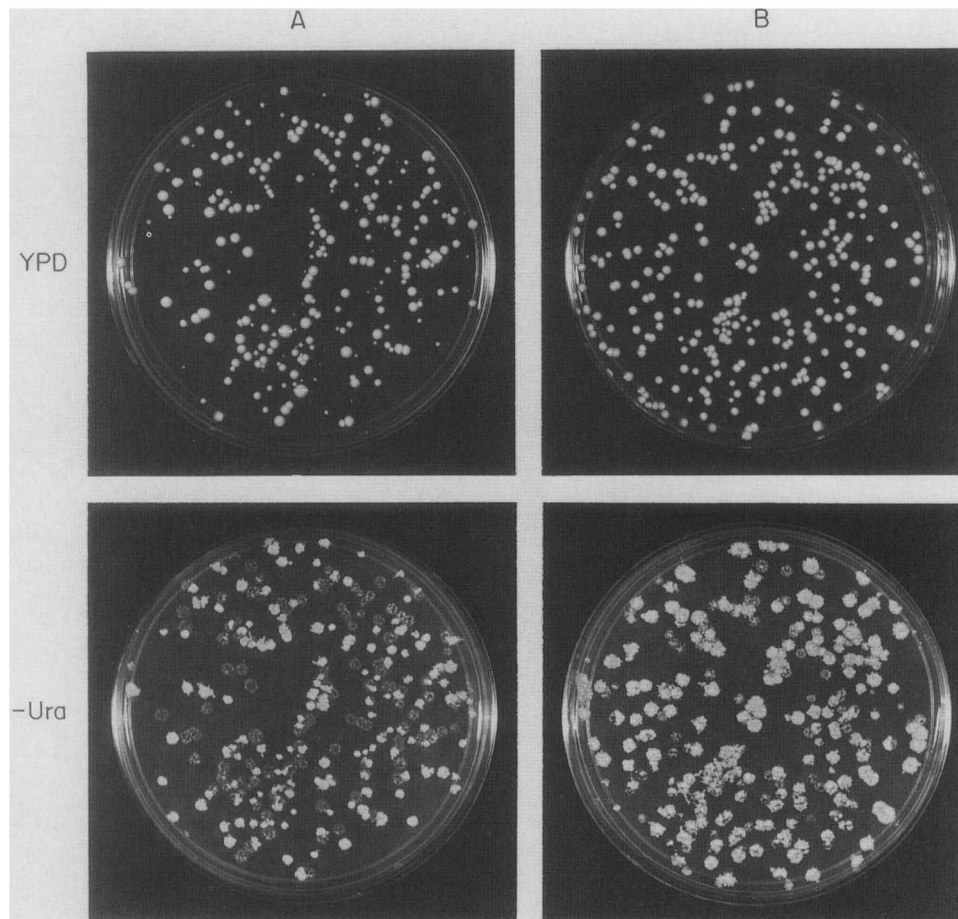
Figure 6. Suppression of the *sch9⁻* growth defect by *bcy1⁻*. The strain S25-31C (see Table 1), a *ura3⁻* strain that lacks both the *SCH9* gene and the gene for the regulatory subunit of the cAPK (*BCY1*), was transformed by multicopy plasmids carrying the *URA3* gene (*B*) or both the *URA3* and the *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 *Hind*III 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 *Hind*III 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 *Hind*III/*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 *Hind*III/*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 *Hind*III 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(*SCH9A9*), 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/*Hind*III fragment containing the complete coding sequence of *SCH9* was removed from pUC(*SCH9A9*) and treated with the large fragment of DNA polymerase. This fragment was then ligated into a *Hind*III cut, vector, pAD-1, which had been treated with the large fragment of DNA polymerase I. pAD-1 is a *LEU2-2*μ-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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