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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 cdc25a 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 CYRI 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 G1 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, cdc25a and cdc35a, which cause G1 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 CYRI, 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 cdc25a 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 the cAMP pathway. Overexpression of SCH9 suppresses the growth defects that result from loss of CDC25, both RAS genes, CYRI, 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 Yepl213 (Sherman et al. 1986; Toda et al. 1987a). Trans-
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 cdc25a 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 cdc25a strain TT25-6 (see Table 1) and examined for complementing activity (Fig. 1). The 6.5-kb HindIII fragment was shown to be capable of suppressing cdc25a, but the 3.0-kb PvuII 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 gene SCH9 would encode a protein of 824 amino acid residues. An in-frame stop codon appears 48 nucleotides upstream of that ATG. Disruption of this open reading frame would destroy 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

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### Table 1. Strain description

| Strain   | Genotype and derivation                                                                 | Source                                      |
|----------|----------------------------------------------------------------------------------------|---------------------------------------------|
| TT25-6   | Mata leu2 ura3 trp1 can1 cdc25-1                                                       | Broek et al. [1987]                         |
| SP1      | Mata his3 leu2 trp1 ade8 can1                                                          | Cold Spring Harbor Laboratory collection   |
| DC124    | Mata his4 leu2 trp1 ade8 can1                                                          | Cold Spring Harbor Laboratory collection   |
| TTSD-1   | A diploid strain formed by mating SP1 and DC124                                       | Broek et al. [1987]                         |
| TTA1-1   | Mata his3 leu2 trp1 ade8 cdc25::URA3, containing pCDC25(TRP1)-1                         | Broek et al. [1987]                         |
| KPPK-1   | Mata/Mata his3 his3 his3/ura3 ura3 trp1/trp1 ade8/+ + can1/+ ras1::HIS3/+ ras2::URA3/+  | Toda et al. (1985)                         |
| KPPK-1T  | A transformant of KPPK-1 with pTPK1-TRP1                                              | A segregant of tetrads from KPPK-1T         |
| SPK-3T   | Mata his3 leu2 ura3 trp1 ade8 can1 ras1::HIS3 ras2::URA3, containing pTPK1-TRP1         | Transformant of TTSD 1 with 2.3-kb BgIII fragment of pcyr1::URA3* |
| T158     | Mata/Mata his3/+ his4/+ leu2/leu2 ura3/ura3 trp1/trp1 ade8/ad8 can1/+ cyr1::URA3/+      | A segregant of tetrads from T158-T          |
| T158-T   | A transformant of T158 with pTPK1-TRP1                                                | A segregant of tetrads from T158-T          |
| T158-5AT | Mata his3 leu2 ura3 trp1 ade8 cyr1::URA3 containing pTPK1-TRP1                         | Toda et al. (1987a)                         |
| T168     | MATa/Mata his3 his3 his3/ura3 ura3 trp1/trp1 ade8/ad8 + tpk1::URA3/+ + tpk2::HIS3/+ + tpk3::TRP1/+ | A segregant of tetrads from T168-T          |
| T168-T   | A transformant of T168 with YRpTPK1-ADE8                                             | A segregant of tetrads from T168-T          |
| T168-6BT | Mata his3 leu2 ura3 trp1 ade8 tpk2::URA3 tpk3::TRP1 containing pTPK1-TRP1               | A transformant of TTSD 1 with the PvuII fragment of pcyr1::ADE8* |
| T198     | Mata/Mata his3/+ his4/+ leu2/leu2 ura3/ura3 trp1/trp1 ade8/ad8 can1/+ sch9::ADE8/+      | A segregant of tetrads from T198            |
| T198-8B  | Mata his3 leu2 ura3 trp1 ade8 sch9::ADE8                                               | A segregant of tetrads from TTSD 1          |
| TT152    | Mata his3 leu2 ura3 trp1 ade8                                                          | A diploid strain formed by crossing T198-8B and TT152 |
| T213     | MATa/ma his3 his3 leu2/ura3/ura3 trp1/trp1 ade8/ad8 sch9::ADE8/+                       | A segregant of tetrads from T213            |
| T213-4A  | Mata his3 leu2 ura3 trp1 ade8 sch9::ADE8                                               | S. Cameron et al. (in prep.)                |
| S13-3A   | MATa/ma his3 his3 leu2/ura3/ura3 trp1/trp1 ade8/ad8                                 | Diploid strain formed by crossing T198-8B and S13-3A |
| S25      | MATa/ma his3 his3 leu2/ura3/ura3 trp1/trp1 ade8/ad8                                 | Segregant from diploid strain S25          |
| S25-31C  | MATa his3 leu2 ura3 trp1 ade8 tpk2::HIS3 tpk3::TRP1 bcy1::LEU2/+ sch9::ADE8/+          | Segregant from diploid strain S25          |

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ATP-binding site (ZoUer et al. 1981; Kamps et al. 1984; found: Asp Phe Gly, shown with closed circles in Figure 2. Downstream of the ATP-binding site, two other protein kinase consensus sequences are triangles in Figure 2. The sequence of the 5CH9-gene product was used in a computer search of GenBank and the NBRF [PIR] database. As expected, significant homology was found between the 5CH9-gene product and the entire family of protein kinases. The 5CH9 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 5CH9 is closer to the cAPK catalytic domains than any other protein kinase we have encountered. Homologies with other protein kinases were also apparent. The 5CH9 protein has lower but very significant homology to the catalytic domains of protein kinase C and cGPK. The 5CH9 protein shows less homology to various other protein kinases. An alignment of the putative catalytic domain of 5CH9 with that of the bovine and yeast cAPKs is seen in Figure 3.

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 5CH9-gene product was used in a computer search of GenBank and the NBRF [PIR] database. As expected, significant homology was found between 5CH9 and the entire family of protein kinases. The 5CH9 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 5CH9 is closer to the cAPK catalytic domains than any other protein kinase we have encountered. Homologies with other protein kinases were also apparent. The 5CH9 protein has lower but very significant homology to the catalytic domains of protein kinase C and cGPK. The 5CH9 protein shows less homology to various other protein kinases. An alignment of the putative catalytic domain of 5CH9 with that of the bovine and yeast cAPKs is seen in Figure 3.

Based on these results, we presume that 5CH9 encodes a protein kinase. Like protein kinase C and cGPK, the 5CH9 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 5CH9 carried on a multicopy plasmid

A multicopy plasmid carrying the 5CH9 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 5CH9-gene product to the cAMP pathway, we tested the ability of a multicopy plasmid carrying 5CH9 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 TPK1 genes (T168-6BT). The strains were viable because they contained suppressor plasmids 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 5CH9 gene to replace, or "exchange," for the resident suppressor plasmid. The results are shown in Table 3. The multicopy plasmid containing the 5CH9 gene could exchange for the resident suppressor plasmid in all cases. Overexpression of the 5CH9-gene product therefore appears to compensate for disruption of all the components of the cAMP effector pathway we tested.

### Cells that overexpress 5CH9 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. 1987; Toda et al. 1987a). The above experiments suggest that the 5CH9-gene product has functions that overlap those of the yeast cAMP pathway. We therefore asked whether overexpression of 5CH9 results in a heat-shock-sensitive phenotype. For this purpose, we constructed a plasmid, YEPAH-SCH9, in which the 5CH9-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 YEPAH-SCH9, and independent transformants were tested for their ability to grow at 37°C.
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 BamH1/BglII ADE8 marker [White et al. 1985] into the BglII 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* 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 G1 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](image-url) Amino acid sequence comparison between SCH9 and cAPK catalytic subunits. A portion of the amino acid sequence of SCH9 is compared to that of TPK 1, one of three yeast genes that encode the catalytic subunits of the cAPK (Toda et al. 1987a), and the bovine Ca catalytic subunit [Shoji et al. 1981; Showers and Mauver 1986]. Identical amino acids are shadowed.

![Figure 2](image-url) Nucleotide sequence and deduced amino acid sequence of SCH9. The sequence starts at the HindIII site at -509 bp and continues through the second PvuII 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 2. Protein kinases homologous to SCH9

| Protein kinase | Source |
|---------------|--------|
| Yeast cAPK (TPK1) | Lisziewicz et al. (1987) |
| Bovine cAPK | Shoji et al. (1981) |
| Bovine cGPK | Takio et al. (1984) |
| Rat protein kinase C-type I | Knopf et al. (1986) |
| Rabbit muscle phosphorylase kinase | Reimann et al. (1984) |
| Yeast STE7 | Teague et al. (1986) |
| Yeast CDC28 | Lorincz et al. (1983) |

Figure 1. 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 Ca catalytic subunit (Shoji et al. 1981; Showers and Mauver 1986). Identical amino acids are shadowed.

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

| Viability of cells carrying extrachromosomal high copy gene* | Viability of cells with chromosomal genotype |
|-------------------------------------------------------------|--------------------------------------------|
| cdc25^-  | ras1^-  | ras2^-  | cyr1^-  | tpk1^-  | tpk2^-  | tpk3^-  |
| phi      | +       | -       | -       | NT      |
| CDC25    | +       | -       | -       | NT      |
| RAS2     | -       | +       | -       | NT      |
| RAS2Val19| +       | +       | -       | 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. TTA1-1, SPK-3T, T158-5AT, and T168-6BT were used for cdc25^-, ras1^- ras2^-, cyr1^-, and tpk1^- tpk2^- tpk3^-, respectively [Table 1]. * pCDC25 [LEU2]-2 [Broek et al. 1987], YEpRAS2-1 [Powers et al. 1984], pRAS2Val19 [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, phi, 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 RAS2Val19, 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,
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 alleles, which suppress all of the defects observed in strains lacking the Bcy1 gene (Cameron et al. 1988). bcy1- strains containing the tpk alleles (bcy1-tpk alleles) 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 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 lowercase letters, followed by a superscript minus sign, such as sch9-. A strain that has a temperature-sensitive recessive mutation is denoted 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 sequencing method (Sanger et al. 1977) using [a-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 PruvII fragment (see Fig. 1) was subcloned into the Smal site of pUC18 [Yanisch-Perron et al. 1985] in both orientations. BamHI and Sph1 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-

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

| Strain | Genotype | Doubling time [D] | Fraction un budded [F] | G1 | D - G1 |
|--------|----------|-----------------|-----------------|----|--------|
| T198-8B | sch9- | 207 | 0.67 | 122 | 85 |
| T213-4A | sch9- | 252 | 0.77 | 176 | 77 |

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 un budded cells (cells in G1) during exponential growth was determined by the microscopic examination of 200 cells. The duration of G1 was determined using the formula G1 = D[1 - log(2 - F]/log 2] [Rivin and Fangmann 1980]. The difference (D - G1) represents the duration of the cell cycle, excluding G1. Time values are in minutes.
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).

Isolation of genes that can suppress a temperature-sensitive $cdc25$

The temperature-sensitive $cdc25^{68}$ strain, TT25-6 (see Table 1), was transformed with a yeast genomic library that was constructed by inserting yeast DNA partially digested with HindIII 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 PKK-1T. PKK-1T was derived from the diploid PKK-1 [Toda et al. 1985] by transformation with \( pTPK1-TRPl \). PKK-1T is a diploid heterozygous at both of the \( RAS \) genes [Toda et al. 1985]. \( pTPK1-TRPl \) contains the 2.4-kb HindIII/SphI fragment of \( TPK1 \) [Toda et al. 1987a] and the 1.4-kb EcoRI fragment of \( ARSl-TRPl \) [Tschesnuker 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. (2) T158-5AT [see Table 1] is a tetrad segregant from a diploid T158-T derived from the diploid T158 by transformation with \( pTPK1-TRPl \). T158 is a diploid heterozygous at the \( CYR1 \) locus, which encodes adenylyl cyclase [Kataoka et al. 1985a]. The \( CYR1 \) gene was disrupted by using pcyr1 :: \( URA3 \), which was constructed as follows. The entire coding sequence of the \( CYR1 \) gene [Kataoka et al. 1985a] was removed by digesting pcyr1-2 [Kataoka et al. 1985a] with \( PvuII \) and \( BglII \). These enzymes cleave in the 5' and 3' flanking regions of the \( CYR1 \) gene, respectively. The \( PvuII \) to \( BglII \) region was replaced with the 1.0-kb HindIII/Smal fragment of \( URA3 \). A 2.3-kb \( BglII \) 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 \( pTPK1-ADE8 \). 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]. \( pTPK1-ADE8 \) was constructed by inserting the 3.4-kb BamHI/BgIII fragment of \( ADE8 \) [White et al. 1985] into the BamHI site of \( pTPK1-TRPl \). 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 genotypes: \( sch9^+ \) \( bcy1^- \) \( TPK1 \) \( tpk2^- \) \( tpk3^- \)] 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 \( psc9h :: ADE8 \), as follows. The 6.5-kb HindIII 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 BglIII site located in the middle of the coding region of \( SCH9 \) [see Fig. 1], and the 3.4-kb BamHI/BgIII fragment of \( ADE8 \) [White et al. 1985] was inserted, creating \( psc9h :: ADE8 \). The 6.4-kb PvuII fragment of \( psc9h :: ADE8 \), which contains \( ADE8 \) flanked by sequences of \( SCH9 \), was used for gene disruption by transforming a diploid TTSD1.

### 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 \( EcoRI \) site of \( pUC9 \) [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 \( YEpaDH-SCH9 \). A 6.4-kb EcoRI/HindIII 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 HindIII cut, vector, \( pAD-1 \), which had been treated with the large fragment of DNA polymerase I. \( pAD-1 \) is a LEU2-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, \( YEpaDH-SCH9 \), contains the \( SCH9 \) gene under the control of the \( ADH1 \) gene promoter.

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