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SDC25, a *CDC25*-Like gene Which Contains a RAS-Activating Domain and Is a Dispensable Gene of *Saccharomyces cerevisiae*

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In the yeast *Saccharomyces cerevisiae*, the *CDC25* gene product activates adenylate cyclase through *RAS1* and *RAS2* gene products. We have recently described the cloning of a DNA fragment which suppresses the *cdc25* mutation but not *ras1*, *ras2*, or *cdc35* mutations. This fragment contains a 5′-truncated open reading frame which shares 47% identity with the C-terminal part of the *CDC25* gene. We named the entire gene *SDC25*. In this paper, we report the cloning, sequencing, and characterization of the complete *SDC25* gene. The *SDC25* gene is located on the chromosome XII close to the centromere. It is transcribed into a 4-kb-long mRNA that contains an open reading frame of 1,251 codons. Homology with the *CDC25* gene extends in the N-terminal part, although the degree of similarity is lower than in the C-terminal part. In contrast with the C-terminal part, the complete *SDC25* gene was found not to suppress the *CDC25* gene defect. A deletion in the N-terminal part restored the suppressing activity, a result which suggests the existence of a regulatory domain. The *SDC25* gene was found to be dispensable for cell growth under usual conditions. No noticeable phenotype was found in the deleted strain.

In the yeast *Saccharomyces cerevisiae*, the *CDC25* gene product activates adenylate cyclase encoded by the *CYR1* gene (allelic to the *CDC35* gene) through *RAS1* and *RAS2* gene products (3, 5, 37). As a result of this activation, cyclic AMP (cAMP) stimulates the cAMP-dependent protein kinase (A kinase), whose regulatory subunit is encoded by the *BCY1* gene and whose catalytic subunit is encoded by the three interchangeable genes *TPK1*, *TPK2*, and *TPK3* (47, 48). This cAMP-dependent protein kinase pathway plays a key role in the nutritional control of the G1/G0 switch (2). The *RAS1* and *RAS2* genes of *S. cerevisiae* encode GDP- and GTP-binding proteins with an intrinsic GTPase activity and are closely related to the *ras* genes of higher eucaryotic organisms. By analogy to transducins G_s and G_i, RAS proteins are believed to be transducers which activate their effectors when bound to GTP but not when bound to GDP (43). In *S. cerevisiae*, biochemical evidence of the activity of the GTP-bound form as a positive effector of adenylate cyclase has been presented (15). The product of the *CDC25* gene is required for cAMP production (5). This effect is mediated by RAS proteins, as deduced from the existence of different mutations in the *RAS2* gene that suppress the *cdc25* mutations. One is a Gly → Val-19 substitution which mimics oncogenic variants and leads to a lower GTPase activity (25). The second is a spontaneous mutation selected as a suppressor of a *cdc25* mutation which corresponds to a Thr → Ile-152 substitution (4). This mutation leads to spontaneous GDP-GTP exchange by increasing the guanyl nucleotide exchange rate on RAS proteins (9). This finding suggests that the *CDC25* gene product is a positive regulator which acts upstream of RAS proteins, most likely as a GDP-GTP exchange factor.

We have recently described the cloning of a DNA fragment that suppresses the *cdc25* mutation but not the *ras1*, *ras2*, and *cdc35* mutations. This fragment codes for a 5′-truncated open reading frame (ORF) that shares 47% identity with the C-terminal part of the *CDC25* gene at the amino acid level. The product of this fragment has been expressed in *Escherichia coli*. Partially purified protein strongly enhances the release of GDP from the *S. cerevisiae* RAS2-GDP or c-Ha-*ras* p21-GDP complex and then promotes faster GDP-GTP exchange (10). We had previously named the corresponding gene *SCD25* (1), but since this name was already used, we changed *SCD25* to *SDC25*.

In this report, we describe the cloning of the N-terminal part of the *SDC25* gene and further characterize the complete gene. Homology with the *CDC25* gene is also present in the N-terminal part, although this homology is weaker than that found in the C-terminal part. In contrast with the C-terminal part, the complete gene on a multicopy plasmid did not suppress the *CDC25* gene defect, although it was transcribed and translated. The suppressing property was restored by a deletion in the N-terminal part. The physiological role of the *SDC25* gene product is discussed.

MATERIALS AND METHODS

Strains and media. The yeast strains used are described in Table 1. YEPD (1% yeast extract, 2% peptone, 2% glucose) and YNB minimal medium (0.17% Difco yeast nitrogen base without amino acids and ammonium sulfate, 0.5% ammonium sulfate, 2% glucose) were used for growth of *S. cerevisiae* strains. In minimal medium, auxotrophies were supplemented with L-leucine (250 μg/ml), L-histidine (100 μg/ml), L-tryptophan (100 μg/ml), uracil (50 μg/μl), and adenine (50 μg/ml). Sporulation medium contained 0.5% yeast extract, 0.5% Difco Bacto-Peptone, and 2% potassium acetate. Solid media contained 3% agar. Sporulation was performed on solid sporulation medium at 29°C from cells pregrown on YEPD.

E. coli DH1, DH5α, and JM109, were used for M13 cloning and for single-strand preparation for DNA sequencing (39).

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TABLE 1. Yeast strains

Strain	Genotype	Source or reference
FDL1.2A[a]	*MAT α ade2 his3 leu2 trp1 ura3 sdc25::HIS3*	This study
FDL1.5C[a]	*MAT***a** *ade2 his3 leu2 trp1 ura3 sdc25::HIS3*	This study
FDL31.21A[b]	*MAT***a** *ade his3 leu2 lys1 trp1 ura3 sdc25::HIS3 cdc25::HIS3*(YEp-RAS2$^{\text{ile-152}}$)	This study
FDL35.1A[c]	*MAT***a** *ade2 his3 leu2 trp1 sdc25::HIS3*	This study
GRF18	*MATα his3 leu2*	G. Fink collection
JCL300-3A[d]	*MAT***a** *ade leu2 lys1 ura3cdc25::HIS3*(YEp-RAS2$^{\text{ala-22}}$)	This study
OL136	*MAT***a** *cdc25-5 cdc35-10 his7 ura1 rca1 ICY1*	Our laboratory
OL501[e]	*MATα ade2 can1-100 trp1-1 leu2-3-112 his3-11,15 ura3 RAS2*$^{\text{Ala-22}}$*::URA3*	This study
OL971.11B	*MATα cdc25-5 his3 his7 leu2 ura3*	4
ppr1-Δ1	*MATα his3 ppr1-Δ1*	29
S150-2B	*MAT***a** *leu2-3,112 Δhis3 trp1-298 ura3-52*	11
SP1	*MAT***a** *ade8 leu2 his3 ura3 trp1 can1*	Gift from M. Wigler
T139-5A.6A	*MATα ade2 his3 leu2 lys1 trp1 can1 cdc25::HIS3*(YEp-*CDC25*)	Gift from M. Wigler
W303	*MAT***a** */MATα ade2-1/ade2-1 can1-100/can1-100 trp1-1/trp1-1 leu2-3,112/leu2-3,112 his3-11,15/his3-11,15 ura3/ura3*	33
W303-1B/D	*MATα ade2 can1-100 trp1-1 leu2-3,112 his3-11,15 ura3*	33

[a] Haploid segregant from the diploid FDL1 (see text).

[b] Haploid segregant from FDL1.2A × JCL300-3A cross. The presence in this strain of the two wild-type *HIS3* copies in the *SDC25* and *CDC25* loci was checked by analysis of the *HIS3* segregation in the progeny of the cross between this strain and W303-1B/D.

[c] Haploid segregant from FDL1.5C × GRF18 cross.

[d] Haploid segregant from SP1 × T139-5A.6A cross. Plasmid YEp-CDC25 was chased from this strain and replaced by plasmid YEp-RAS2$^{\text{ile-152}}$.

[e] Obtained by transformation of W303-1B/D with the integrative plasmid YIp-RAS2$^{\text{ala-22}}$ cut with *Cla*I in the *RAS2*$^{\text{Ala-22}}$ sequence (35).

Cell transformation. *E. coli* and yeast cell transformations were performed as previously described (22).

Vectors and genomic library. We used shuttle vectors YRp7 (50) and YEp352 (17), which harbor the *S. cerevisiae TRP1* and *URA3* genes, respectively. pLBO is a pUC18 (39) derivative that contains a 1.8-kb *Bam*HI DNA fragment carrying the *S. cerevisiae HIS3* gene (gift from M. Labouesse). YIp-RAS2$^{\text{ala-22}}$ contains the dominant allele *RAS2*$^{\text{Ala-22}}$ (35). p20V3/4 is a pBR322-derived plasmid that contains the 7-kb *Sal*I-*Pvu*II fragment of yeast DNA containing the entire *CDC25* gene.

Disruption of the *SDC25* gene. First we inserted the 0.52-kb *Eco*RI.1-*Bam*HI.1 fragment of pDLR (Fig. 1) into the multicloning sites of plasmid pTZ19R (Pharmacia), generating plasmid pEB3. Then plasmid pFD1 was constructed by inserting the 1.7-kb *Bam*HI.4-*Bgl*II.4 fragment of YRPSDC25a into the *Bam*HI site of pEB3 in the same orientation. The 1.8-kb *Bam*HI fragment of pLBO that encompasses the *HIS3* gene was cloned into the *Bam*HI site of pFD1, replacing the *Bam*HI.1-*Bam*HI.4 fragment of the *SDC25* gene (see Fig. 8) and generating plasmid pFD1.3. The *Eco*RI.1-*Eco*RI.2 DNA fragment of pFD1.3, containing the recombinant DNA, was purified and used to transform the homozygous *his3/his3* diploid strain (W303).

DNA preparations and analysis. Yeast genomic DNA for use in Southern blot analysis was prepared as previously described (5). Yeast plasmid DNA was prepared as described by Jacquet et al. (22). For analysis, DNA was digested with restriction enzymes and after electrophoresis was transferred to nylon membranes (Pall Biodyne) and hybridized by standard procedures (39).

Radioactive probes. DNA probes for Southern or Northern (RNA) blot hybridizations were purified by electroelution and Elutip-d (Schleicher & Schuell) and labeled by nick translation (36). For all labeling reactions, [α-^{32}P]dCTP (22.2 Bq/mmol; Amersham) was used.

DNA sequence analysis. DNA sequences were determined by the chain termination method (39), using an Amersham sequencing kit and [^{35}S]dATP (18.5 Bq/mmol; Amersham). The cyclone deletion method of Dale et al. (12) was used in some instances.

RNA preparations and analysis. Yeast RNA was prepared as previously described (5). Poly(A)$^+$ RNA was purified on oligo(dT)-cellulose (Pharmacia) as instructed by the supplier. For Northern blots, 10 μg of total or poly(A)$^+$ RNA was denaturated with glyoxal, electrophoresed on a horizontal agarose gel, and transferred to a nylon membrane (Pall Biodyne). Prehybridization and hybridization were performed in 3× SSC (SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7.6)–5× Denhart solution (39)–50% formamide–2 × 10^{-2} M sodium phosphate (pH 7.5)–0.1% sodium dodecyl sulfate (SDS)–10^{-3} M EDTA at 42°C. After 36 h of hybridization, membranes were washed two times for 15 min each time at room temperature in 2× SSC–0.1% SDS, followed by three 30-min washes at 58°C in 0.1× SSC–0.1% SDS.

S1 mapping of transcripts. The 679-bp *Sau*3AI-*Cla*I DNA fragment (see Fig. 4) was cloned into the *Bam*HI-*Cla*I sites of pUT332 plasmid (gift from G. Tiraby). This plasmid was designated pCS12. For use in S1 mapping, pCS12 was digested by *Cla*I and labeled at the 5′ end with [γ-^{32}P]dCTP (185 TBq/mmol) by polynucleotide kinase (39). Double-stranded DNA thus labeled was digested with *Eco*RI at the pUT332 *Eco*RI site located 20 bp upstream the *Bam*HI-*Sau*3AI site. The *Eco*RI-*Cla*I fragment labeled at the 5′ end of the *Cla*I site was purified by agarose gel electrophoresis and used to determine the 5′ terminus of the *SDC25* transcript. A 40-μg sample of RNA and 30 ng of the radiolabeled DNA fragment were used in each hybridization experiment to obtain a large molar excess of probe over the *SDC25* mRNA. RNA (40 μg) was hybridized at 47°C with the ^{32}P-labeled DNA probe (30 ng) as described previously (39). The mixture was treated with S1 nuclease (Sigma) for 30 min at 25°C. S1-protected fragments were separated by electrophoresis alongside G+A sequence ladders generated from the same labeled fragment by the Maxam-Gilbert technique (39).

Selection of protein-β-Gal fusions. The pRG3 plasmid was introduced by transformation into *E. coli* MC4100::(Mucts)::(MudIIPR13) (11). A mixed-phage stock was produced by thermoinduction of Mu *c*ts and used in a plasmid transduction experiment as described previously (11). Insertions of Mu dIIPR13 in pRG3 were selected on LB plates containing

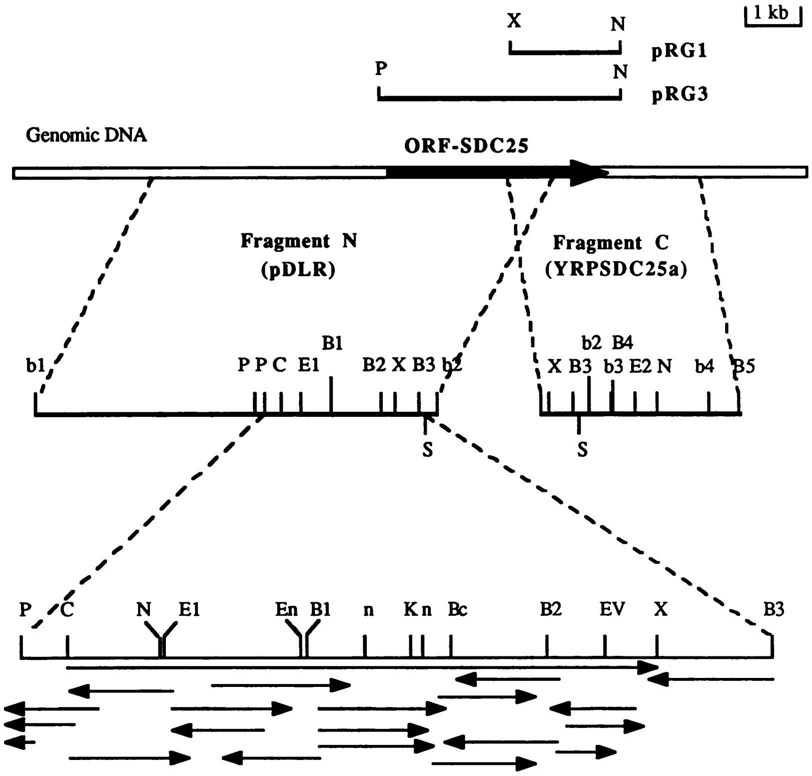

FIG. 1. Strategy for cloning and sequencing of the *SDC25* gene. The *SDC25* ORF is indicated by a large arrow in the genomic DNA. The restriction map of the two cloned DNA fragments N and C is represented. The C fragment has been cloned in the YRp7 vector to give plasmid YRPSDC25a (4). To isolate the N fragment inserted in YRp7 (plasmid pDLR), the *Xba*I-*Sma*I fragment from the C fragment was used as a probe. The two DNA fragments present in plasmids pRG1 and pRG3 are represented at the top. The sequencing strategy of the *Pst*I-*Bam*HI.3 part of N fragment is shown. The arrows indicate the portions actually sequenced. Restriction sites (numbered from the 5' end to the 3' end): B, *Bam*HI; b, *Bgl*II; C, *Cla*I; E, *Eco*RI; EV, *Eco*RV; En, *Eco*NI; K, *Kpn*I; N, *Nru*I; n, *Nde*I; P, *Pst*I; S, *Sma*I; X, *Xba*I.

ampicillin (50 μg/ml) and chloramphenicol (25 μg/ml). To select directly protein fusions expressed in yeast cells, plasmid DNA from a pool of 7,000 transductants was extracted and used to transform yeast strain S150-2B. Ura$^+$ transformants were replica plated on YEPD containing 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal; 40 μg/ml).

Nucleotide sequence accession number. The sequence reported has been assigned GenBank accession number M31771.

RESULTS

Cloning and sequencing strategy. The *SDC25* gene was cloned as two overlapping DNA fragments designated N and C (Fig. 1). Cloning and sequencing of the C fragment containing the C-terminal part of the ORF have already been described (1). Restriction mapping of the *SDC25* locus performed by Southern blot analysis suggested that the remaining part of the *SDC25* gene was included in the 7-kb *Bgl*II.1-*Bgl*II.2 fragment. This fragment was cloned by in situ colony hybridization of a minigenomic library of 6- to 9-kb *Bgl*II DNA fragments prepared from yeast strain OL136 and inserted in the YRp7 vector. The sequencing strategy for the N fragment is presented in Fig. 1. The 3' part of this N fragment overlaps 793 nucleotides of the previously published sequence (1). The sequence of 3,883 nucleotides starting 127 nucleotides before the first in-frame ATG and ending at the stop codon of the *SDC25* ORF is given in Fig. 2. This sequence contains a large ORF, starting at nucleotide +1 and ending at nucleotide +3,753. This sequence does not contain the canonical sequence TACTAAC for splicing of yeast introns (46). The calculated molecular mass for the gene product of 1,251 amino acids is 145 kDa. The complete gene was reconstructed on a plasmid (pRG3) as well as a truncated version containing the C-terminal domain (pRG1) (Fig. 1).

Genetic localization of the *SDC25* gene. The *SDC25* gene was localized on chromosome XII by hybridization (data not shown) to contour-clamped homogeneous electric field-separated chromosomes (6). This localization was confirmed by genetic crossing using the disrupted *SDC25*::*HIS3* allele (see below for construction of the disruption). From analysis of 112 tetrads from the cross **a** *ade2 his3 leu2 trp1 sdc25*::*HIS3* × α *his3 ppr1*-Δ*1* (**a** FDL35.1A × α *his3 ppr1*-Δ*1*), the *SDC25* gene was located 5.5 centimorgans (cM) from the chromosome XII centromere on the left arm (using *trp1* and *leu2* as centromeric markers) and 8.7 cM from the *PPR1* gene located on the right arm of the same chromosome at 3.2 cM from the centromere. The distance values are based on the following tetrad analysis data (parental ditype:nonparental ditype:tetratype): *trp1-ppr1*, 45:56:11; *trp1-sdc25*::*HIS3*, 46: 49:17; *sdc25*::*HIS3-ppr1*, 93:0:19; *leu2-sdc25*::*HIS3*, 33:60: 19; and *leu2-ppr1*, 37:59:16.

Expression of the *SDC25* gene. (i) Transcription studies. The RNA products of the *SDC25* gene were analyzed by Northern blotting (Fig. 3). An RNA of 4,000 nucleotides was

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                                                            CTGCAGG

 -120 CTCGCAAAATTTAAGGTTCCCTTCTACAATAGTAGTCAAAATTGCTTTTTTGCATATAAC

                       ↓               ↓           ↓
  -60 AAAGTGAAAAAAAAAAATATGAGAGACATATCTAAAAGACATATATAATCTGCCACCATA

    1 ATGAGTTGCACTGCGTCATATGCCGGCATGACAACTCCGGTGAAAGATAAGGAAGGCCAC
    1  M  S  C  T  A  S  Y  A  G  M  T  T  P  V  K  D  K  E  G  H

   61 GGGATTCCATGCTTACAACCTATCGATGTAGTGGAATGTACCTATCAATATTTTACAAAA
   21  G  I  P  C  L  Q  P  I  D  V  V  E  C  T  Y  Q  Y  F  T  K

  121 TCACGGAATAAACTGTCTTTAAGGGTAGGCGATTTGATTTACGTACTCACTAAAGGTTCT
   41  S  R  N  K  L  S  L  R  V  G  D  L  I  Y  V  L  T  K  G  S

  181 AATGGCTGGTGGGATGGTGTTCTTATCAGACACAGCGCTAATAATAATAATAATAATTCG
   61  N  G  W  W  D  G  V  L  I  R  H  S  A  N  N  N  N  N  N  S

  241 TTGATACTAGACAGAGGTTGGTTCCCCCCTTCTTTTACACGGTCCATTCTAAACGAACTA
   81  L  I  L  D  R  G  W  F  P  P  S  F  T  R  S  I  L  N  E  L

  301 CACGGGGTGCCTGACATCGGTAATGAATTGGAAATATTTCAAGCGGGTCTTAATCTTAAA
  101  H  G  V  P  D  I  G  N  E  L  E  I  F  Q  A  G  L  N  L  K

  361 CTGGAATTATCAAGCAACCCAGTGATCTTATCATTGGAAGACTTTTTAGACTGCTGTCGC
  121  L  E  L  S  S  N  P  V  I  L  S  L  E  D  F  L  D  C  C  R

  421 GATATTGAATTCAAGGAACAACTGGCTTGGTCACCTACTCCCGTCCACGAAAGGAAAGGC
  141  D  I  E  F  K  E  Q  L  A  W  S  P  T  P  V  H  E  R  K  G

  481 TGCTGTGAGCTGCTGTACTATAACCAGGATTTAGATGTTTATTGTCGCACGTTACCATAT
  161  C  C  E  L  L  Y  Y  N  Q  D  L  D  V  Y  C  R  T  L  P  Y

  541 TTACCACAAAATCAAGTTGAAACCGTGAACGACTATTCGTCTTTTCCTGCAATATCGAAG
  181  L  P  Q  N  Q  V  E  T  V  N  D  Y  S  S  F  P  A  I  S  K

  601 ATTGCTGGTAAAAAGATGCCTATAACGTCAAGCCCCGATCTGTTCTATCTCAATGATTGT
  201  I  A  G  K  K  M  P  I  T  S  S  P  D  L  F  Y  L  N  D  C

  661 GATGTCGTCTATTGGTATGACCTCACTCGCTTAGTGTGTCATTATGTTAATTTAACAGAG
  221  D  V  V  Y  W  Y  D  L  T  R  L  V  C  H  Y  V  N  L  T  E

  721 CGCGACCTATTGGCAAATGAACGGGAAAAGTTTCTAACTTCCTTGGATTTATTAACAGCT
  241  R  D  L  L  A  N  E  R  E  K  F  L  T  S  L  D  L  L  T  A

  781 CAAATAACCTATGTTTATATGCTTTTCAGGAATCTCCGTTTAGTTGAAGATAGTTTCAAA
  261  Q  I  T  Y  V  Y  M  L  F  R  N  L  R  L  V  E  D  S  F  K

  841 AAAACCCTCAAAAAACTAATTTACACCTTGTCTAGGTTTTCAATAAATGCAAATATTTGG
  281  K  T  L  K  K  L  I  Y  T  L  S  R  F  S  I  N  A  N  I  W

  901 TTTCATTCCACATCGTTTGAAGAAAGAGAAGCCATAGCCTCCCAGAAGGATCCAGAAAGA
  301  F  H  S  T  S  F  E  E  R  E  A  I  A  S  Q  K  D  P  E  R

  961 AGATCCCCTCTTCTACAGTCAATCCTAGGAACCTTCCAAAAATTTCATTTTCTACTGCGT
  321  R  S  P  L  L  Q  S  I  L  G  T  F  Q  K  F  H  F  L  L  R

 1021 CTACTACATTTCCTCTCAAATCCTAACGAACTTACAATACTGCCTCAATTGACTCCTCGA
  341  L  L  H  F  L  S  N  P  N  E  L  T  I  L  P  Q  L  T  P  R

 1081 TTTTTCAAGGATTCTTTCAATACAATTTCATGGAATAACCCGTTTTTGCGTACAGTCTTC
  361  F  F  K  D  S  F  N  T  I  S  W  N  N  P  F  L  R  T  V  F

 1141 AACCAGCATATGTCCATGACCTTACCGAGACAGATGATTAAAGCCGTTGCTGGCGCTTCA
  381  N  Q  H  M  S  M  T  L  P  R  Q  M  I  K  A  V  A  G  A  S

 1201 GGAATTGTTGCGGAAAATATTGATGAAATTCCAGCTTCCAAACAGGGCACTTTCATCTCG
  401  G  I  V  A  E  N  I  D  E  I  P  A  S  K  Q  G  T  F  I  S

 1261 TCAGAAACGTCTCACCATTCACCATCAGCCCCGTTTCAAAGAAGGAGAAGAGGTACCATT
  421  S  E  T  S  H  H  S  P  S  A  P  F  Q  R  R  R  R  G  T  I

 1321 TTCTCTAATGTGTCAGGAAGTTCCGATGAGTCTGACACCATATGGTCCAAAAGGAAAAAA
  441  F  S  N  V  S  G  S  S  D  E  S  D  T  I  W  S  K  R  K  K

 1381 CCATACCCGCTAAATGAAGAAACTCTAAGCCTTGTAAGGGCCAGGAAGAAGCAGCTTGAT
  461  P  Y  P  L  N  E  E  T  L  S  L  V  R  A  R  K  K  Q  L  D

 1441 GGTAAACTAAAACAAATGATCAAAAGTGCTAATGAATATCTCAGTAACACGGCTAATTTC
  481  G  K  L  K  Q  M  I  K  S  A  N  E  Y  L  S  N  T  A  N  F

 1501 AAAATGTTGAATTTTGAAATGAACTTCAAAACCTACGAAGAAGTAAGCGGAACAATTCCT
  501  K  M  L  N  F  E  M  N  F  K  T  Y  E  E  V  S  G  T  I  P

 1561 ATAATTGATATTCTGGAAAACCTAGATTTAACTATTTTTCTAAACTTGAGAGAGTTGGGA
  521  I  I  D  I  L  E  N  L  D  L  T  I  F  L  N  L  R  D  L  G

 1621 GATGAGAATAGAGTTTTTGACGAAGATGTCTTTGACGAAGATGTCGCTATTGGTGATGAA
  541  D  E  N  R  V  F  D  E  D  D  F  D  E  D  V  A  I  G  D  E

 1681 GATAAAGAGTTTTTGAAACACTCTTTATCATCCCTATCGTATATCTTATCCGACTATTTT
  561  D  K  E  F  L  K  H  S  L  S  S  L  S  Y  I  L  S  D  Y  F

 1741 AATATGAAGCAATATTTTCATGAATTGTCGCCCACGCATTTGACATTAGAGGATCCTTTC
  581  N  M  K  Q  Y  F  H  E  L  S  P  T  H  L  T  L  E  D  P  F

 1801 GTTTTCTCGCCAATGCAAAACGACTTGCCTACCGGTTATTATGAACCAATGAAACCTTCA
  601  V  F  S  P  M  Q  N  D  L  P  T  G  Y  Y  E  P  M  K  P  S

 1861 TCCTTGAATTTAGATAATGCCAAGGATAAGAAGAATGGGAGCCAAAATACTGATATCCAA
  621  S  L  N  L  D  N  A  K  D  K  K  N  G  S  Q  N  T  D  I  Q

 1921 GAGGAGGAAGATGAATATGAGCCAGACCCGGATAGTCTTATTCTCTTCCACAACCTCATC
  641  E  E  E  D  E  Y  E  P  D  P  D  S  L  I  L  F  H  N  L  I

 1981 AATCAAGATTCTGATTTCAATGATCTAAAGTTTTTTAATCTCGCCCACGTTTTTAAAAAA
  661  N  Q  D  S  D  F  N  D  L  K  F  F  N  L  A  H  V  F  K  K

 2041 TCCTGTGATGACTATTTTGATGTGCTTAAACTAGCCATTGAGTTCGTGAATCAATTAATT
  681  S  C  D  D  Y  F  D  V  L  K  L  A  I  E  F  V  N  Q  L  I

 2101 CTAGAAAGAGAGAATTTGTTAAATTATGCTGCTAGAATGATGAAAAACAATATCACGGAA
  701  L  E  R  E  N  L  L  N  Y  A  A  R  M  M  K  N  N  I  T  E

 2161 TTGCTATTGCGCGGGGAAGAAGGCTATGGGTCCTATGACGGCGGTGAAACTGCCGAAAAA
  721  L  L  L  R  G  E  E  G  Y  G  S  Y  D  G  G  E  T  A  E  K

 2221 AGTGACACGAATGCTGTTTATGCAGATTCAGATACTAAAGACAATGACGAATGGCGTGAC
  741  S  D  T  N  A  V  Y  A  D  S  D  T  K  D  N  D  E  W  R  D

 2281 AGCCAAGTCAAATTACCGAGGTATTTGCAGCGCGAGTATGACAGTGAACTGATTTGGGGC
  761  S  Q  V  K  L  P  R  Y  L  Q  R  E  Y  D  S  E  L  I  W  G

 2341 TCTAACAATAGGATTAAAGGTGGTTCTAAACACGCACTGATCTCTTACTTGACAGATAAT
  781  S  N  N  R  I  K  G  G  S  K  H  A  L  I  S  Y  L  T  D  N

 2401 GAAAAGAAGGACCTATTTTTCAATATTACTTTTTTAATCACTTTCAGAAGCATCTTTACT
  801  E  K  K  D  K  F  F  N  I  T  F  L  I  T  F  R  S  I  F  T

 2461 ACAACGGAGTTTTTAAGCTACTTGATCTCGCAATATAATTTGGATCCACCAGAGGATTTG
  821  T  T  E  F  L  S  Y  L  I  S  Q  Y  N  L  D  P  P  E  D  L

 2521 TGCTTTGAAGAATACAATGAATGGGTGACGAAAAAGCTTATACCGGTTAAATGTAGGGTG
  841  C  F  E  E  Y  N  E  W  V  T  K  K  L  I  P  V  K  C  R  V

 2581 GTTGAGATTATGACAACCTTTTTCAAGCAATATTGGTTCCCGGGCTATGATGAGCCCGAT
  861  V  E  I  M  T  T  F  F  K  Q  Y  W  F  P  G  Y  D  E  P  D

 2641 CTTGCGACCCTAAATCTGGATTATTTTGCGCAAGTAGCAATCAAGGAAAATATAACAGGA
  881  L  A  T  L  N  L  D  Y  F  A  Q  V  A  I  K  E  N  I  T  G

 2701 TCTGTGGAATTACTAAAGGAGGTCAATCAGAAGTTTAAACTAGGTAATATACAAGAAGCG
  901  S  V  E  L  L  K  E  V  N  Q  K  F  K  L  G  N  I  Q  E  A

 2761 ACTGCACCAATGAAAACGTTAGATCAACAGATCTGCCAGGACCATTACTCGGGCACTTTA
  921  T  A  P  M  K  T  L  D  Q  Q  I  C  Q  D  H  Y  S  G  T  L

 2821 TACTCTACCACGGAATCCATTTTGGCCGTCGATCCAGTTTTATTTGCCACTCAATTAACG
  941  Y  S  T  T  E  S  I  L  A  V  D  P  V  L  F  A  T  Q  L  T

 2881 ATACTAGAGCATGAAATTTATTGTGAGATAACCATTTTTGATTGTTTGCAAAAAATTTGG
  961  I  L  E  H  E  I  Y  C  E  I  T  I  F  D  C  L  Q  K  I  W

 2941 AAGAACAAGTATACAAAATCGTATGGGGCTTCACCGGGTTTGAACGAGTTTATCAGTTTT
  981  K  N  K  Y  T  K  S  Y  G  A  S  P  G  L  N  E  F  I  S  F

 3001 GCCAATAAACTGACAAATTTCATATCCTACTCTGTTGTAAAGGAGGCTGATAAAAGTAAG
 1001  A  N  K  L  T  N  F  I  S  Y  S  V  V  K  E  A  D  K  S  K

 3061 CGGGCCAAGCTACTCTCTCATTTTATTTTTATCGCAGAATATTGTAGGAAATTCAATAAC
 1021  R  A  K  L  L  S  H  F  I  F  I  A  E  Y  C  R  K  F  N  N

 3121 TTTTCTTCCATGACTGACATCATTTCAGCATTATATTCTTCACCAATTTATCGTTTAGAG
 1041  F  S  S  M  T  D  I  I  S  A  L  Y  S  S  P  I  Y  R  L  E

 3181 AAAACCTGGCAGGCAGTTATTCCTCAAACGAGAGATCTATTGCAGTCACTGAACAAGTTG
 1061  K  T  W  Q  A  V  I  P  Q  T  R  D  L  L  Q  S  L  N  K  L

 3241 ATGGATCCCAAGAAAAATTTCATAAATTACAGAAACGAGCTGAAGTCTTTACATAGCGCT
 1081  M  D  P  K  K  N  F  I  N  Y  R  N  E  L  K  S  L  H  S  A

 3301 CCCTGCGTACCGTTTTTCGGCGTTTATTTATCTGATCTAACCTTTACTGATTCCGGAAAT
 1101  P  C  V  P  F  F  G  V  Y  L  S  D  L  T  F  T  D  S  G  N

 3361 CCGGATTATCTTGTCTTGGAACATGGTTTAAAGGGTGTCCATGATGAGAAGAAATATATA
 1121  P  D  Y  L  V  L  E  H  G  L  K  G  V  H  D  E  K  K  Y  I

 3421 AACTTCAACAAAAGGAGCAGACTTGTTGATATCTTACAAGAGATCATATATTTCAAGAAA
 1141  N  F  N  K  R  S  R  L  V  D  I  L  Q  E  I  I  Y  F  K  K

 3481 ACACATTATGATTTCACTAAAGATCGGACGGTAATTGAATGTATATCAAATTCATTGGAA
 1161  T  H  Y  D  F  T  K  D  R  T  V  I  E  C  I  S  N  S  L  E

 3541 AACATCCCCCATATTGAGAAACAATACCAATTATCATTAATTATTGAACCAAAACCAAGA
 1181  N  I  P  H  I  E  K  Q  Y  Q  L  S  L  I  I  E  P  K  P  R

 3601 AAGAAAGTCGTTCCGAATTCCAATTCGAATAATAAATCACAAGAAAAATCCAGGGATGAC
 1201  K  K  V  V  P  N  S  N  S  N  N  K  S  Q  E  K  S  R  D  D

 3661 CAAACCGATGAAGGAAAAACATCCACTAAGAAAGACAGATTTCCAAAATTTCAATTACAT
 1221  Q  T  D  E  G  K  T  S  T  K  K  D  R  F  P  K  F  Q  L  H

 3721 AAGACAAAGAAAAAAGCTCCCAAGGTTTCTAAGTAA
 1241  K  T  K  K  K  A  P  K  V  S  K  *
```

FIG. 2. Nucleotide sequence of the *SDC25* gene. The deduced amino acid sequence of the *SDC25* ORF is indicated under the DNA sequence. At the 5′ end, arrows indicate transcriptional start points. The asterisk indicates the translational stop codon. Six corrections on the first sequence already described (1) have been made: one addition A at position 2038, the deletion of one T between positions 2072 and 2073, the addition of the triplet GCC at position 2212, and the replacement of one C by one T at position 2915.

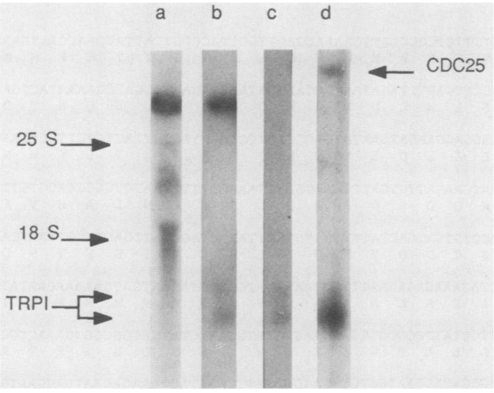

FIG. 3. Northern blot analysis of the *SDC25* transcript. RNA was extracted from cells grown on minimal medium supplemented with the required amino acids. A 10-μg sample of total RNA from strain OL971.11B containing pRG3 (lane a) or 10 μg of poly(A)$^+$ RNA from strain OL971.11B (lane b) and the *sdc25*::*HIS3* disrupted strain FDL1.2A (lanes c and d) were electrophoresed on a 1% agarose gel. This material was transferred to a Pall Biodyne nylon membrane and hybridized to the labeled *SDC25* DNA fragment (0.8-kb *Bam*HI.3-*Bam*HI.4) (lanes a to c) and to the labeled *CDC25* fragment (1.7-kb *Bgl*II fragment from plasmid p20V3/4) (lane d). Labeled *TRP1* DNA (1.45-kb *Eco*RI fragment from YRp7) was added as a control in these hybridization experiments. Positions of 25S and 18S rRNAs determined by ethidium bromide staining and of *CDC25* and *TRP1* transcripts are indicated. Exposure times were 1 day for lane a, 3 days for lanes b and c, and 7 days for lane d.

detected in the wild-type strain (lane b). This RNA was absent in a strain that contains the *sdc25*::*HIS3* disrupted allele (see below for its construction) (lane c). The *CDC25* DNA used as a probe hybridized with an RNA of 5,200 nucleotides (lane d), as previously described (5). Under the stringent conditions of hybridization used here, no cross-hybridization was observed between the *SDC25* and *CDC25* transcripts. Since the specific radioactivity of the three probes used in this experiment was of the same order of magnitude (6×10^8 dpm/μg of nucleotide), quantitative comparison can be done. The *TRP1* transcripts were used as internal standards (27) to compare the relative amounts of the *SDC25* and *CDC25* mRNAs. The *SDC25* transcripts were approximately three times more abundant than the *TRP1* transcripts (lane b), whereas the *CDC25* transcripts were at least three times less abundant than *TRP1* RNAs (lane d). Therefore, the *SDC25* mRNA can be estimated to be 10-fold more abundant than the *CDC25* mRNA.

The 5′ ends were determined by S1 mapping, using the *Sau*3AI-*Cla*I 5′-labeled fragment overlapping the start of the ORF with 594 bp of the upstream region of the *SDC25* gene and 85 bp of the coding region (Fig. 4). Three major fragments, corresponding to 5′ ends at positions −42, −27, and −15, were protected from S1 nuclease digestion. Minor bands corresponding to positions −41, −26, −14, and −13 were also visible. RNA starting at position −42 was the most abundant (Fig. 4).

(ii) Expression of the complete *SDC25* gene reconstructed on a plasmid. The complete gene was reconstructed in the multicopy plasmid YEp352, yielding the recombinant plasmid pRG3 (Fig. 1). Northern blot analysis and 5′-end determination were performed on RNA extracted from cells transformed with pRG3. The *SDC25* mRNA was much more abundant in this transformed strain than in the wild-type strain, as indicated by the results of the nuclease S1 mapping experiment (Fig. 4B). The RNA size and 5′ ends were the same as in the wild-type strain (Fig. 3 and 4). Therefore, the *SDC25* gene is actively transcribed from its own promoter in plasmid pRG3.

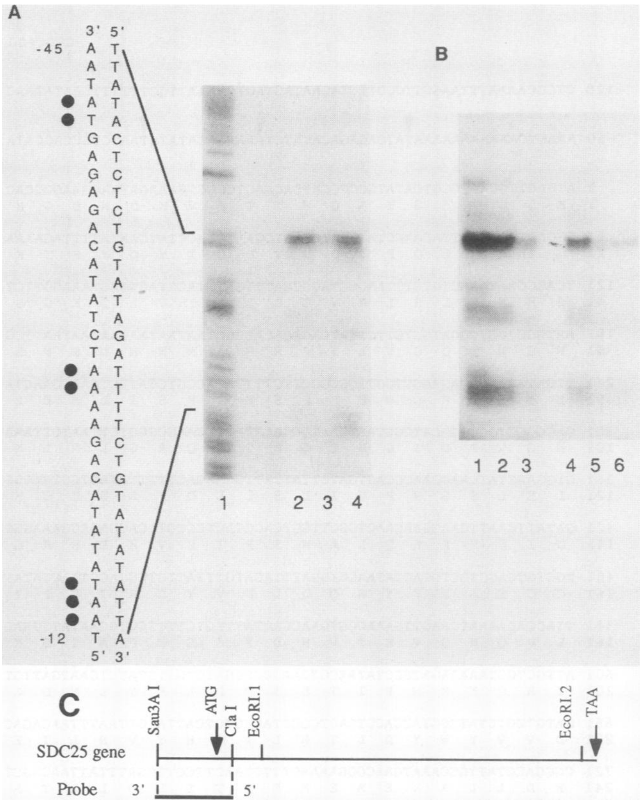

FIG. 4. Determination of *SDC25* 5′ ends. S1 mapping of the *SDC25* transcription start point was performed as indicated in Materials and Methods, using as a probe the *Sau*3AI-*Cla*I DNA fragment shown in panel C. (A) Lane 1 shows the sequence ladders (A+G) derived from the *Sau*3AI-*Cla*I labeled fragment. Hybridization mixtures containing 40 μg of poly(A)$^+$ RNA from strain OL971.11B grown on minimal selective medium were submitted to 100 (lane 2), 200 (lane 3), and 400 (lane 4) U, respectively, of S1 nuclease per ml. Circles on the sequence indicate the deduced transcriptional start points, taking into account the 1.5-bp discrepancy in the migration rate versus the normal sequence (41). (B) Hybridization mixtures containing 40 μg of total RNA from OL971.11B transformed with pRG3 (lanes 1 to 3) and 40 μg of poly(A)$^+$ RNA from strain OL971.11B (lanes 4 to 6). A 100-U/ml concentration of S1 nuclease was used in lanes 1 and 4. In this case, 1,500 cpm from the 50,000 cpm added with the probe was protected from nuclease S1 digestion by poly(A)$^+$ RNA from OL971.11B (lane 4), compared with 12,000 cpm in the case of total RNA from OL971.11B transformed with pRG3 (lane 1). S1 nuclease was used at 200 U/ml in lanes 2 and 5 and 400 U/ml in lanes 3 and 6. In lanes 1 to 3, 1/10 of the material was loaded; in lanes 4 to 6, all of the material was loaded.

To test the ability of the *SDC25* transcript to be translated from plasmid pRG3, SDC25–β-galactosidase (βGal) protein fusions were selected. We used the defective mini-Mu phage Mu dIIPR13, which is capable of producing in vivo protein-βGal fusions when the defective phage is inserted in frame into an ORF (11). A collection of pRG3 plasmids containing Mu dIIPR13 was produced in *E. coli*. The plasmids containing protein fusions were directly selected in yeast cells by their ability to hydrolyze X-Gal. Among 8,000 yeast Ura$^+$

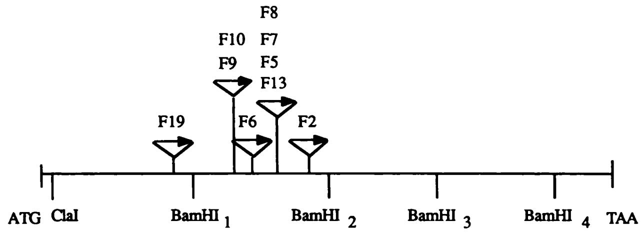

FIG. 5. *SDC25*-βGal fusion. Localization and orientation of mini-Mu phage Mu dIIPR13 insertions in the *SDC25* ORF were determined by *Cla*I and *Bam*HI restriction analysis, owing to the presence of the unique *Bam*HI site in the mini-Mu phage Mu dIIPR13 at 120 bp from the 5′ end and the unique *Cla*I site at 950 bp from the 5′ end. Insertions are drawn as triangles above the map; arrows indicate the orientation of the *lacZ* gene.

transformants, 25 were capable of hydrolyzing X-Gal. Plasmid DNA was extracted from 11 transformed yeast clones and amplified in *E. coli*. Mini-Mu insertions were found to be located within the *SDC25* ORF in 9 of 11 plasmids analyzed by restriction mapping. All were in the same orientation as the *SDC25* ORF (Fig. 5). In the case of fusion F5, we confirmed by nucleotide sequence determination that the fusion had occurred in frame at position 1339. These results demonstrate that the *SDC25* ORF present in pRG3 can be translated in yeast cells.

***cdc25*-suppressing properties of the *SDC25* gene. (i) Effect of the complete gene cloned on a multicopy plasmid.** Since the C domain is able to suppress the *cdc25-5* mutation, we tested the *cdc25-5*-suppressing ability of the complete gene present in pRG3. In contrast to plasmid pRG1, which contains the C-terminal part (Fig. 1), plasmid pRG3 did not suppress the thermosensitivity of the *cdc25-5* strain (Fig. 6). However, the amount of *SDC25* mRNA transcribed from plasmid pRG3 was high (see above) and similar to that of the *SDC25* mRNA transcribed from plasmid pRG1 (data not shown).

(ii) Activity of the C domain of *SDC25* on *RAS*. The C-terminal part on plasmid pRG1 not only suppressed *cdc25-5* thermosensitivity but also led to associated phenotypes: lack of glycogen accumulation (as shown by the lack of iodine staining), heat shock sensitivity (Fig. 6), and a sporulation defect in diploid strains. These phenotypes were observed not only in strain OL971.11B but also in other strains containing a wild-type *CDC25* allele (data not shown). This pleiotropic phenotype, also described as associated with $RAS2^{\text{Val-19}}$ (25) and *bcy1* (30) mutations, is characteristic of deregulated adenylate cyclase and protein kinase A activities. None of these phenotypes are observed in the presence of the complete *SDC25* gene on plasmid pRG3.

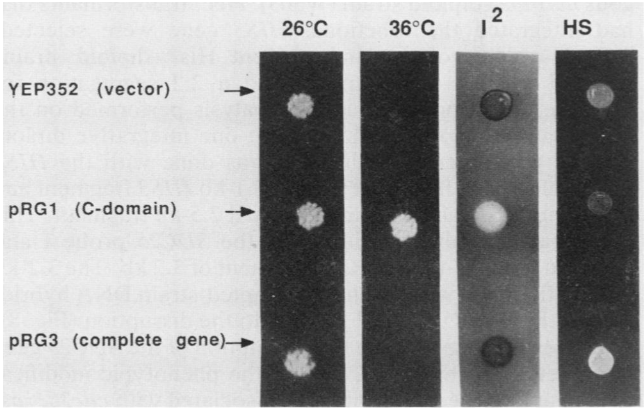

FIG. 6. Phenotypes associated with the truncated and complete *SDC25* gene. Strain OL971.11B (*cdc25-5 ura3*) was transformed with plasmids YEp352 (control), pRG1, and pRG3. The Ura$^+$ transformants were tested for the ability to grow on selective minimal medium at 26 and 36°C. Iodine staining was done by pouring a solution of 0.2% I_2–0.4% KI on cell patches after 2 days of growth on selective medium at 26°C. To test heat shock sensitivity (HS), cells grown in selective medium until stationary phase were diluted to 10^7 cells per ml, incubated at 52°C for 18 min, spotted on selective medium, and grown at 26°C.

The Ala-22 (35) mutation in the *RAS2* gene leads to a dominant thermosensitive phenotype. This block can be overcome by overexpression of *CDC25* in the presence of a wild-type *RAS* gene. This result has been interpreted as a trapping of the *CDC25* gene product by the $\text{RAS2}^{\text{Ala-22}}$ protein. If the SDC25 C domain performs the same biochemical function as the *CDC25* gene product, then it should be capable of suppressing the growth defect due to the $RAS2^{\text{Ala-22}}$ mutation at the restrictive temperature in the presence of a wild-type *RAS* gene. To perform this experiment, we constructed strain OL501, containing the $RAS2^{\text{Ala-22}}$ allele, by integrative transformation with the $\text{YIp-RAS2}^{\text{ala-22}}$ integrative vector (Table 1). This strain is thermosensitive for growth. This thermosensitivity was reversed by the SDC25 C domain present on plasmid YRPSDC25a. In a control experiment, we checked that a plasmid containing the CDC25 C domain was also capable of suppression whereas the YRp7 vector was not. These results indicate that the SDC25 C domain can replace the *CDC25* gene product in activating the adenylate cyclase through functional wild-type RAS1 or RAS2 protein (integration of $RAS2^{\text{Ala-22}}$ leads to duplication of the wild-type *RAS2* gene).

(iii) The N-terminal part of *SDC25* inhibits the C domain. To investigate the role of the region within the N-terminal part of the *SDC25* gene which prevents the *SDC25* gene product from suppressing the *cdc25-5* mutation, a set of deletions obtained by BAL 31 digestion starting at the *Eco*NI site was screened for the ability to suppress the *cdc25-5* thermosensitive mutation. Plasmid pRG3-9, which contains a deletion of 1,032 bp (Fig. 7), was purified from a thermoresistant transformant. We confirmed by nucleotide sequence determination that the deletion result in an in-frame junction between amino acids at positions 263 and 608. To ensure that activation of the suppressing activity of *SDC25* was due to this deletion, the 2-kb *Cla*I-*Xba*I fragment was replaced by the deleted 1-kb *Cla*I-*Xba*I fragment to create plasmid pRG3-9*. To differentiate it from plasmid pRG3-9, pRG3-9* was constructed by inserting the deleted fragment in pRG3*, in which we have destroyed the *Pst*I site. Both pRG3-9* and pRG3-9 suppressed the thermosensitivity of OL971.11B (Fig. 7) and led to a lack of glycogen accumulation (data not shown), as did plasmid pRG1. Thus, a deletion within the *Cla*I-*Xba*I fragment in the N-terminal part of the *SDC25* ORF is able to activate the *SDC25* gene product to suppress the *CDC25* gene defect. Two other deletions within the *SDC25* coding region were made by restriction enzyme deletion. The first deletion lacks the *Nru*I-*Sma*I fragment that codes for 734 amino acids (plasmid pRG4) and leads to a junction between amino acids at positions 140 and 874; the second lacks a *Hin*dIII fragment containing the *SDC25* promoter and coding for the 853 N-terminal amino acids (plasmid pRG5) (Fig. 7). These two deleted plasmids, in contrast to pRG3-9, failed to suppress the *cdc25-5* ther-

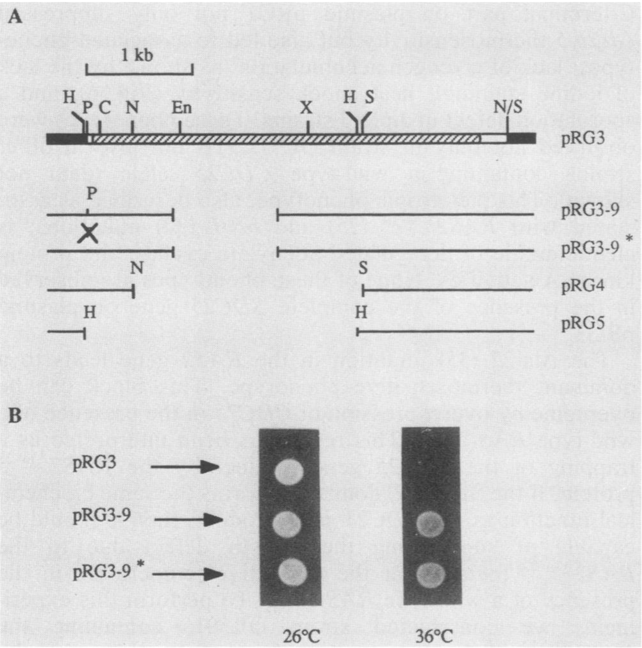

FIG. 7. Deletion within the N-terminal part of the *SDC25* ORF which activates *cdc25*-suppressing activity. (A) Constructs used. At the top is shown a restriction map of part of plasmid pRG3, including the *Pst*I-*Nru*I insert. Symbols: ■, vector sequences; ▨, *SDC25* ORF. Below, the deletion within the *SDC25* coding sequence present on deleted plasmids is represented by the interrupted lines. Plasmid pRG3-9 was constructed in the following way. Plasmid pRG3 was linearized at its unique *Eco*NI site and incubated with nuclease BAL 31 (0.5 U/μg of DNA). Aliquots were taken after 0, 3, 6, 9, 15, and 18 min, and the reaction was stopped by phenol extraction. After treatment with the Klenow fragment of DNA polymerase I and T4 DNA ligase, the ligation product was used to transform yeast strain OL971.11B (*cdc25-5 ura3*). Prototrophic Ura$^+$ transformants were isolated at the permissive temperature, replica plated, and incubated at the restrictive temperature of 36°C. Plasmid pRG3-9 was recovered from one of the thermoresistant transformant strains by passage in *E. coli*, and the extent of the deletion in this plasmid was determined by restriction mapping. Plasmid pRG3-9* was constructed in the following way. First, the unique *Pst*I site in pRG3 situated at the junction between the YEp352 vector and the insert was destroyed by digesting this plasmid with *Pst*I, removing the resultant protruding nucleotides with T4 DNA polymerase, and recircularizing by using T4 DNA ligase. Second, the 2-kb *Cla*I-*Xba*I fragment of this plasmid was replaced by the deleted 1-kb *Cla*I-*Xba*I fragment of pRG3-9, generating plasmid pRG3-9*. The deletions present in pRG4 and pRG5 result from the ligation of pRG3 previously digested by *Nru*I-*Sma*I and *Hin*dIII, respectively. (B) Ability of the *SDC25* deletion to rescue the temperature-sensitive *cdc25* allele. Strain OL971.11B (*cdc25-5 ura3*) was transformed with plasmids pRG3, pRG3-9, and pRG3-9*. The Ura$^+$ transformants were tested for the ability to grow on selective medium at 26 and 36°C.

mosensitive mutation, which indicates that not every deletion leads to activation of the *SDC25* gene product to suppress the *cdc25* mutation.

Disruption of the *SDC25* gene and associated phenotypes. In our search for a function, we disrupted the *SDC25* gene by the one-step gene disruption procedure (38), The internal *Bam*HI.1-*Bam*HI.4 fragment of the *SDC25* gene was removed and replaced by the *HIS3* gene (Fig. 8). The *Eco*RI.1-*Eco*RI.2 fragment containing the recombinant DNA (see Materials and Methods) was used to transform the homozygous *his3*/*his3* diploid strain (W303). His$^+$ transformants that had integrated the functional *HIS3* gene were selected. Tetrad analysis of five independent His$^+$ diploid strains resulted in four viable spores and a 2:2 segregation for histidine prototrophy. Southern analysis performed on the four haploid meiotic products from one integrative diploid showed that when hybridization was done with the *HIS3* probe, all strains had the resident 10.1-kb *HIS3* fragment and the disrupted strains had an additional 2.5-kb fragment. This latter fragment also hybridized to the *SDC25* probe 1 and replaced the wild-type *SDC25* fragment of 3.2 kb. The 3.2-kb *SDC25* fragment was absent in disrupted-strain DNA hybridized to the *SDC25* probe 2 internal to the disruption (Fig. 8). These results demonstrate that disruption of the *SDC25* gene is not lethal for the cell. None of the phenotypic modifications that have been described as associated with *cdc25*, *ras*, and *cdc35* mutations (20, 25, 45) were observed in the *sdc25*::*HIS3* disrupted strain: the cAMP level was the same as in the wild-type strain; no significant difference in glycogen accumulation, tested either by iodine staining or by measurement of the intracellular glycogen, was observed between disrupted and wild-type strains; and growth was not altered on glycerol medium. Other phenotypes, such as generation time on fermentable and nonfermentable carbon sources, cellular density in stationary phase, efficiency of

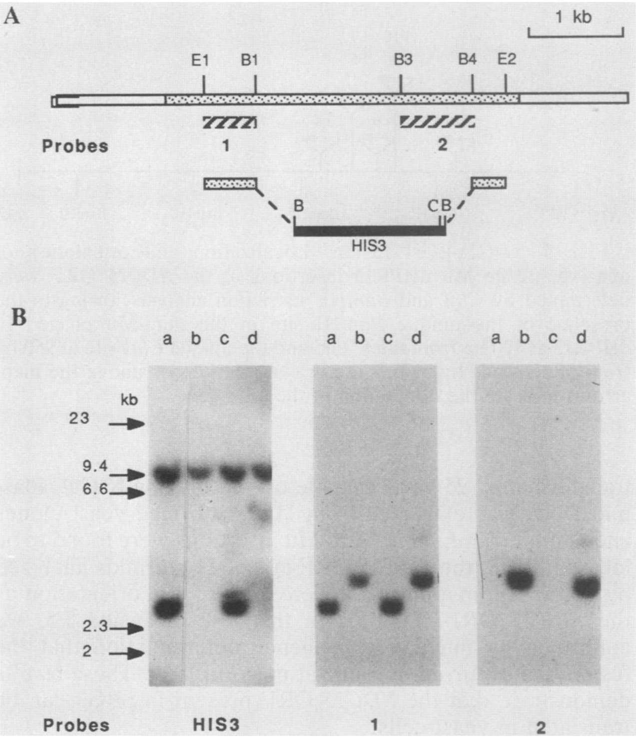

FIG. 8. Disruption of the *SDC25* gene. (A) Schematic representation of the construction of the disrupted gene (see text). Symbols: ▦, *SDC25* ORF; ■, *HIS3* DNA fragment; ▨, *SDC25* probe. (B) Southern analysis of four meiotic products of the His$^+$ integrative diploid FDL1. Lanes: a and c, His$^+$ spores (FDL1.2A and FDL1.2C); b and d, His$^-$ spores (FDL1.2B and FDL1.2D). Genomic DNA was extracted, digested with *Eco*RI, and subjected to agarose gel electrophoresis. The DNA was transferred to a nylon membrane (Pall Biodyne) and hybridized with a ^{32}P-labeled *HIS3* probe (1.6-kb *Bam*HI fragment from plasmid pLBO) or *SDC25* probes 1 (*Eco*RI.1-*Bam*HI.1) and 2 (*Bam*HI.3-*Bam*HI.4). Standard DNA fragment sizes are shown on the left side.

sporulation, efficiency of conjugation, cryosensitivity and thermosensitivity, and secretion by measurement of the secreted invertase, were tested, and no significant differences from the wild-type strain were noticed.

A second disruption, consisting of replacement of the *Bgl*II.2-*Bgl*II.3 fragment by the *HIS3* gene, was also made. The same results were obtained.

To test the possibility that the wild-type *CDC25* gene suppresses the defect of the *SDC25* disruption in a pathway different from that for cAMP production, we constructed a double disruptant, *sdc25*::*HIS3 cdc25*::*HIS3* (FDL31.21A). This strain was viable in presence of the allele $RAS2^{\mathrm{Ile}\text{-}152}$, which rescues the cAMP defect (4). Thus, a possible essential role of *SDC25* which can be rescued by *CDC25* seems to be excluded. However, we cannot exclude the possibility that *SDC25* is an activator of RAS in a function other than the activation of adenylate cyclase, and use of the activated allele $RAS2^{\mathrm{Ile}\text{-}152}$ would overcome the requirement for *SDC25* or *CDC25*. To test possible physiological interrelationships between *CDC25* and *SDC25* gene products, we compared the thermosensitivity of the double mutant *sdc25*::*HIS3 cdc25-5* with that of the *cdc25-5* mutant. Three strains of each genotype were grown at 26, 29, 33, and 36°C on glucose medium and glycerol medium. No difference in thermosensitivity was observed.

DISCUSSION

In this report, we present the cloning, sequencing, and characterization of the complete *SDC25* gene. This gene was first identified because a DNA fragment present on a plasmid and containing its 3′ 584 codons is capable of suppressing *cdc25* mutations. The N-terminal part has been cloned, and the complete *SDC25* gene has been reconstituted and sequenced. *SDC25* contains an ORF of 1,251 codons. The putative gene product would have a molecular mass of 145 kDa. The *SDC25* gene does not contain the canonical sequence TACTAAC conserved in yeast introns (46) and therefore is likely to be unspliced. Only one sequence similar to the consensus TATA box was found: 5′-ATATAA-3′ (positions −67 to −62). The predicted amino acid sequence of *SDC25* presents one putative phosphorylation site for the cAMP-dependent protein kinase (position 439) (8). The codon adaptation index, which measures the synonymous codon usage bias (40), is low (0.145), a feature common to several poorly expressed genes and to *CDC25*. The *SDC25* gene has been located on chromosome XII by hybridization to electrophoretically separated chromosomes. Genetic studies confirmed this localization and showed that it is located 5.5 cM from the centromere on the left arm. The *CDC25* gene has been localized on the same chromosome (23), but it is not associated with the centromere.

The *SDC25* gene is transcribed into a 4-kb-long mRNA during exponential growth. Its transcript is at least 10-fold more abundant than the *CDC25* mRNA transcript. Downstream of the stop codon, sequences which fit very closely to the consensus sequence for polyadenylation have already been described (1). At the 5′ end of the gene, three major transcriptional start points have been identified. The first seems to be the main transcriptional start point used during exponential growth. This mRNA starts with the sequence AUG, which defines an ORF of nine amino acids ending with TAA at position −15 that is in frame with the main ORF. The occurrence of a small ORF before the main ORF has been reported in the 5′ upstream regions of several genes (18, 29), but in all reported cases the first codon AUG was located several nucleotides after the transcriptional start point. Since the first nucleotide is modified by capping, it should not be recognized as part of an initiation codon. Moreover, it lacks the consensus for efficient translation. In contrast, the sequence surrounding the AUG of the main ORF (5′-CAUAAUGAGU-3′) is very similar to the canonical sequence of the yeast initiator region (5′-[A/Y]A[A/U]AAU GUCU-3′) defined by Cigan and Donahue (7). In any case, efficient translation of the *SDC25* ORF is demonstrated by production of active βGal in the in-frame fusions promoted by transposition of the mini-Mu phage.

The *SDC25* ORF is slightly smaller than the *CDC25* ORF (1,251 residues instead of 1,589). The *SDC25* and *CDC25* gene products are closely related, since the relative amino acid compositions are very similar except for serine, which is 8.2% in the case of *SDC25* and 13% in the case of *CDC25*. The amino acid sequences of the *SDC25* and *CDC25* ORFs were compared by using the FASTA program (Fig. 9A). The *SDC25* amino acid sequence shows similarities throughout its length with that of the *CDC25* ORF. In the N-terminal part of each ORF (*SDC25* positions 1 to 650 and *CDC25* positions 1 to 980), five segments are partially homologous and are separated by nonhomologous sequences which contain most of the 338 additional amino acids of the *CDC25* ORF. The two first nonhomologous sequences of *CDC25* are noteworthy for their serine-rich content (positions 1 to 56 and 130 to 190) (5). The *SDC25* ORF from positions 22 to 101 and the *CDC25* ORF from positions 54 to 130, which correspond to the first homologous segment, are both related to the SH3 consensus sequence (Fig. 9B). This SH3 sequence has been identified in proteins that associate with the membrane cytoskeleton such as products of proto-oncogenes c-*src* (26) and c-*abl* (21), phospholipase Cγ (42), α-spectrin (28), myosin I (24), and the ABP1 *S. cerevisiae* protein (14). It has been proposed that the SH3 domain is involved in actin binding and could serve to bring together signal transduction proteins and their targets or regulators. Thus, the *SDC25* gene product could be associated with a membrane fraction as has been shown for the *CDC25* gene product (16). The *CDC25* ORF contains putative phosphorylation sites by the cAMP-dependent protein kinase at positions 77, 135, 142, 151, 174, 825, and 826 (8), which are not conserved in the *SDC25* ORF, and the unique putative phosphorylation site for the cAMP-dependent protein kinase (position 439) is present in a nonconserved region. The C-terminal parts of the two ORFs are more strongly related. From amino acids 650 to 1200 of the *SDC25* ORF, an optimal alignment with the *CDC25* amino acid sequence (positions 980 to 1544) leads to 47% identical residues. A hydrophobic domain which has been postulated to be a transmembrane domain in *CDC25* (13) (positions 1455 to 1469) is conserved in the C-terminal part of the *SDC25* ORF (positions 1102 to 1116). The last 50 C-terminal amino acids of the *SDC25* ORF are not related to the last 45 C-terminal amino acids of the *CDC25* ORF, although both sequences are very polar.

It has been shown that the C domain of SDC25, expressed in *E. coli*, directly acts in vitro as an exchange factor on the purified RAS2 protein and on the human c-Ha-*ras*-encoded p21 protein. Thus, the SDC25 carboxyl-terminal domain can enhance the regeneration of the active form of RAS proteins (10). In yeast cells, this SDC25 C domain suppresses the *cdc25* defect and the *RAS2*(Ts) dominant mutation in *RAS1 RAS2* cells, and it leads to an unregulated cAMP-activating cascade as in the $RAS2^{\mathrm{Val}\text{-}19}$ (25) and *bcy1* (30) mutants. These results in conjunction with to the high level of homology between the two C domains of CDC25 and SDC25

A

```
CDC25   54  LTSIRPIGIVVAAYDFNYPIKKDSSSQLLSVQQGETIYILNKNSSGWWDGLVIDDSNGK-------VNRGWFPQNFGRP-LRDSH
            .  . :: .:   :.. .    ..    ::.  :. ::.: : : :::::..:  :          ..:::::  : :  :  .:
SDC25   22  IPCLQPIDVVECTYQY-FTKSRNK----LSLRVGDLIYVLTKGSNGWWDGVLIRHSANNNNNNSLILDRGWFPPSFTRSILNELH

CDC25  272  ILPLEEIEMIINGIRSNIASTWSPIPLITKTSDYKLVYYNKDLDIYCSELP
            :: ::.       :       ::: :.  .     :.::: :::.::  ::
SDC25  129  ILSLEDFLDCCRDIEFKEQLAWSPTPVHERKGCCELLYYNQDLDVYCRTLP

CDC25  669  FSKFLRHVQLLYFVLQSSVFSDDNTLPQLLPRFFKGSFSGGSWTNPF     CDC25  805  SKRKKKYPLTVDTLNTMKKKSSQI
            : ::     :: :.           :::: ::::: ::   :: :::                 ::::: :::  .::  .. .  :.
SDC25  332  FQKFHF-RRLLHFLSNPNELT---ILPQLTPRFFKDSFNTISWNNPF     SDC25  455  SKRKKPYPLNEETLSLVRARKKQL

CDC25  859  TYEQINQNVLLLEILENLDLSIFINLKNLIKTPSIL----------LDLESEEFLVHAMSSVSSVLTEFFDIKQAFHDIVIRLIMTTQQT
            :::..   . ...::::::: ::.::. :     ..          .  :  ::: : .::.: .: ..:..:: ::.      .   .
SDC25  511  TYEEVSGTIPIIDILENLDLTIFLNLRELGDENRVFDEDVFDEDVAIGDEDKEFLKHSLSSLSYILSDYFNMKQYFHE------LSPTHL

CDC25  939  TLDDPYLFSSMRSNFP
            ::.::..:: :  ..:
SDC25  595  TLEDPFVFSPMQNDLP

CDC25  980  QLALSLFHVLVSQDVEFNNLEFLNNSDDFKDACEKYVEISNLACIIVDQLIEERENLLNYAARMMKNNLTAELLKGEQEKWFDIYSEDYS
               : ::: :. :: .::.: :.:    ::  :. : ..  ::   :.::: ::::::::::::::::.:  ::.::.   .  :
SDC25  650  PDSLILFHNLINQDSDFNDLKFFNLAHVFKKSCDDYFDVLKLAIEFVNQLILERENLLNYAARMMKNNITELLLRGEEG--YGSYDG--G

CDC25 1070  DD-DSENDEAIIDDELGSEDYIERKAANIEKNLPWFLTSDYETSLVYDSRGKIRGGTKEALIEHLTSHELVDAAFNVTMLITFRSILTTR
            .  .  .  :.  :     :  :..   .   ::..:  .:.  :.  :  .:.:: : :::  :: .:  :  ::.: :::::::.::
SDC25  736  ETAEKSDTNAVYADS-DTKDNDEWRDSQV--KLPRYLQREYDSELIWGSNNRIKGGSKHALISYLTDNEKKDLFFNITFLITFRSIFTTT

CDC25 1159  EFFYALIYRYNLYPPEGLSYDDYNIWIEKKSNPIKCRVVNIMRTFLTQYWTRNYYEPGIP-LILN-FAKMVVSEKIPGAEDLLQKINEKL
            ::.  ::  ::: ::: : ...:: :. ::  :.::::: :: ::. :::   : :: .  : :. :: . . : : :  .::  .:.:.
SDC25  823  EFLSYLISQYNLDPPEDLCFEEYNEWVTKKLIPVKCRVVEIMTTFFKQYWFPGYDEPDLATLNLDYFAQVAIKENITGSVELLKEVNQKF

CDC25 1247  INENEKEPVDP-KQ-QDSVSAVV-QTTKRDNKSPIHMSSSSLPSSASSAFFRLKKLKLLDIDPYTYATQLTVLEHDLYLRITMFECLDRA
               :  :   : :     .       :       :                       : .::  .:::::.:::..:  ::.:.::..
SDC25  913  KLGNIQEATAPMKTLDQQICQDHYSGTLYSTTESI-----------------------LAVDPVLFATQLTILEHEIYCEITIFDCLQKI

CDC25 1334  WGTKYC-NMGGSPNITKFIANANTLTNFVSHTIVKQADVKTRSKLTQYFVTVAQHCKELNNFSSMTAIVSALYSSPIYRLKKTWDLVSTE
            :  ::    : :: .  ::  :: ::::.:  .::.::   : ::   :. .:. :. .::::::: :.::::::::::: :::. :  .
SDC25  980  WKNKYTKSYGASPGLNEFISFANKLTNFISYSVVKEADKSKRAKLLSHFIFIAEYCRKFNNFSSMTDIISALYSSPIYRLEKTWQAVIPQ

CDC25 1423  SKDLLKNLNNLMDSKRNFVKYRELLRSVTDVACVPFFGVYLSDLTFTFVGNPDFL---------HNSTNIINFSKRTKIANIVEEIISFK
             .:::  :: ::: :.::. ::  :.:   . :::::::::::::::  ::::.:         :.    ::: :: .. .:..::: ::
SDC25 1070  TRDLLQSLNKLMDPKKNFINYRNELKSLHSAPCVPFFGVYLSDLTFTDSGNPDYLVLEHGLKGVHDEKKYINFNKRSRLVDILQEIIYFK

CDC25 1504  RFHYKLKRLDDIQTVIEASLENVPHIEKQYQLSLQVEPRSG
            . :: . .   .   :  ::::.::::::::::: .::..
SDC25 1160  KTHYDFTKDRTVIECISNSLENIPHIEKQYQLSLIIEPKPR
```

B

```
Consensus   ALYDY------D-----●-●--GD-●-●●------WW---------------------G●●?--Y●--
              F F      E           E                                        F
CDC25 (65)  AAYDFnypikkDsssqiLsVqqGEtIyILnknssgWWdgividdsngk-------vnrGWFPqnFGrp
SDC25 (33)  ctYqYftksrnk-----LsLrvGDlIyVLtkgsngWWdgvlirhsannnnnnslildrGWFPpsFtrs
```

FIG. 9. Comparison of the *SDC25* and *CDC25* amino acid sequences. (A) Optimized alignment (52) of the *SDC25* and *CDC25* ORFs. Double dots indicate identity; single dots indicate a conservative change. (B) SH3 consensus sequence. The amino acid position of the first residue of each sequence is given in parentheses. Hydrophobic residues are represented by circles; conserved amino acids in the consensus are represented by capital letters.

strengthen the hypothesis that the SDC25 C domain can replace the *CDC25* gene product to activate the adenylate cyclase by activating the *RAS* gene products.

An interesting result is the inability of the complete *SDC25* gene to suppress *cdc25* deficient strains even on a multicopy plasmid, although the truncated gene can do so. This result does not seem to be due to the lack of expression, since (i) the level of RNA is high and approximately the same in cells transformed with the truncated gene and the complete gene on a multicopy plasmid and (ii) the ORF is translated, as shown by βGal fusion experiments. Rather, it could be explained by the effect of specific elements present in the N-terminal part. The deletion of 344 amino acids in the N-terminal part of the gene product (plasmid pRG3-9), which has been selected by its ability to restore the suppressing property of the *SDC25* gene, strongly suggests that the corresponding part of the protein contains some element regulating the exchange factor domain. This region contains many sequences not conserved between *SDC25* and *CDC25* ORFs. A second deletion of 734 amino acids (plasmid pRG4), encompassing the first deletion, does not have the same property. The failure of this construction to suppress the defect of the *cdc25* mutation can be interpreted as giving rise to a deficient protein. The difference between the amino acid sequences in the N-terminal parts of these two deleted gene products should lead to different conformations, one of which (in pRG3-9) restores the capability to interfere with the RAS proteins. Such an activation by deletion is reminiscent of many oncogenes which have been shown to be truncated versions of normal genes, having lost their regulatory domains. As an example, truncation of the N-terminal sequence in the c-*erbB* gene encoding the epidermal growth factor receptor removes the regulatory domain of the receptor and leads to constitutive tyrosine kinase activity of the oncogene v-*erbB* (51).

The *SDC25* gene appears to be dispensable for cell growth under tested conditions: deletions of the gene lead to viable cells, and no phenotype was detected after either disruption or overexpression of the *SDC25* gene. The lack of phenotype for a deleted gene is often explained by the existence of a redundant gene. Several examples of such redundancy already exist in the cAMP pathway: *RAS1* and *RAS2* genes are functionally interchangeable (25, 45), and the same is true for *TPK1*, *TPK2*, and *TPK3* (48), for *PDE1* and *PDE2* (34), and most likely for *IRA1* and *IRA2* (44). However, a strict redundancy between *CDC25* and *SDC25* for the activation of adenylate cyclase through *RAS* must be eliminated because (i) the lack of a functional *CDC25* gene can be rescued neither by the genomic *SDC25* nor by *SDC25* on a multicopy plasmid and (ii) the possibility that *CDC25* could compensate the defect due to deletion in *SDC25* was examined, but no difference in growth capabilities was detected in cells harboring a *SDC25* disruption in a *cdc25* disrupted background. Thus, if the *SDC25* gene product interferes with the RAS-cAMP pathway in vivo, it should do it under conditions that have not yet been identified. It seems more likely that *SDC25* is involved in another system as a dispensable regulator. Many small GTP-binding proteins have been described in *S. cerevisiae*. These proteins might also require an exchange factor. By the activity of the C domain, the *SDC25* gene product could act on a *ras*-related GTP-binding protein, the N-terminal part of the molecule being involved in regulation or targeting. Therefore, the *SDC25* gene product can be considered a new member of the family of the CDC25-like proteins. This product contains the first domain for which a GDP-to-GTP exchange activity on RAS protein has been demonstrated, a domain that is under regulation in the complete protein. Recently another member of this new family of proteins has been described in *Schizosaccharomyces pombe*: the *STE6* gene product, which is involved in *RAS1* activation (19).

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ADDENDUM IN PROOF

Nucleotides 3137 and 3138 of the *SDC25* gene sequence (Fig. 2) should be C and A, respectively, instead of A and C as shown. This results in codon 1046 reading GCA, and thus coding for Ala instead of Asp.

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