Components Required for Cytokinesis Are Important for Bud Site Selection in Yeast

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Abstract. Polarized cell division is a fundamental process that occurs in a variety of organisms; it is responsible for the proper positioning of daughter cells and the correct segregation of cytoplasmic components. The *SPA2* gene of yeast encodes a nonessential protein that localizes to sites of cell growth and to the site of cytokinesis. *spa2* mutants exhibit slightly altered budding patterns. In this report, a genetic screen was used to isolate a novel ochre allele of *CDC10*, *cdc10-10*; strains containing this mutation require the *SPA2* gene for growth. *CDC10* encodes a conserved potential GTP-binding protein that previously has been shown to localize to the bud neck and to be important for cytokinesis. The genetic interaction of *cdc10-10* and *spa2* suggests a role for *SPA2* in cytokinesis. Most importantly, strains that contain a *cdc10-10* mutation and those containing mutations affecting other putative neck filament proteins do not form buds at their normal proximal location. The finding that a component involved in cytokinesis is also important in bud site selection provides strong evidence for the cytokinesis tag model; i.e., critical components at the site of cytokinesis are involved in determining the next site of polarized growth and division.

In a wide variety of organisms, the specific orientation and/or asymmetry of cell division is a critical factor in the determination of distinct developmental fates (for review see Hyman and Stearns, 1992; Strome, 1993). Polarized divisions can be important for directing appropriate cell–cell contacts, mediating growth in a specific direction, or establishing intrinsic cytoplasmic differences between two daughter cells (examples can be found in Koch et al., 1967; Hyman and White, 1987; Hyman, 1989; Quatrano, 1978; Allen and Kropf, 1992). While the events involved in establishing cellular division planes have been characterized to some degree, the mechanisms through which cells orient their divisions have not been elucidated.

Saccharomyces cerevisiae undergoes polarized cell division and is a useful organism for studying this process. Yeast cells grow by budding, and the position where the bud forms ultimately determines the plane of cell division. The location of the bud site depends upon the mating type locus and the pedigree of the cell (Freifelder, 1960; Snyder, 1989). Haploid *MATa* and *MATα* cells undergo axial budding in which mother cells form buds adjacent to the previous bud site and daughter cells bud adjacent to the birth scar (i.e., proximal sites). Diploid *MATa*/MATα cells exhibit a different pattern: mother cells usually bud adjacent to the old bud site, but daughter cells preferentially form buds on the opposite side of the cell (i.e., distal sites).

Several models have been proposed to explain the specific positioning of bud sites in yeast. One hypothesis, the cytokinesis tag model (Snyder et al., 1991; Madden et al., 1992), proposes that critical components at the previous site of cytokinesis initiate assembly of factors involved in bud formation at proximal sites (Chant and Herskowitz, 1991; Snyder et al., 1991; Madden et al., 1992). Loss or modification of these sites might result in bud formation at secondary sites, perhaps adjacent to the spindle pole body (SPB)[1] (the yeast microtubule organizing center), and/or its associated microtubules (Byers, 1981; Snyder et al., 1991; Madden et al., 1992). For cells that exhibit proximal budding, support for the concept of a cytokinesis tag derives from three lines of evidence: (*1*) the budding patterns themselves demonstrate that new buds form adjacent to previous sites, (*2*) after cytokinesis, components from the bud neck remain at the cortex; proteins involved in forming the next bud assemble near these components (Ford and Pringle, 1991; Kim et al., 1991; Snyder et al., 1991), and (*3*) in haploid cells, components involved in bud formation begin assembly at the cortex during G1 while the microtubule organizing center resides on the side of the nucleus distal to the nascent bud site (Snyder et al., 1991). This last observation indicates that polarity begins at the cortex, rather than the SPB (Snyder et al., 1991). Direct evidence for the cytokinesis tag model would come from the identification of critical protein(s) involved in forming the tag.

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1. *Abbreviations used in this paper*: ORF, open reading frame; SPB, spindle pole body.

Genes important for bud site selection have been identified through genetic screens and other approaches. The *BUD3* and *BUD4* genes are necessary for selection of proximal sites in haploid cells; mutations in these genes often result in budding at distal sites (Chant and Herskowitz, 1991). The *CDC24*, *RSR1/BUD1*, *BUD2*, *BUD5*, *PFY1*, and *CAP* genes are necessary for selection of both proximal and distal sites (Sloat et al., 1981; Bender and Pringle, 1989; Chant and Herskowitz, 1991; Chant et al., 1991; Haarer et al., 1990; Vojtek et al., 1991); mutations in these genes can lead to bud formation at random sites. How these different genes function in bud site selection is not known (see Discussion).

The *SPA2* gene of yeast also participates in bud site selection (Snyder, 1989). In unbudded cells, the SPA2 protein localizes as a patch on the edge of the cell at the incipient site of bud formation, and in budded cells, it is at the bud tip (Snyder, 1989; Snyder et al., 1991). The SPA2 protein is at the neck in cells undergoing cytokinesis (Snyder et al., 1991). Deletion of the *SPA2* gene reveals that it is not essential for cell growth (Snyder, 1989). *spa2* mutants exhibit a reduced fidelity of budding at proximal sites; distal budding is unaffected. *spa2* mutants are defective in shmoo formation during mating and exhibit a minor defect in cytokinesis (Gehrung and Snyder, 1990; Snyder et al., 1991).

Other proteins have been identified that localize to the incipient bud site and site of cytokinesis. These include actin (Adams and Pringle, 1984; Kilmartin and Adams, 1984) and the *CDC3*, *CDC10*, *CDC11*, and *CDC12* gene products (Haarer and Pringle, 1987; Ford and Pringle, 1991; Kim et al., 1991; Kim, H. B., B. K. Haarer, and J. R. Pringle, personal communication). These latter components each localize in a ring near the cell surface before bud emergence; they remain as a ring at the neck as the bud forms and grows, and persist as a ring after cytokinesis. The CDC3, CDC10, CDC11, and CDC12 proteins are related to each other in primary sequence (25–37% identity; Haarer, B. K., S. R. Ketcham, S. K. Ford, D. J. Ashcroft, and J. R. Pringle, personal communication) and are thought to form the 10-nm filaments located under the plasma membrane at the bud neck (Byers, 1981). At the restrictive temperature, temperature-sensitive *cdc3*, *cdc10*, *cdc11*, and *cdc12* mutants lack these filaments (see Byers, 1981), are defective in cytokinesis, and form long multi-budded cells (Hartwell, 1971).

In this report, we describe a search for genes that genetically interact with *SPA2*. Using a screen for synthetic lethal mutants (Costigan et al., 1992; Bender and Pringle, 1991), we have uncovered a new mutation in the *CDC10* gene, *cdc10-10*. Yeast strains carrying this mutation require the *SPA2* gene for growth. The genetic interaction between *CDC10* and *SPA2* suggests that the *SPA2* gene plays a role in cytokinesis. Furthermore, yeast strains containing the *cdc10-10* allele exhibit abnormal budding patterns indicating that proteins important for cytokinesis are involved in bud site selection.

Materials and Methods

Yeast Strains and Standard Methods

Yeast strains are listed in Table I. Standard procedures for cloning (Sambrook et al., 1989) and manipulating yeast (Sherman et al., 1986) were used. Growth rates of Y574 and Y834, and Y835 strains were determined at 24.5°C by diluting log phase cultures to OD (600) = 0.3 and measuring the OD (600) at various time points over the ensuing 7-h period.

Isolation of Mutants that Require the SPA2 Gene for Growth

SPA2-dependent mutants were isolated using the procedure described by Costigan et al. (1992). Briefly, a *spa2-Δ1 ade2-101* strain containing a *SPA2/SUP4/CEN4* plasmid (Y574) was mutagenized to 48% viability with ultraviolet light. Of 2.5×10^5 colonies screened, four mutants were obtained that segregated 2:2 for the *SPA2*-dependent phenotype after a backcross to Y554. Each of these mutant strains formed colonies with red sectors after addition of a second *SPA2*-containing plasmid that lacked *SUP4* (see Costigan et al. 1992).

Backcrossing the *cdc10-10* mutant strain to Y554 (a *spa2-Δ1* strain containing the *SPA2 SUP4* plasmid) yielded *cdc10-10* segregants (with the plasmid) that exhibited wild-type growth, but a budding pattern defect. However, backcrosses with *SPA2* strains lacking the suppressor Y603 or Y837 produced *cdc10-10 SPA2* segregants with a cytokinesis defect. All 17 *cdc10-10 SPA2* segregants analyzed (14 for crosses with Y603 and three for Y837) displayed the cytokinesis defect. To reduce the probability of acquiring second site suppressor mutations, *cdc10-10 SPA2* strains lacking the *SUP4* plas-

Table I. Yeast Strains

	Strains
S288C	
Y574	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ1 leu2-Δ98 spa2-Δ1::TRP1* + pSPA2/TRP1/SUP4/CEN
Y554	*MATα ura3-52 lys2-801 ade2-101 trp1-Δ1 his3-Δ200 spa2-Δ1::TRP1* + pSPA2/TRP1/SUP4/CEN
Y603	*MATα ura3-52 lys2-801 ade2-101 trp1-Δ1 his3-Δ200*
Y831, Y832, Y833	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ1 leu2-Δ98 spa2-Δ1::TRP1 cdc10-10* + pSPA2/TRP1/SUP4/CEN
Y834, Y835	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ1 leu2-Δ98 cdc10-10*
Y836	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ1 leu2-Δ98 spa2-Δ1::TRP1 cdc10-10* + pSPA2/URA3/CEN
Y837	*MATα ura3 trp1 his4 HMRα HMLα can1 MAL2 bud5::URA3*
Y270	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ1 his3-Δ200* *MATα ura3-52 lys2-801 ade2-101 trp1-Δ1 his3-Δ200*
A364A	
Y145	*MATa ura1 his7*
Y524	*MATa ura1 lys2 ade2 ade1 gal1 his7 tyr1 cdc3-1*
Y526	*MATa ura1 lys2 ade2 ade1 gal1 his7 tyr1 cdc10-1*
Y528	*MATa ura1 lys2 ade2 ade1 gal1 his7 tyr1 cdc11-1*
Y529	*MATa ura1 lys2 ade2 ade1 gal1 his7 tyr1 cdc12-1*
Y172	*MATa ura3-52 ade2-101 leu2-3,112 trp1-289 his7 can1 sap1*

Y831 is the original mutant. Y832 and Y833 are independent segregants obtained through backcrosses.

mid (Y834 and Y835) were also prepared by another method. A plasmid containing the *SPA2* gene (p203, Gehrung and Snyder, 1990) deleted for the *CEN4* and *ARS1* by digestion with SmaI and BglII) was cleaved with SacI and transformed into strain Y831 in order to achieve integration at the *spa2-Δ1* locus. The Ura$^+$ colonies produced red sectors on the transformation plates. Cells lacking the *SUP4* plasmid were transferred to plates containing 5-FOA which selects for *ura3* cells that have lost the integrated *SPA2* plasmid through homologous recombination. Most Ura- segregants were also Trp$^-$, indicating that they recombined in such a manner as to lose the *spa2-Δ1* allele. *cdc10-10 SPA2* strains obtained by the integration/excision procedure exhibited identical phenotypes to those obtained through backcrosses.

Complementation of the cdc10-10 Nonsectoring Phenotype

A YEp24 plasmid carrying a 4.1-kb insert with the *CDC10* gene was kindly provided by the Pringle laboratory. A 1.7-kb XmnI/KpnI fragment containing the *CDC10* gene as its only ORF (Oliver et al., 1992) was subcloned into pRS316, a *CEN/URA3* vector (Sikorski and Hieter, 1989) to produce p238. Both the CDC10/CEN and CDC10/YEp24 plasmids were transformed (Chen et al., 1992) into the *cdc10-10 spa2-Δ1* strain, Y831; control CEN plasmids containing either the *BUD3* or *BUD5* genes (Chant and Herskowitz, 1991; Chant et al., 1991) and appropriate vectors (YEp24 and pRS316) were transformed into the same strain.

Staining with Calcofluor, Hoechst 33258, and Anti-SPA2 Antibodies

Staining with Calcofluor and Hoechst 33258 was described by Madden and Snyder (1992) and Snyder and Davis (1988), respectively. Immunofluorescence with affinity-purified anti-SPA2 antibodies was performed on strains Y574, Y831, Y832, Y833, and Y834 as described previously (Snyder, 1989; Gehrung and Snyder, 1990; Snyder et al., 1991). Rat monoclonal anti-tubulin antibody YOL1/34 was included as a positive control. Rabbit antibodies were detected with Texas red–conjugated goat anti–rabbit antibodies and rat antibodies were detected with FITC-conjugated goat anti–rat antibodies.

Sites of bud formation were quantitated for cells grown in rich YPD medium similar to that described by Madden and Snyder (1992). Cells with a single bud scar and one bud were grouped into three classes: those with a bud proximal to the bud scar (within 45°; Class I), those with a bud distal to bud scar (the distal 90° sector; Class III), and those with the bud at an intermediate distance from the bud scar (the middle region, see Fig. 6; Class III). A similar scheme was used to quantify bud formation sites in cells with two buds. In instances where the bud appeared to lie on a boundary, one half of the cells were scored as one class, and the other half were counted as the other. Classifications were rarely ambiguous for *cdc10-10* cells (without *SUP4*), *cdc3-1*, *cdc10-1*, *cdc11-1*, and *cdc12-1* cells because the buds that formed at the proximal position were nearly always closely adjacent to the previous buds and buds that formed on the opposite side usually lay 180°C from the bud scar (or other bud).

Projection Formation Assays

2-ml cultures of strains Y574, Y832, and Y833 were grown at 30°C to OD (600) = 0.3 and treated with 5.0 μg/ml α-factor. After 50 min, another aliquot of α-factor was added, and the cells were incubated for an additional 45 min. A 1-ml aliquot was then withdrawn and fixed for 1 h with 3.7% formaldehyde. The other ml was incubated for 35 min more, and then fixed. The number of cells forming projections and the size of the projections were evaluated using differential interference microscopy. There appeared to be no significant difference between cells in mutant cultures and those in wild-type cultures at either time point.

Sequencing the cdc10-10 Mutation

The *CDC10* gene was sequenced from strain Y574 (the parent strain), strain Y831 (the original *cdc10-10* mutant), and strain Y832 (a *cdc10-10* strain derived from backcrosses). Minipreparations of yeast DNA were prepared from each of these strains (Sherman et al., 1986), and a 1.4-kb segment was amplified using primers derived from the 5′ and 3′ regions that flank the *CDC10* open reading frame (ORF) (Saiki et al., 1985). The sequences of the primers were 5′-GTGAAGGATTTCCTAGCGGTACCAAC-3′ and 5′-CCTCTAGAGCAATACCTGTACCTCTAGTTTTC-3′. The amplified fragments were cleaved with KpnI and XbaI, gel purified and cloned into the Bluescript SK$^+$ vector (Stratagene, La Jolla, CA). The inserts were sequenced (Sanger et al., 1977; Sambrook et al., 1989) using seven primers spaced 180–200 bp apart throughout the *CDC10* gene. For each primer, the four reactions (G, A, T, and C) were loaded on a gel adjacent to the equivalent reactions from the other two strains (to ensure accurate comparisons of the sequence).

Of the 1,305 bp sequenced, one deviation was found between the wild-type Y574 sequence and that reported by Steensma and van der Aart (1991). We assume that this difference represents a PCR/cloning error in our sequence, because the sequence from the other two *cdc10-10* strains matched the published sequence at this position. Four changes from the wild-type sequence were found in the sequence derived from mutants Y831 (two differences) and Y832 (three differences). One change was present in both mutant strains, and presumably represents the bona fide *cdc10-10* mutation. The other differences were not common to both mutants. Presumably these mutations are due to PCR/cloning artifacts, since at least one mutant allele matched the wild-type sequence at each of these positions. Mutations due to PCR/cloning have been well-documented (Saiki et al., 1988).

Disruption of the CDC10 Gene

The *cdc10-Δ1* and *cdc10-Δ2* alleles were constructed by digesting a SK$^+$ plasmid containing a 1.4-kb KpnI/XbaI fragment of *CDC10* with BamHI and BglII and filling in the ends with the large fragment of DNA polymerase I in the presence of dNTPs. A 1.1-kb fragment containing the *URA3* gene was obtained by cleaving YEp24 (Botstein et al., 1979) with HindIII; the ends were filled in, and the gel-isolated fragment was ligated to the cleaved *CDC10*-containing plasmid. For *cdc10-Δ1*, the *URA3* gene is transcribed in the opposite direction as *CDC10*; for *cdc10-Δ2*, the *URA3* gene is transcribed in the same direction. The plasmids containing the *cdc10-Δ1* and *cdc10-Δ2* mutant alleles were cleaved with KpnI and XbaI, and transformed into diploid strain Y270. The resulting *cdc10-Δ/CDC10* heterozygous diploids were sporulated and tetrads analyzed. For *cdc10-Δ1* heterozygotes, 50 tetrads were analyzed: 34 segregated 4 live: no dead and 16 segregated 3 live: one dead progeny. For *cdc10-Δ2* heterozygotes, 40 tetrads were analyzed: 35 segregated 4 live: no dead and 5 segregated 3 live and one dead progeny. 2:2 segregation of the *URA3* marker was deduced from each of these tetrads (~half the dead cells in the 3:1 tetrads were deduced to be Ura$^-$). There was one exception: one tetrad from the *cdc10-Δ1* heterozygotes yielded 3 Ura$^+$ colonies and 1 Ura$^-$ colony; this segregation pattern is presumed to have occurred because of a gene conversion event. All Ura$^+$ colonies were temperature-sensitive for growth. Proper substitution of *cdc10-Δ1* and *cdc10-Δ2* at the *CDC10* locus was deduced by three different criteria: (*1*) DNA gel blot analysis using a *CDC10* probe, (*2*) failure of *cdc10-Δ* cells to complement the temperature sensitive *cdc10-10* growth defect after appropriate matings, and (*3*) linkage of the *URA3* marker to *MAT*.

Results

Isolation of New Mutants that Require the SPA2 Gene for Growth

To identify genes involved in the same processes as *SPA2*, we searched for mutants that require the *SPA2* gene for growth using a screen described previously (Costigan et al., 1992). Briefly, a *spa2-Δ1 ade2-101* yeast strain carrying a centromeric plasmid containing both *SUP4* and a wild-type copy of *SPA2* was constructed. *ade2-101* is an ochre allele; yeast strains containing this mutation are red. In the presence of *SUP4*, an ochre suppressing tRNA, the cells are white (Goodman et al., 1977; Shaw and Olson, 1984; Costigan et al., 1992). When the *spa2-Δ1 ade2-101* strain containing the *SPA2/SUP4* plasmid is plated on nonselective medium, the cells form white colonies with many red sectors because the centromeric plasmid is lost at a high frequency and the *SPA2* gene is not essential. The starting strain was mutagenized and plated on nonselective medium at 25°C; mutants that cannot survive without the plasmid were identified as homogeneous white colonies (see Fig. 1). Four mutants that segregated the *SPA2*-dependent phenotype as a single Mendelian locus were found (see Materials and

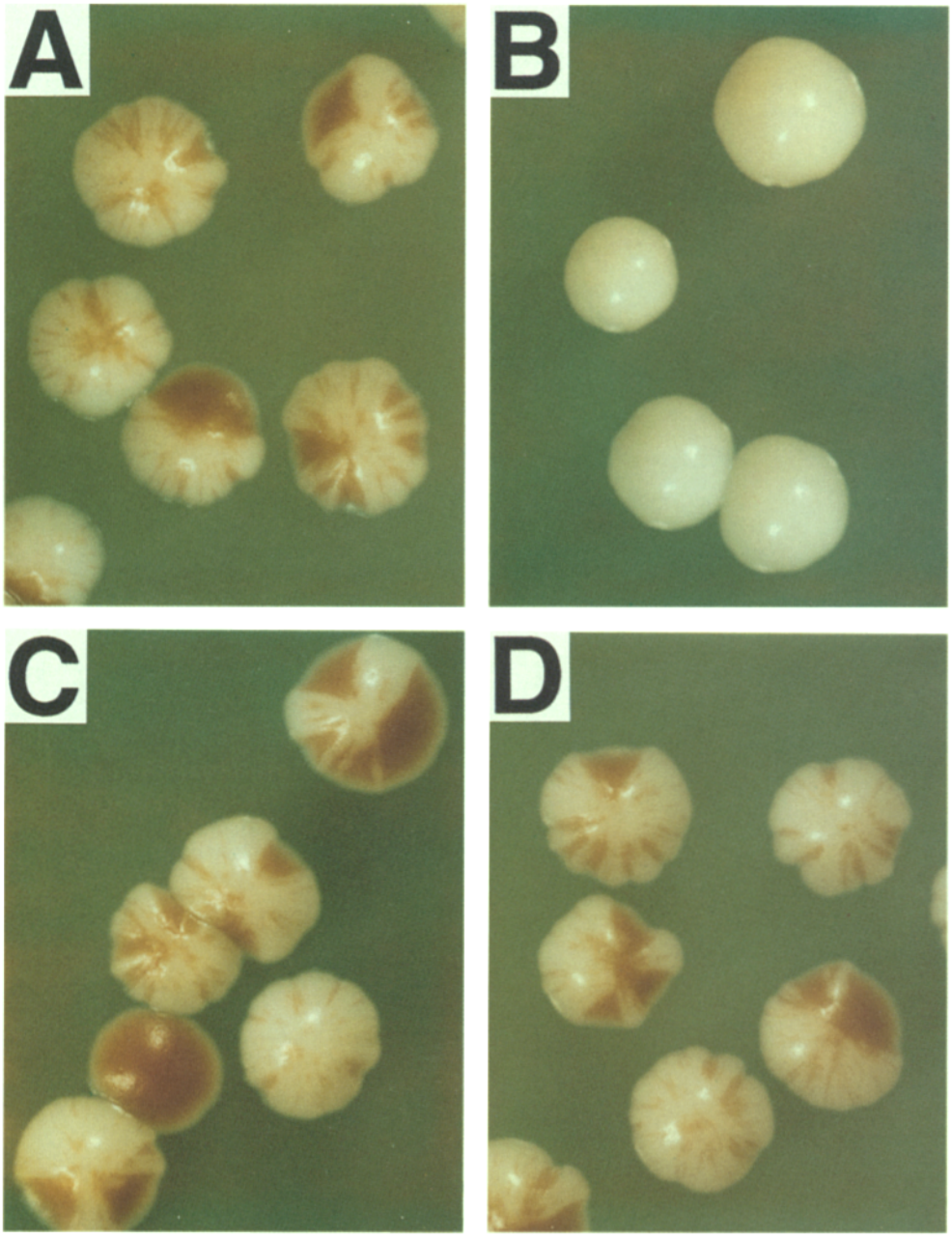

Figure 1. SPA2 plasmid loss in *CDC10* and *cdc10-10* strains as measured by a colony color assay. (*A*) The parental *spa2-Δ1 CDC10* strain carrying a *SPA2/SUP4/CEN* plasmid (Y574). (*B*) The *spa2-Δ1 cdc10-10* strain carrying a *SPA2/CEN* plasmid (Y831). (*C*) Y831 carrying the *CDC10/CEN* plasmid. (*D*) Y831 carrying the *SPA2/CEN* plasmid (p203). The cells were plated on nonselective medium containing low levels of adenine (Hieter et al., 1985; Costigan et al., 1992). Dark (red) sectors indicate cells from which the plasmid has been lost.

Methods). Each of these mutants can lose the *SPA2/SUP4* plasmid in the presence of a second plasmid containing *SPA2*, demonstrating that the mutant requires *SPA2* and not some other gene on the original plasmid (Fig. 1 *D*). One mutant strain was shown to contain a novel allele of *CDC10*, *cdc10-10*; this strain has been characterized in detail. The other three *SPA2*-dependent mutants are not alleles of *CDC10* and will be described further elsewhere.

Loss of the *SPA2* plasmid was not detected in a *cdc10-10* mutant using the colony color assay (Fig. 1). However, sporulation and tetrad analysis of a *cdc10-10/CDC10 spa2-Δ1/spa2-Δ1* strain revealed that segregants that do not contain the plasmid form very small colonies on rich medium after long incubation periods (data not shown). These segregants were deduced to be *spa2-Δ1 cdc10-10* double mutants based on the segregation of markers in other members of the tetrads. *spa2-Δ1 cdc10-10* microcolonies failed to reach a detectable density when grown in liquid medium for 1 wk. Thus, *spa2-Δ1 cdc10-10* cells have an extremely severe growth defect.

cdc10-10 Is an Allele of CDC10

During backcrosses, the *cdc10-10* mutation was found to be tightly linked to *LEU2* and *MAT*. A heterozygous *MATα/MATα cdc10-10/CDC10 leu2/LEU2* strain was sporulated and tetrads were dissected. Of 12 tetrads that segregated 2:2 for both *LEU2* and *cdc10-10*, 11 were parental ditypes and one was a tetratype, suggesting that *cdc10-10* is ~4.5 cm from *leu2*. Eleven tetrads segregated 2:2 for both *cdc10-10* and *MATα*; these included 8 parental ditypes and 3 tetratypes. These limited data suggested that the *cdc10-10* muta-

tion was located at or near the *CDC10* locus, which lies in the interval between *LEU2* and *MAT* (Mortimer and Schild, 1985).

Several tests were performed to determine whether the *cdc10-10* mutation is an allele of *CDC10*. First, a plasmid containing the *CDC10* gene as the only ORF was constructed and transformed into the *cdc10-10 spa2-Δ1* strain (Fig. 1 *C*). Control plasmids containing either vector sequences or the *BUD3* or *BUD5* genes were transformed into the same strain. The *CDC10* plasmid complemented the *SPA2*-dependent phenotype of *cdc10-10* (Fig. 1 *C*), whereas the control plasmids did not. Next, the *cdc10-10 spa2-Δ1* strain was mated to a *cdc10-1* strain. The *cdc10-10* mutation does not fully complement the *cdc10-1* mutation as evidenced by a cytokinesis defect in the *cdc10-10/cdc10-1* diploid cells (see below). Third, sequence analysis of the *cdc10-10* allele indicates the presence of a single bp mutation in the *CDC10* coding region (see below). Thus, we conclude that the *cdc10-10* mutation is within the *CDC10* gene.

CDC10 Is Predicted to Encode a GTP-binding Protein

The sequence of the *CDC10* gene has been determined previously (Steensma and van der Aert, 1991; Pringle, J. R., personal communication) and is predicted to encode a protein 322 amino acid residues in length. The predicted CDC10 protein sequence is very similar (40–42% identity) to those of several other predicted proteins in the Genbank database, including one protein from *Drosophila* and two from mice (Nottenburg et al., 1990; K. Kato, [Genbank accession no. X61452] D. C. Hayward, S. J. Delaney, and G. L. Miklos [Genbank accession no. X67202]; Fig. 2). Inspection of each of these sequences reveals that they are nearly 100% identical in regions containing a GXXXXGKS--DXXG--TKXD motif. This sequence is conserved in GTP-binding proteins (Dever et al., 1987), suggesting that the CDC10 protein and its homologs bind GTP. Since the CDC10 protein is thought to be a component of the 10-nm neck filaments, its potential to form a coiled-coil was analyzed using the program of Lupas et al. (1991). No significant potential for coiled-coil formation was predicted by this program ($p \leq 1 \times 10^{-3}$).

The cdc10-10 Allele Contains an Ochre Mutation at Codon 133

To further understand the nature of the *cdc10-10* mutation, the *CDC10* region was cloned using PCR from two different yeast strains containing the *cdc10-10* allele (the original mutant and a segregant) and the *CDC10* wild-type parent (Saiki et al., 1985). The sequences of the cloned DNAs were determined as described in Materials and Methods. The sequence of the wild-type strain matched that previously reported (Steensma and van der Aert, 1991) except for one nucleotide difference, which is attributed to a PCR/cloning artifact (see Materials and Methods). A single bp change was found in both *cdc10-10* strains; this mutation is a C–T transition at position 397 that predicts a UAA ochre codon (Fig. 2) at codon 133. Thus, the *cdc10-10* strain is expected to produce a protein whose length is only 40% that of the wild-type protein. The truncated protein is expected to lack one of the highly conserved motifs found in GTP-binding proteins (Fig. 2).

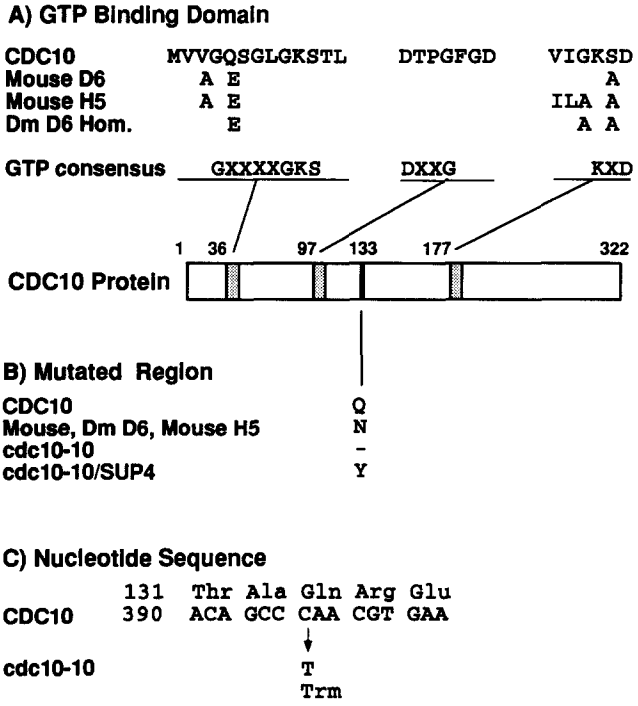

Figure 2. (*A*) The conserved GTP consensus sequence in CDC10, Mouse D6, Mouse H5, and Drosophila D6 genes. (*B*) The predicted amino acid sequence of the altered region and its sequence similarity to homologs in other species. A tyrosine residue is expected to be inserted in the *SUP4 cdc10-10* strain (Goodman et al., 1977). (*C*) The one bp difference between *cdc10-10* and wild-type *CDC10* (see Materials and Methods). The Mouse D6 (Diff 6) sequence is from Nottenburg et al. (1990). The Mouse H5 mRNA and Drosophila Diff 6 sequences are unpublished sequences present in GenBank (Mouse, K. Kato; Drosophila, D. C. Hayward, S. J. Delaney, and G. L. Miklos).

cdc10-10 Mutants Exhibit Defects in Cytokinesis

The phenotypes of *cdc10-10* strains were analyzed both in the absence and presence of an ochre suppressing tRNA. *cdc10-10* strains that possess a wild-type copy of *SPA2* but lack *SUP4* were constructed (see Materials and Methods) and analyzed. *cdc10-10* strains live, but grow significantly slower at 25°C than wild-type cells (doubling time in rich medium at 25°C = 5.8–6.8 h vs 3.3 h for isogenic wild-type strains). *cdc10-10* strains plated at 30°C exhibit little, if any, growth, and no colonies are observed at 37°C (Fig. 3). Microscopic examination of cells grown at 25°C reveals that they usually have an elongated cell shape and exhibit a cytokinesis defect; the cells appear as long chains of connected cells, and individual modules sometimes contain multiple nuclei (Fig. 4; the elongated cell shape is more apparent in Fig. 5). The average number of "modules" per chain is 2.7 (339 cells and 912 modules counted). Digestion of the cell walls with zymolyase reduces the length of the chain; however, most cells remain connected, indicating that internal parts of the cell are still attached. Staining with Calcofluor, a fluorescent dye that binds chitin (and bud scars, see below), reveals that chitin staining is usually much more delocalized along the cell surface of *cdc10-10* cells than in wild-type cells (Fig. 4). Although some chitin staining appears at the neck between cells, it is not as sharply concentrated as in wild-type (see

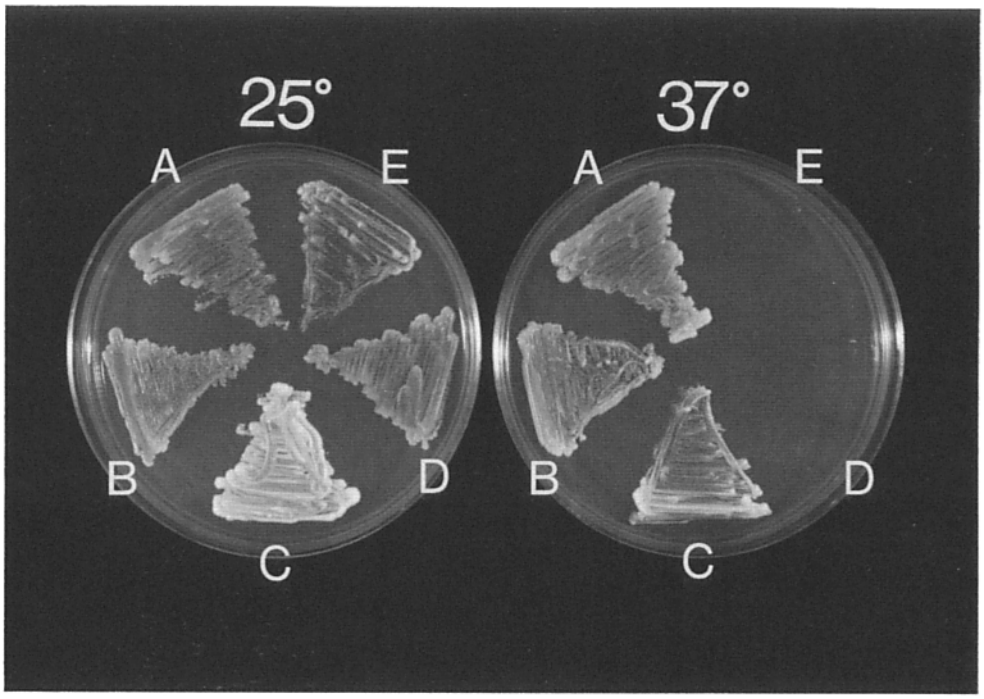

Figure 3. cdc10-10 mutants exhibit a temperature-sensitive growth defect. *cdc10-10* and wild-type cells were streaked on two plates containing rich medium. One plate was incubated at 25°C and the other at 37°C. The following strains were tested: (*A*) Wild-type (Y603), (*B*) *spa2-Δ* (Y574 without the plasmid), (*C*) *cdc10-10 SUP4*, and (*D* and *E*) two independent *cdc10-10 SPA2* isolates. Growth on a plate incubated at 30°C was identical to that seen at 37°C.

below). *cdc10-10* cells were also grown at the permissive temperature, shifted to 37°C and examined after either a 2 or 4-h incubation at this temperature. The defects for cells incubated at the restrictive temperature appear more severe than those for cells grown at 25°C; the cells at 37°C become highly elongated and the lengths of the chains are longer (data not shown). In general, these results are similar to those previously observed with temperature-sensitive *cdc10-1* strains at the restrictive temperature (Hartwell, 1971).

The localization of the SPA2 protein was determined in *cdc10-10* and *cdc10-1* cells grown at 25°C by indirect immunofluorescence with anti-SPA2 antibodies. In vegetatively growing wild-type cells or *cdc10-1* cells grown at 25°C, the SPA2 protein localizes to bud tips; in cells undergoing cytokinesis, the SPA2 protein is tightly concentrated in the neck region (Fig. 5 *C*) (Snyder et al., 1991). In *cdc10-10* cells, most bud tips stain with anti-SPA2 antibodies (Fig. 5). However, many *cdc10-10* cells that appear to be in the process of attempting cytokinesis often have a long thick fiber of SPA2 staining that extends between the cytoplasms of the mother and daughter cells. Approximately 70% of the mutant cells that stain at the neck possess this thick SPA2 bar, and the remainder display broad staining at the neck region (not shown). *cdc10-1* cells were also stained with anti-SPA2 antibodies after incubation at 37°C for 2.5 h. The SPA2 protein localized to bud tips, but no staining was observed at the neck (data not shown). These results indicate that the SPA2 protein exhibits aberrant localization in *cdc10-10* mutants at 25°C and fails to localize to the neck in *cdc10-1* cells at 37°C.

Bud Site Selection Is Altered in cdc10-10 Strains

In wild-type haploid cells, a bud forms adjacent to the previous site of cytokinesis. In contrast, haploid *cdc10-10* strains often form buds opposite to the previous cytokinesis site. The position where buds form on *cdc10-10* cells was analyzed quantitatively using two methods. First, the sites of bud formation were analyzed relative to previous sites of cytokinesis. Wild-type and *cdc10-10* strains were stained with Calcofluor, which recognizes chitinous bud scars, annular structures left from previous cytokinesis events. Cells with a single bud and a single bud scar (Fig. 5 *F*) were classified according to the scheme shown in Fig. 6 (see also Madden and Snyder, 1992). In Class I cells, the bud was located next to the bud scar. Class III cells formed a bud on the opposite side of the cell, and Class II cells contained a bud in the central region. The relative cell surface area of the central region in Class II cells is estimated to be several-fold larger than that for Class I and III domains (Madden and Snyder, 1992). As shown in Fig. 6, >96% of the cells from the parental strain budded adjacent to the previous cytokinesis site (Class I cells), consistent with previous results (Snyder, 1989; Madden and Snyder, 1992). In contrast, while many *cdc10-10* cells budded at proximal sites (52%), a large percentage budded at distal sites (38%). Few Class II cells (10%) were apparent.

In a complementary approach, cells that had two buds (which represent 25% of the total population) were scored for the relative location of their buds using a scheme similar to that described above (Table II). Consistent with the results above, in the majority of cells with two buds, the buds are adjacent (48%) or on opposite sides of the cell (44%). Very few cells formed buds at intermediate positions (8%).

In the Presence of an Ochre Suppressing tRNA, cdc10-10 Strains Appear Normal but Exhibit a Budding Pattern Defect

In the presence of the *SUP4* ochre-suppressing tRNA, *cdc10-10* strains are expected to produce a protein with a tyrosine at position 133 instead of the conserved glutamine/asparagine residue in the wild-type CDC10 protein and its homologs (Fig. 2). This substitution causes relatively few detect-

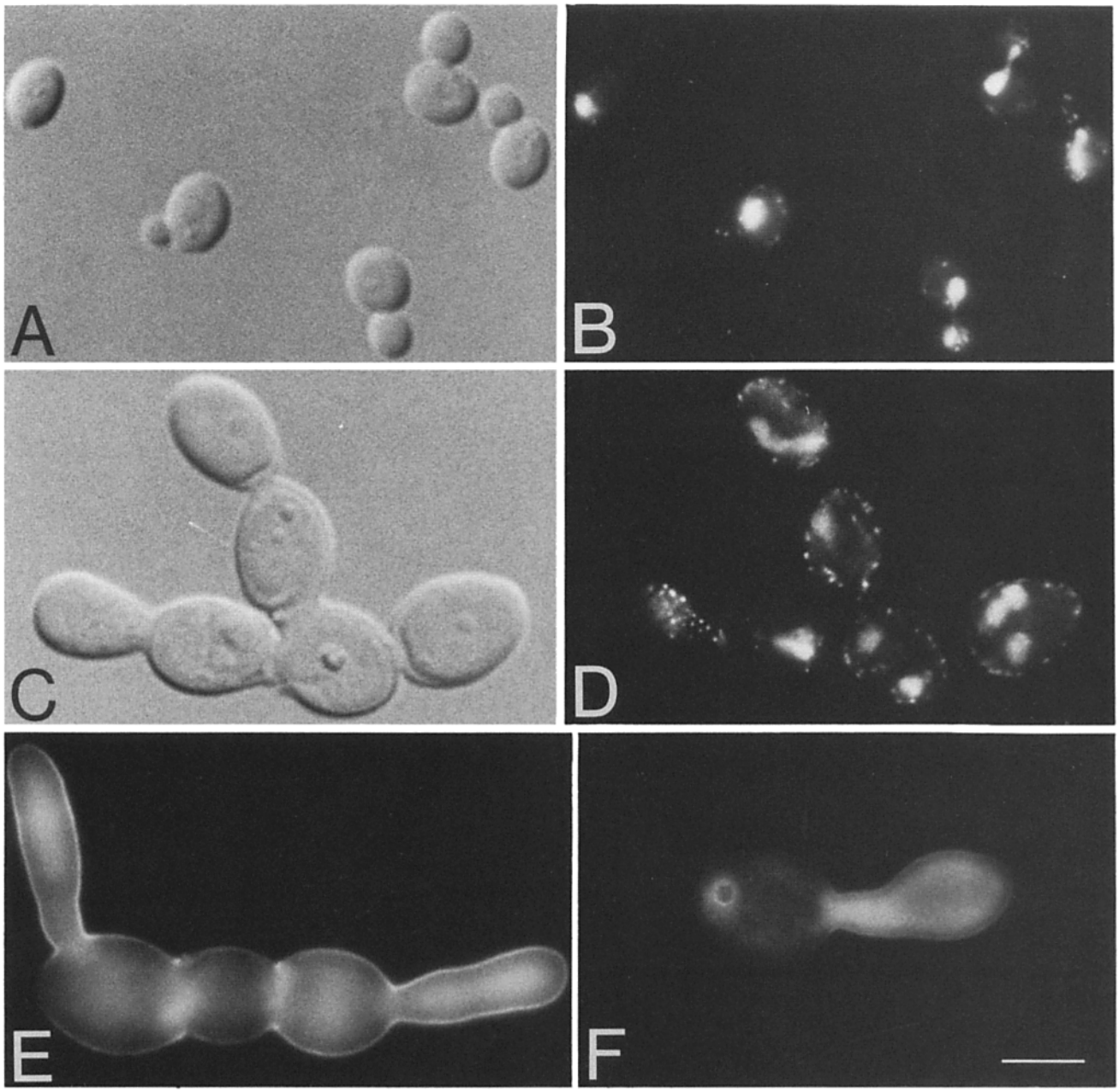

Figure 4. The cytokinesis defect of *cdc10-10* cells. *cdc10-10* cells appear as connected chains containing multiple nuclei. (*A* and *C*) Cells viewed by differential interference contrast microscopy. (*B* and *D*) Hoechst 33258 DNA stain of the cells shown in *A* and *C*, respectively. (*E* and *F*) Calcofluor which stains chitin. *A* and *B* show wild-type cells, and *C*, *D*, *E*, and *F* show *cdc10-10* cells. Note that chitin staining is more diffuse in mutant cells than in the wild-type and *cdc10-10 SUP4* cells shown in Fig. 7. Bar, 5 μm.

able defects. At 18, 25, 30, and 37°C, *cdc10-10 SUP4* cells form colonies that are comparable in size to those of wild-type cells. *cdc10-10 SUP4* cells appear morphologically normal, and no cytokinesis defect is evident. Staining of *cdc10-10 SUP4* cells with anti-SPA2 antibodies reveals a staining pattern identical to that of wild-type cells; the SPA2 protein localizes to the edge of the cell in most unbudded cells, to the bud tip in budded cells, and to the neck in cells undergoing cytokinesis (data not shown). Finally, *cdc10-10 SUP4 MATa* cells treated with α-factor form projections of similar size and shape to those of wild-type cells (data not shown). Thus, in most respects, *cdc10-10 SUP4* cells appear normal.

However, staining with Calcofluor reveals that the budding pattern of *cdc10-10 SUP4* cells is altered. In a wild-type haploid cell, bud scars almost invariably cluster near one end or form a continuous line across the cell (Fig. 7 *A*). However, in a *cdc10-10* cell, the bud scars often appear randomly distributed around the cell (Fig. 7 *B*). The position of bud formation was quantitated in cells containing a single bud and a single bud scar as described in the previous section. The *cdc10-10 SUP4* strain showed a preference for proximal budding, but a large number of cells (35%) budded at non-proximal sites. Interestingly, the cells that did not bud at proximal sites selected relatively random bud sites; they did

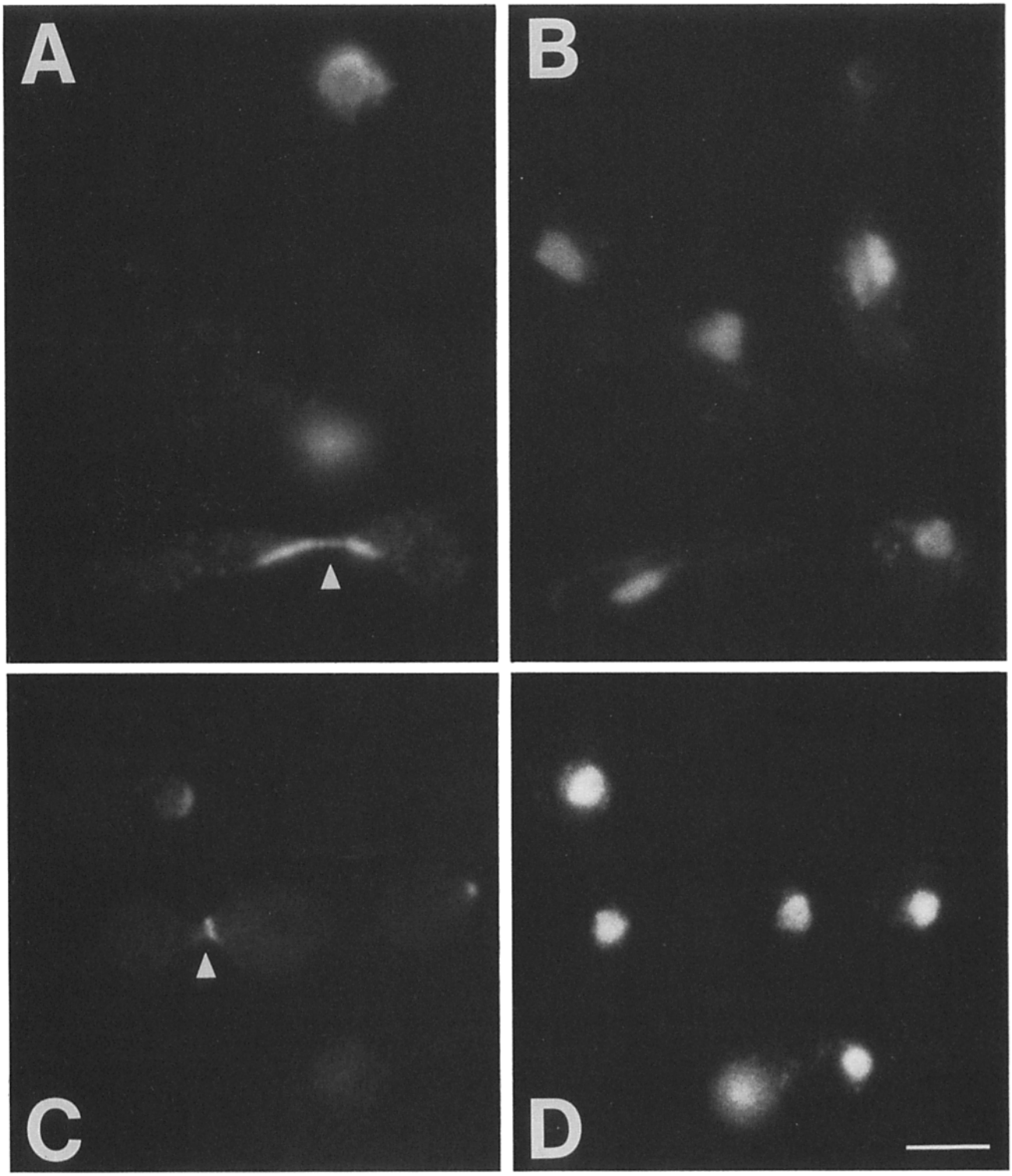

Figure 5. Localization of the SPA2 protein in *cdc10-10* cells. (*A*) *cdc10-10* cells stained with antibodies; (*B*) Hoechst stain of the same cells; (*C*) Wild-type cells (Y145) stained with anti-SPA2 antibodies; and (*D*) Hoechst stain of the same cells. Bar, 3 μm.

not bud primarily at distal sites as did *cdc10-10* cells in the absence of the suppressor. These observations indicate that the *CDC10* gene is important for proper bud site selection in yeast.

The CDC10 Gene Is Not Essential for Yeast Cell Growth

Yeast strains containing the *cdc10-10* allele are expected to

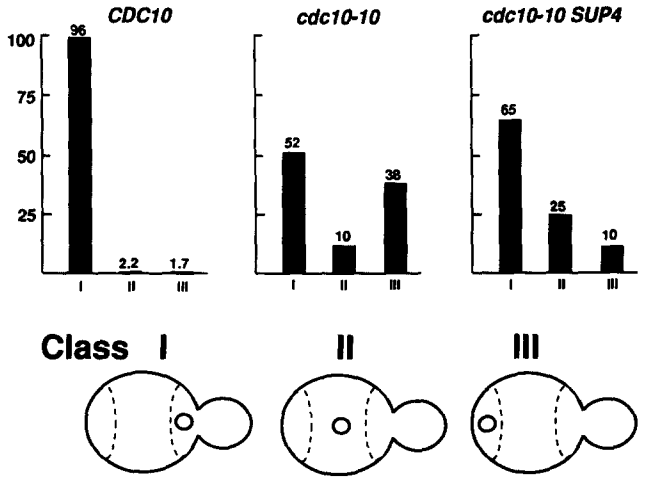

Figure 6. Sites of bud formation in wild-type, *cdc10-10*, and *cdc10-10 SUP4* strains. The position of the bud was determined relative to the bud scar for wild-type (Y574 containing the *SPA2/SUP4* plasmid; 405 cells counted), *cdc10-10* (Y834; 100 cells counted), and *cdc10-10 SUP4* strains (Y832; 423 cells counted; identical results were obtained for strain Y833). Cells were assigned to *Class I, II,* or *III* as depicted in the bottom of the figure.

lack a large segment of the *CDC10* protein in vivo, yet they are still viable. To determine if *CDC10* is essential for yeast cell growth, strains carrying a large deletion of the gene were constructed. In both *cdc10-Δ1* and *cdc10-Δ2*, a DNA fragment that contains the *URA3* gene was substituted for 668 bp of *CDC10* coding sequences. In each case, only the initiator codon and one other *CDC10* codon lies upstream of the *URA3* insertion. In *cdc10-Δ2*, the *URA3* gene is transcribed in the same direction as *CDC10*; for *cdc10-Δ1*, the two genes are transcribed in opposite directions. The mutant alleles were transformed into diploid yeast and the heterozygous strains were sporulated. Dissection of tetrads revealed that for each allele, four viable spores were produced when plates were incubated at 24.5°C; two were Ura^+ and two were Ura^-. (90 tetrads were analyzed: 50 for *cdc10-Δ1* and 40 for *cdc10-Δ2* (see Materials and Methods).) Thus, *CDC10* is not essential for yeast cell growth at 24.5°C.

cdc10-Δ1 and *cdc10-Δ2* cells display phenotypes similar to *cdc10-10*. At 25°C, the cells are usually elongated and connected and form multibudded chains. Furthermore, *cdc10-Δ* cells do not form colonies at 37°C, and when cultures are shifted to the restrictive temperature, the cells become even more elongated, and the number of connected cells increases (data not shown). Thus, *CDC10* is necessary for cell growth (and cytokinesis) at higher temperatures.

Table II. Budding Patterns of Cytokinesis Mutants at Semipermissive Temperatures

Class	I	II	III	N
cdc10-10	48	8	44	120
Wild type*	99	0	1	200
cdc3-1	33	11	56	109
cdc10-1	50	12	37	115
cdc11-1	39	12	49	118
cdc12-1	44	14	42	123

cdc strains were grown at 30°C, and cells containing two buds were classified according to the Class I, II, III scheme. Numbers indicate the percentage of cells in each class. The strains scored were: *cdc10-10* (Y834), *cdc3-1* (Y524), *cdc10-1* (Y526), *cdc11-1* (Y528), and *cdc12-1* (Y529).
* For the isogenic wild-type strain, Y172, cells containing a single bud and bud scar were scored.

cdc10-10, cdc3-1, cdc11-1, and cdc12-1 Cells Often Form Buds at Distal Sites

Since *cdc10-10* cells exhibit altered budding patterns, the fidelity of bud site selection was examined in cells with mutations in the other genes believed to encode neck filament proteins. Haploid *cdc10-1*, *cdc3-1*, *cdc11-1*, and *cdc12-1* cells were grown at the permissive temperature (25°C), semipermissive temperature (30°C) or restrictive temperature (37°C), and stained with Calcofluor and Hoechst 33258.

In general, mutant cells grown at 25°C have normal cell shapes, as determined by differential interference microscopy, and normal budding patterns, as determined by Calcofluor staining (data not shown). *cdc3-1*, *cdc10-1*, *cdc11-1*, and *cdc12-1* cells grown at the semipermissive temperature often exhibit elongated shapes, and many cells are connected, indicative of a cytokinesis defect (Pringle and Hartwell, 1981; Fig. 8). The unseparated cells are connected in tandem linear array or reside adjacent to one another, suggesting that cells grow buds primarily at proximal and distal sites. The position of bud site formation was quantitated in cells with two buds using the classification scheme described above. For each cytokinesis mutant, *cdc3-1*, *cdc10-1*, *cdc11-1*, and *cdc12-1*, the two buds were either adjacent to one another (33–50%; Class I) or opposite one another (37–56%; Class III) (Fig. 8 and Table II) in the vast majority of the cells. Few cells budded at other sites (11–14%; Class II). These results suggest that when cells contain a defect in one of the putative neck filament proteins, new buds form at either proximal sites or distal sites.

Discussion

In this report, a new allele of *CDC10*, *cdc10-10*, is described. The *cdc10-10* mutation causes a severe growth defect in combination with *spa2-Δ1*, suggesting a genetic interaction between the *CDC10* and *SPA2* genes. *cdc10-10* cells (in the presence or absence of *SUP4*) form buds at non-proximal sites, indicating that *CDC10* plays a role in bud site selection in yeast.

The CDC10 Gene Is Not Essential for Cell Growth in Yeast

The *CDC10* gene was disrupted and shown to be nonessential for cell growth. Most of the genes defined by thermal-sensitive *cdc* mutations that have been analyzed thus far encode essential proteins (e.g., Johnson et al., 1985; Johnston et al., 1991); thus, *CDC10* is somewhat unusual in that it is required for growth only at higher temperatures. Since the CDC3, CDC11, and CDC12 proteins are each predicted to be related in sequence to CDC10, it is likely that CDC10 function is to some extent redundant with that of one or more of the other putative neck filament proteins. Consistent with this possibility, 2 μm plasmids containing either *CDC3* or *CDC12* weakly suppress the *cdc10-10 spa2-Δ1* nonsectoring

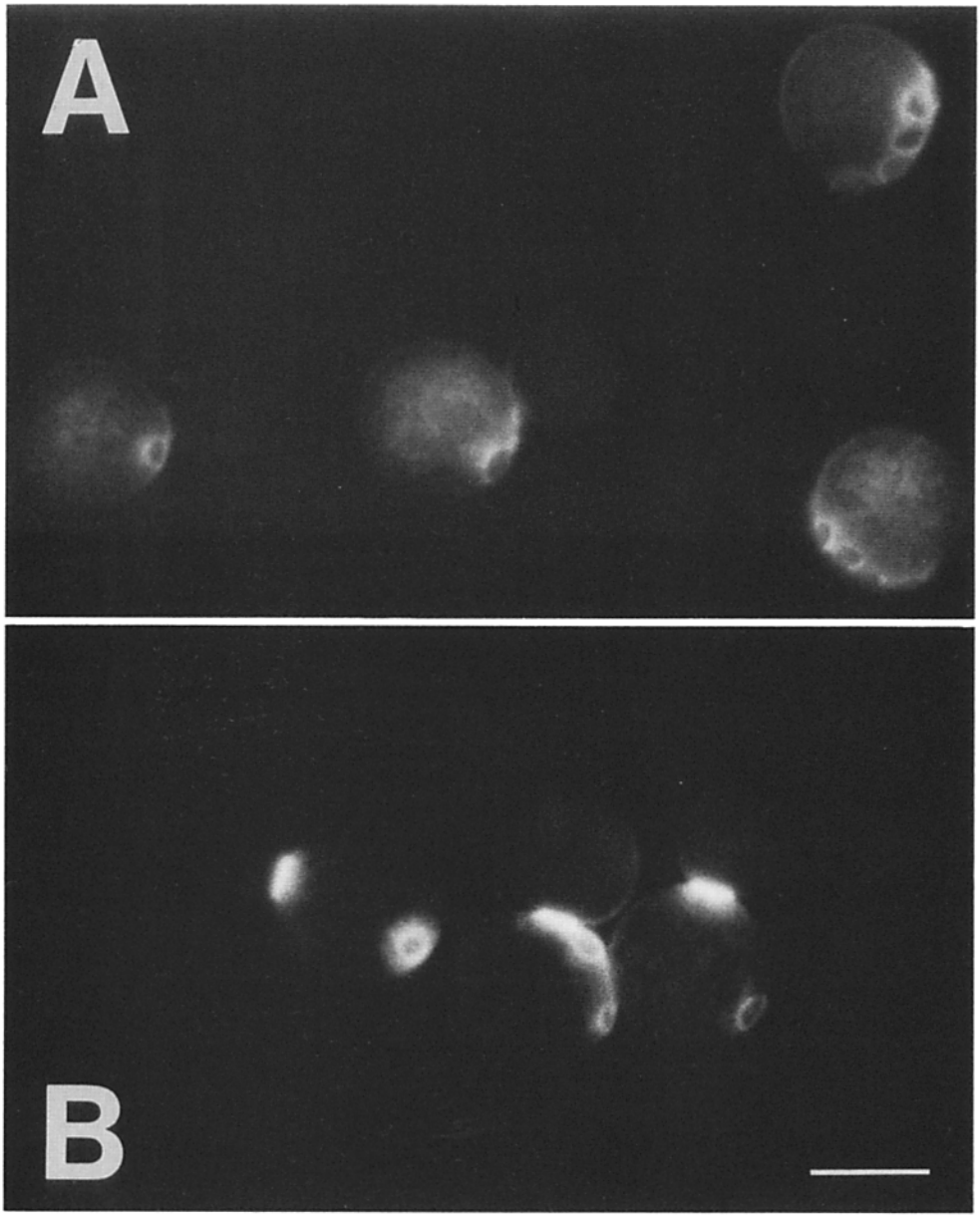

Figure 7. Sites of bud formation in wild-type and *cdc10-10 SUP4* cells stained with Calcofluor. (*A*) Wild-type cells (Y574 containing the *SPA2* plasmid). The cell in the center has a bud scar that is out of the plane of focus. The scar lies adjacent to the bud and the visible scar. (*B*) *cdc10-10 SUP4* cells (Y831). Bar, 5 μm.

phenotype (Flescher, E.G., K. Madden, and M. Snyder, unpublished results).

The *cdc10-10* allele contains an ochre mutation at codon 133. In the absence of an ochre-suppressing tRNA, a protein less than half of the size of the wild-type protein is expected to be produced in *cdc10-10* cells. No downstream methionine codons are present near the ochre codon, ruling out the possibility of translation reinitiation and the restoration of CDC10 function via two truncated CDC10 proteins. Since the phenotypes conferred by the *cdc10-10* mutation are similar to those of the *cdc10-Δ* alleles, it is likely that *cdc10-10* is a null mutation.

In the presence of *SUP4*, the *cdc10-10* mutation is largely suppressed, as expected for an ochre mutation. The CDC10 protein in *SUP4*-suppressed strains is expected to contain a tyrosine at position 133, whereas the wild-type CDC10 protein and its homologs in other species contain glutamine or asparagine at that position. Thus, substituting a residue with a neutral polar hydroxyl side chain for one containing a neutral polar amide group does not significantly affect CDC10 function.

A Genetic Interaction between SPA2 and CDC10 Suggests a Role for SPA2 in Cytokinesis

Before this study, two observations suggested that *SPA2* plays a role in cytokinesis. First, the SPA2 protein localizes at the bud neck in cells that are undergoing cytokinesis; second, *spa2-Δ* cells exhibit a very slight cytokinesis defect (Snyder et al., 1991). Since *CDC10* is important for cytokinesis, the severe growth defect/lethality of the *spa2-Δ1 cdc10-10* double mutant provides additional strong evidence that *SPA2* plays a role in this process. It is unlikely that the growth defect of *spa2-Δ1 cdc10-10* cells is due to the combined effect of two mutations that individually cause poor growth; *spa2-Δ1* mutants exhibit normal growth rates (Snyder, 1989; Costigan et

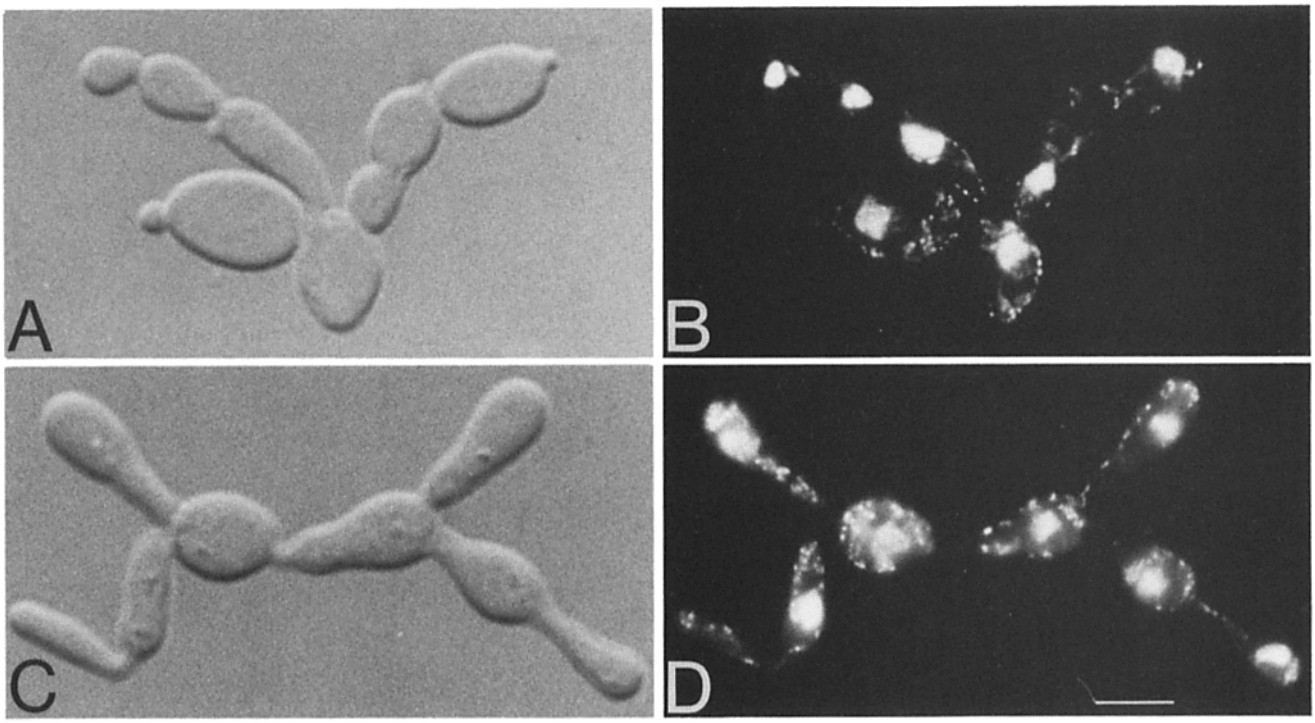

Figure 8. Morphology of *cdc11-1*, and *cdc12-1* cells grown at a semipermissive temperature (30°C). *A* and *B*, *cdc11-1* cells; *C* and *D*, *cdc12-1* cells. The left panels shows cells viewed by differential interference microscopy; the right panels show the Hoechst 33258 staining of the same cells. Bar, 5 μm.

al., 1992). Since both SPA2 and CDC10 proteins localize at the site of cytokinesis, it is possible that these two proteins interact physically.

It is unlikely that *SPA2* has redundant functions with *CDC10* or other putative neck filament genes; the *SPA2* gene when present on a high copy number 2 μm plasmid does not suppress the *cdc10-1*, *cdc3-1*, or *cdc12-1* growth defects (unpublished results). In addition, the SPA2 protein and the putative neck filament proteins have slightly different localization patterns: CDC3, CDC10, CDC11, and CDC12 each form a very tight ring at the neck, whereas the SPA2 protein localizes as a patch or very diffuse ring. Thus, it is likely that both SPA2 and the neck filament proteins contribute to the process of cytokinesis, but in different ways.

Bud Site Selection in Yeast

Cytokinesis tag models have been proposed previously to account for bud site selection in yeast (see Introduction). It has been suggested that, in cells which undergo proximal budding, critical components at the previous site of cytokinesis serve to nucleate assembly of components involved in forming the new bud at an adjacent site, and/or serve as a source of components for initiating bud formation at an adjacent site (Fig. 9) (Chant and Herskowitz, 1991; Snyder et al., 1991; Madden et al., 1992). In *MATa*/MATα diploid daughter cells, which preferentially undergo distal budding, the critical components are lost or modified and bud formation occurs at a secondary site, perhaps near the SPB or its associated microtubules (Snyder et al., 1991; Madden et al., 1992), or at previous sites where growth components have assembled. Since haploid and diploid strains homozygous at the mating locus (e.g., *MATa*/MAT*a* strains) differ in their budding patterns from *MATa*/MATα strains, the overall budding patterns must be controlled by the mating locus (see Chant and Herskowitz, 1991; Chant et al., 1991).

The timing of progression through the cell cycle can explain why diploid mother cells preferentially bud at proximal sites, while diploid daughters usually form buds at distal sites (Fig. 9). Diploid daughters undergo a longer G1 period than mother cells. If the tag directing bud site selection persists for only a limited time after cytokinesis, a long G1 period increases the probability that the tag will be lost or modified before budding initiates. Consequently, diploid daughter cells bud at secondary sites. Several observations correlate cell cycle delay with non-proximal budding patterns. For example, diploid mother cells that have budded several times ("old mothers") have been shown to have a longer G1 than mother cells that have budded only once (Egilmez and Jazwinski, 1989), and old mothers exhibit a greater preference for non-proximal sites (Snyder, 1989). Yeast cells that exit the cell cycle and enter stationary phase also use the secondary sites when they resume growth (Madden and Snyder, 1992).

The Role of the CDC10 Gene Product in Bud Site Selection

Like diploid daughter cells, *cdc10-10* mutants often bud at distal sites. This suggests that CDC10 is directly or indirectly involved in bud site selection in yeast. We speculate that CDC10 and the other neck filament proteins are components of (or interact with) the cortical tag that directs bud site selection. Supporting this hypothesis, these proteins localize to the neck and persist at the cortex from one cell cycle to the next (Ford and Pringle, 1991; Haarer and Pringle, 1987; Kim

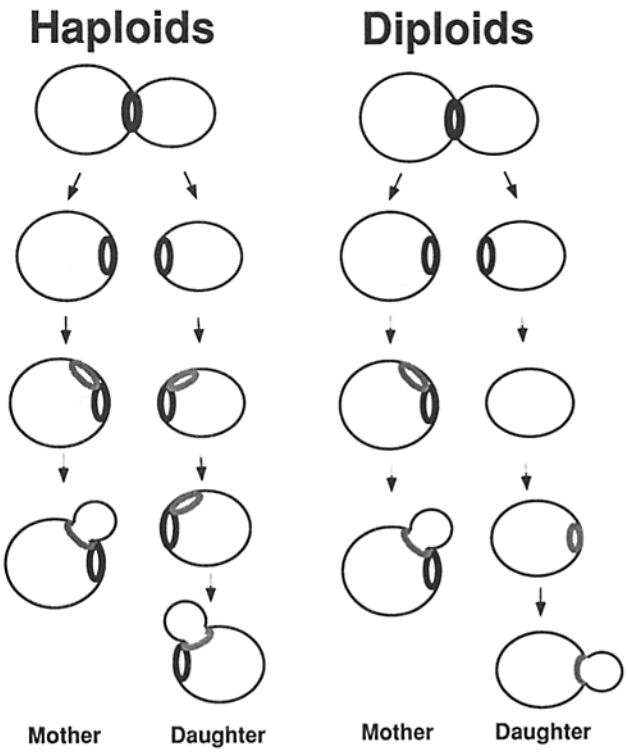

Figure 9. Models for bud site selection. Temporal progression through the cell cycle is from top to bottom. The dark ring indicates the tag remaining from cytokinesis; the material assembling for the new bud is depicted as a light ring. In diploid daughters, the tag is lost and the new material assembles at a secondary site, either opposite the SPB and/or its associated microtubules or a site of previous cell surface growth.

et al., 1991; Kim, H.B., B. K. Haarer, and J. R. Pringle, personal communication). *cdc10-10, cdc3-1, cdc10-1, cdc11-1,* and *cdc12-1* mutants grown at the semipermissive temperature often lose the tag or form a non-functional tag and bud at the secondary (distal) site. Since these cells also bud at proximal sites, the tag may not be entirely lost, either because the cells were grown at the semipermissive temperature and retain some functional product, or because other components that also contribute to the tag are present at the proximal site.

It is also possible that mutations in the putative neck filament proteins delay the cell cycle and thereby reduce the fidelity of tag-directed proximal budding. However, we favor the hypothesis that the neck filament proteins function specifically as part of the cytokinesis tag because of their localization patterns. Furthermore, J. Chant and J. Pringle have found that the BUD3 gene product, which is important in selection of proximal sites, also localizes to the neck at cytokinesis (Chant, J., and J. Pringle, personal communication). *bud3* mutants exhibit a bipolar pattern of budding similar to that of *cdc10-10* and other neck filament mutants (Chant and Herskowitz, 1991).

cdc10-10 mutants containing *SUP4* also have aberrant budding patterns. However, unlike *cdc10-10* cells, *cdc10-10 SUP4* cells often bud at random sites. Immunofluorescence localization studies suggest a possible explanation. In addition to its role in cytokinesis, CDC10 appears as a cortical ring at the bud site before bud emergence (Kim, H. B., S. R. Ketcham, B. K. Haarer, and J. R. Pringle, personal communication), suggesting that CDC10 may participate in the assembly of bud-forming components at the incipient bud site. In *cdc10-10 SUP4* mutants, the defective CDC10 protein might be unable to target to the correct bud site and therefore initiate the assembly of components at random sites. (In addition, the cytokinesis tag at the proximal site might be defective due to the *cdc10-10* mutation.) It is possible that many of the mutants that exhibit a random budding pattern form aberrant complexes that initiate bud formation at random sites (Snyder et al., 1991; Madden et al., 1992).

To summarize, we speculate that mutants defective only in the cytokinesis tag bud in bipolar patterns, while mutants with alterations in the targeting or assembly of proteins at the incipient bud site exhibit random budding patterns. Mutants defective in both the tag and the assembly of bud-forming components exhibit either bipolar or random patterns, depending on the nature of the mutation (i.e., *cdc10, spa2*).

The Role of a GTP-binding Protein in Filament Formation and Bud Site Selection

The CDC10 protein is predicted to bind GTP and is thought to be a component of the highly ordered array of 10-nm neck filaments. Since the putative CDC10 protein is not likely to contain a coiled-coil domain (like that of intermediate filament proteins which form 10-nm filaments), it is possible that the 10-nm neck filaments assemble from nucleotide binding proteins analogous to actin or tubulin filaments (e.g., Mitchison and Kirschner, 1984), and that the hydrolysis of GTP affects the kinetics of assembly of these filaments. Like CDC10, the FtsZ protein of *E. coli* also binds GTP, localizes as a ring at the septum formation site, and is important in cytokinesis (Bi and Lutkenhaus, 1991; RayChaudhuri and Park, 1992; de Boer et al., 1992). Thus, although FtsZ and the putative neck filament proteins are not very similar in their predicted protein sequences, it is possible that cytokinesis in many organisms is mediated by filaments formed from GTP-binding proteins.

The Cytokinesis Tag and the Determination of the Axis of Cell Division

The existence of specialized cortical sites involved in the determination of specific cleavage planes is not unique to the cytokinesis tag of the budding yeast cell. In some higher plants, a ring of microtubules and microfilaments called the preprophase band exists at the division site, and the microfilaments persist after cytokinesis to guide cell growth (Lloyd and Traas, 1988; Lloyd, 1991*a*,*b*). Treatment of *C. elegans* embryos with microtubule inhibitors and laser irradiation of microtubules extending from the centrosome to the cortex have shown that cortical sites of microtubule attachment are required for specification of division planes in early development (Hyman and White, 1987; Hyman, 1989). Several other observations or experimental manipulations in a wide variety of systems have demonstrated the involvement of cortical sites of microtubule attachment in the orientation of cell divisions (e.g., Dan and Inoue, 1987; Lutz et al., 1988).

In yeast, the cytokinesis tag is thought to direct assembly of components involved in bud formation to the cortex. Pre-

sumably, a subset of these components interacts with microtubule ends (Snyder et al., 1991), thus orienting the spindle axis in the proper plane (Page and Snyder, 1993). Elucidation of proteins and mechanisms involved in the establishment of the yeast cortical complex will contribute to the understanding of cleavage plane specification in other organisms.

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