CDP1, a Novel *Saccharomyces cerevisiae* Gene Required for Proper Nuclear Division and Chromosome Segregation

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ABSTRACT

To identify new gene products involved in chromosome segregation, we isolated *Saccharomyces cerevisiae* mutants that require centromere binding factor I (Cbf1p) for viability. One Cbf1p-dependent mutant (denoted *cdp1-1*) was selected for further analysis. The *CDP1* gene encodes a novel 125-kD protein that is notably similar to previously identified mouse, human and *Caenorhabditis elegans* proteins. *CDP1*Δ and *cdp1-1* mutant cells were temperature sensitive for growth. At the permissive temperature, *cdp1-1* and *cdp1*Δ cells lost chromosomes at a frequencies ~20-fold and ~110-fold higher than wild-type cells, respectively. These mutants also displayed unusually long and numerous bundles of cytoplasmic microtubules as revealed by immunofluorescent staining. In addition, we occasionally observed improperly oriented mitotic spindles, residing entirely within one of the cells. Presumably as a result of undergoing nuclear division with improperly oriented spindles, a large percentage of *cdp1* cells had accumulated multiple nuclei. While *cdp1* mutant cells were hypersensitive to the microtubule-disrupting compound thiabendazole, they showed increased resistance to the closely related compound benomyl relative to wild-type cells. Taken together, these results suggest that Cdp1p plays a role in governing tubulin dynamics within the cell and may interact directly with microtubules or tubulin.

THE faithful transmission of genetic information requires that when cells divide, each daughter cell receives one and only one copy of each chromosome. During mitosis in eukaryotic cells, the duplicated chromosomes bind to a microtubule-based spindle apparatus that guides them through a series of movements, culminating in the separation of chromosomes into two complete complements. Each of the chromosome complements is packaged into the nucleus of a daughter cell.

The assembly, function and disassembly of the mitotic spindle are complex processes that are only partially understood. In *Saccharomyces cerevisiae,* the nuclear envelope does not break down during mitosis and the spindle is contained entirely within the nucleus. The microtubule organizing center, or spindle pole body, is embedded in the nuclear envelope. Early in the cell cycle, the spindle pole bodies are duplicated and migrate to positions defining the spindle poles (reviewed in BYERS 1981). Before the onset of mitosis, nuclear microtubules (MT) polymerize from the duplicated spindle pole bodies. A subset of these MTs, called kinetochore MTs, form attachments to the chromosomes. Another subset consists of interdigitating MTs emanating from opposite poles (WINEY *et al.* 1995). Microtubules also project from the spindle pole bodies into the cytoplasm (the equivalent of astral MTs). During mitosis, these cytoplasmic MTs play a role in positioning the nucleus in the bud neck and in orienting the spindle so that it projects from the mother cell into the bud (JACOBS *et al.* 1988; PALMER *et al.* 1992; SULLIVAN and HUFFAKER 1992). Microtubule-based mechanochemical motors are thought to generate the forces that account for the movements of the nucleus and chromosomes during mitosis and that separate the spindle pole bodies (HOYT *et al.* 1992, 1993; ROOF *et al.* 1992; SAUNDERS and HOYT 1992; ESHEL *et al.* 1993; LI *et al.* 1993; MIDDLETON and CARBON 1994; SAUNDERS *et al.* 1995). Following mitosis, nuclear MTs become undetectable, whereas cytoplasmic MTs persist throughout the cell cycle. In *S. cerevisiae,* the function of cytoplasmic MTs during interphase is unknown. The differential regulation of polymerization and depolymerization of the various classes of MTs, the precise coordination of MT-mediated events with progression through the cell cycle, and the diversity of functions performed by MTs suggests that a significant number of as yet unidentified proteins are involved in MT dynamics and function.

In most organisms, spindle MTs attach to chromosomes at the kinetochore, a multiprotein complex assembled at the centromere. In *S. cerevisiae,* the centromere is relatively small, consisting of three conserved DNA elements, CDEI, CDEII and CDEIII, which together span ~125 bp (reviewed in CARBON 1984; CLARKE and CARBON 1985; FITZGERALD 1987; CARBON and CLARKE 1990; CLARKE 1990; MURPHY and FITZGERALD 1990). Several kinetochore constituents have been identified and cloned. The products of the *CTF13, NDC10/CBF2/CTF14* and *CEP3/CBF3b* genes form a

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complex that binds to the CDEIII region of the centromere (LECHNER and CARBON 1991; DOHENY *et al.* 1993; GOH and KILMARTIN 1993; JIANG *et al.* 1993; LECHNER 1994; STRUNNIKOV *et al.* 1995). Although the exact functions of these gene products are currently unknown, they are all essential for viability. The *CBF1/CEP1/CPF1* (hereafter referred to as *CBF1*) gene product binds to the CDEI region of the centromere (BAKER *et al.* 1989; CAI and DAVIS 1989, 1990; JIANG and PHILIPPSEN 1989; BAKER and MASISON 1990; MELLOR *et al.* 1990). Cells lacking Cbf1p are viable, but lose chromosomes at a frequency ~10-fold higher than wild-type cells. In addition, like many mutants defective in chromosome segregation, *cbf1*Δ mutants are more sensitive than wild-type cells to microtubule disrupting drugs (CAI and DAVIS 1990). Therefore, although not essential for growth under laboratory conditions, Cbf1p plays a role maintaining the high fidelity with which chromosomes are segregated.

Cells lacking Cbf1p are also unable to grow in the absence of exogenously provided methionine (BAKER and MASISON 1990; CAI and DAVIS 1990). This methionine auxotrophy is due, at least in part, to a requirement for Cbf1p in transcription of the *MET16* gene (THOMAS *et al.* 1992; O'CONNELL *et al.* 1995) We have shown previously that single amino acid alterations in Cbf1p can preferentially impair either its ability to function in chromosome segregation or its ability to function in transcription (FOREMAN and DAVIS 1993). These results demonstrate that the roles Cbf1p plays in each of these capacities are mechanistically distinct.

Because chromosome segregation is a fundamental aspect of cell division, it is important to identify and define the roles of gene products involved in this process. We have performed a genetic screen for novel gene products that play a role in chromosome segregation. This screen makes use of the fact that chromosome segregation is more efficient in cells that contain Cbf1p than in cells from which the *CBF1* gene has been deleted. Mutations in other genes whose products are involved in chromosome segregation might be expected to exacerbate the effects of a *CBF1* deletion, resulting in inviability. Based on this rationale, we have performed a screen for mutants that can be propagated in the presence of Cbf1p, but that are unable to grow in the absence of Cbf1p. These mutants have been designated *cdp* (Cbf1p-dependent). In this paper, we describe one of these mutants, *cdp1,* which appears to have a defect in microtubule function, resulting in improper chromosome segregation and nuclear division.

MATERIALS AND METHODS

Strains, media and microbiological techniques: Yeast strains used in this study were congenic (W303 derivatives) and are listed in Table 1. Standard molecular cloning techniques (SAMBROOK *et al.* 1989) and yeast genetic techniques and media were used (SHERMAN 1991). Microtubule disrupting drugs were dissolved in DMSO (benomyl) (DuPont) or dimethylformamide (thiabendazole) (Sigma) and added to molten media.

Plasmids: pMW29LYS2 was provided by M. WAHLBERG (University of Texas Southwestern Medical Center). It is a derivative of pMW29 (ZIELER *et al.* 1995), a pUC18-based plasmid containing *CEN4 ARS1 ADE3 LYS2 GAL1* promoter-polylinker-*GAL7* terminator. In YCpCBFAL, the *CBF1* gene is cloned into the polylinker region of pMW29LYS2 along with 254 bp of the *CBF1* promoter. In this configuration the *CBF1* gene is expressed from its own promoter and is not under the control of the *GAL1* promoter. p1C12Δ95–2922 contains an *Eco*RI-*Cla*I (bps −196–95) fragment and a *Bgl*II-*Eco*RI (bps 2922–3695) fragment from the *CDP1* gene joined at the *Eco*RI sites and subcloned into *Cla*I, *Bam*HI-cleaved pRS304 (which contains the *TRP1* marker) (SIKORSKI and HIETER 1989).

Screen for *CBF1*-dependent mutants: Yeast strains YNN534 and YNN535 were mutagenized with ethylmethane sulfonate or UV light to ~30% viability (SHERMAN 1991). UV-mutagenized cells were allowed to recover in the dark for 48 hr. Following mutagenesis, ~50,000 cells were plated on YPD and the resulting colonies were examined for the presence of white sectors. Colonies that appeared to lack sectors were patched to YPD plates and reexamined for sectoring using a dissecting microscope. Three hundred thirty-seven candidates failed to sector in this test and were streaked to medium containing 0.2% α-aminoadipate (Sigma). One hundred thirty-one mutants did not grow on this medium and were transformed in parallel with the plasmid pUN55 (ELLEDGE and DAVIS 1988) or its derivative YCpCBFUS, which differs only in that it contains the *CBF1* gene. In 48 mutants, sectoring and growth on α-aminoadipate-containing medium were restored by YCpCBFUS, but not by pUN55.

Cloning of *CDP1*: *cdp1–1* cells were transformed, using the lithium acetate method (ITO *et al.* 1983), with a yeast genomic library that carries a *URA3* marker on a centromere-based (low copy number) plasmid (RAMER *et al.* 1992). Approximately 20,000 *URA*$^+$ colonies were replica-plated to synthetic minimal media containing necessary supplements as well as uracil and 0.2% α-aminoadipate. After 2 days of growth, replicas were once again made onto fresh α-aminoadipate-containing medium. Surviving colonies were replicated to YPD plates, plates containing 0.1% 5-fluoroorotic acid (5-FOA), and plates lacking methionine. Colonies that were white (confirming that they had lost pCBFAL) and failed to grow on both methionine-free medium (indicating that they were not *CBF1*-containing clones) and 5-FOA-containing medium were chosen for further analysis.

Two plasmids, containing overlapping DNA fragments of ~3.4 and ~3.7 kb were recovered that complemented the *CBF1* dependence, temperature sensitivity and thiabendazole hypersensitivity of the *cdp1* mutant. The plasmids were isolated and transformed into *Escherichia coli* by standard methods (GUTHRIE and FINK 1991).

Sequencing was performed by the Beckman Center PAN facility by thermocycling using custom oligonucleotide primers, dye terminators and Taq DNA polymerase. Reaction products were analyzed using an Applied Biosystems model 373 sequencing system. Both strands were sequenced. The sequence was analyzed using the Intelligenetics Suite (Intelligenetics, Inc., Mountain View, CA), GCG (Genetics computer group, Madison, WI), and NCBI (Bethesda, MD) sequence analysis packages.

To demonstrate linkage of the complementing DNA to the mutant phenotypes, an 897-bp *Eco*RI fragment (bps 2798–3695) containing the 3′-end of the single open reading frame was subcloned into the integrating plasmid pRS304, which carries the *TRP1* marker. (SIKORSKI and HIETER 1989). The

TABLE 1

Yeast strains

Strain	Genotype	Source
YCRY2	*α ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1*	R. FULLER
YCRY3	**a**/*α ade2-1/ade2-1 his3-11,15/his3-11,15 trp1-1/trp1-1 leu2-3,112/leu2-3,112 ura3-1/ura3-1*	R. FULLER
YNN531	*α ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1, cbf1Δ::LEU2*	FOREMAN and DAVIS (1993)
YMW80	**a** *ade2-1 ade3Δ22 his3-11,15 leu2-3,112 trp1-1 ura3-1 lys::hisG*	M. WAHLBERG
YNN532	*α ade2-1 ade3Δ22 his3-11,15 leu2-3,112 trp1-1 ura3-1 lys2::hisG cbf1Δ::LEU2*	This study
YNN533	**a** *ade2-1 ade3Δ22 his3-11,15 leu2-3,112 trp1-1 ura3-1 lys2::hisG cbf1Δ::LEU2*	This study
YNN534	*α ade2-1 ade3Δ22 his3-11,15 leu2-3,112 trp1-1 ura3-1 lys2::hisG cbf1Δ::LEU2* [YCpCBFAL]	This study
YNN535	**a** *ade2-1 ade3Δ22 his3-11,15 leu2-3,112 trp1-1 ura3-1 lys2::hisG cbf1Δ::LEU2* [YCpCBFAL]	This study
YNN536	*α ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 cdp1-1*	This study
YNN537	**a** *ade2-1 ade3Δ22 his3-11,15 leu2-3,112 trp1-1 ura3-1 lys2::hisG cbf1Δ::LEU2 cdp1-1* [YCpCBFAL]	This study
YNN538	**a** *ade2-1 ade3Δ22 his3-11,15 leu2-3,112 trp1-1 ura3-1 lys2::hisG cbfΔ::LEU2 cdp1-1* [YCpCBFUS]	This study
YNN539	*α ade2-1 ade3Δ22 his3-11,15 leu2-3,112 trp1-1 ura3-1 lys2::hisG cbf1Δ::LEU2 CDP1/cdp1::TRP1*	This study
YNN540	**a**/*α ade2-1/ade2-1 his3-11,15/his3-11,15 trp1-1/trp1-1 leu2-3,112/leu2-3,112 ura3-1/ura3-1 cdp1-1/cdp1-1*	This study
YNN541	**a**/*α ade2-1/ade2-1 his3-11,15/his3-11,15 trp1-1/trp1-1 leu2-3,112/leu2-3,112 ura3-1/ura3-1* CFIII(D8B.d) *URA3 SUPP11*	This study
YNN542	**a**/*α ade2-1/ade2-1 his3-11,15/his3-11,15 trp1-1/trp1-1 leu2-3,112/leu2-3,112 ura3-1/ura3-1 cdp1-1/cdp1-1* CFIII(D8B.d) *URA3 SUP11*	This study
YNN543	**a** *ade2-1 his3-11,15 trp1-1 leu2-3,112 ura3-1 cdp1Δ::TRP1*	This study
YNN544	**a**/*α ade2-1/ade2-1 his3-11,15/his3-11,15 trp1-1/trp1-1 leu2-3,112l/eu2-3,112 ura3-1/ura3-1 cdp1Δ::TRP1/cdp1Δ::TRP1*	This study
YNN545	**a**/*α ade2-1/ade2-1 his3-11,15/his3-11,15 trp1-1/trp1-1 leu2-3,112l/eu2-3,112 ura3-1/ura3-1 cdp1Δ::TRP1/cdp1Δ::TRP1* CFIII(D8B.d) *URA3 SUP11*	This study

resulting plasmid, pRS304RI900, was linearized with *Bgl*II, which cleaves within the subcloned fragment, and used to transform strain YNN532. Integration of the plasmid at the *CDP1* locus was confirmed in strain YNN539 (a *TRP1*$^+$ transformant) by Southern blotting. The integration resulted in a partial duplication of *CDP1* without disruption of the wild-type gene. YNN539 was mated to YNN537 and the resulting diploid was sporulated. In all 23 tetrads analyzed, the *cdp1* temperature-sensitive phenotype and the *TRP1* marker segregated away from one another, indicating that the cloned DNA is tightly linked to the *CDP1* gene.

Chromosome fragment loss assay: Strains YCRY3, YNN540, and YNN544 were transformed with the plasmid pYCF5/CEN6 (HEGEMANN *et al.* 1988) linearized at the *Not*I site. The resulting strains, YNN541, YNN542 and YNN545, carried an ~135-kb linear fragment of chromosome *III* bearing *URA3* and *SUP11* markers. Quantitation of the loss frequency of the marked chromosome fragment was performed as described by SHERO *et al.* (1991).

Cell fixation and staining: To count nuclei, cells were fixed with ethanol and stained with 1 μg/ml 4′,6-diamino-2-phenylindole (DAPI) as described (PRINGLE *et al.* 1991). Microtubules were stained with YOL-34 rat anti-tubulin (Accurate Scientific Corporation) antibodies and fluorescein-labeled goat anti-rat secondary antibodies (Cappel) (STEARNS *et al.* 1990).

Disruption of the *CDP1* gene: p1C12Δ95–2922 was linearized with *Eco*RI and used to transform YCRY3. *TRP1*$^+$ transformants were analyzed by Southern blot to confirm that the plasmid had integrated at the *CDP1* locus. One transformant, heterozygous for a deletion of nucleotides 95–2922 of the *CDP1* gene, was sporulated and tetrads were dissected to obtain haploid *TRP1*$^+$ cells lacking *CDP1*.

RESULTS

Identification of yeast mutants that depend on *CBF1* for viability: Under standard laboratory conditions, Cbf1p is a nonessential gene product that increases the fidelity with which chromosomes are segregated. Presumably, certain aspects of the chromosome segregation process are made more efficient by Cbf1p. To investigate which processes might be facilitated by Cbf1p, we performed a screen for mutants in which the *CBF1* gene is essential for viability.

The overall strategy used in this screen is similar to previous studies (KRANZ and HOLM 1990; BENDER and PRINGLE 1991; ZIELER *et al.* 1995). The background strains used (YNN532 and YNN533, which differ from one another only with respect to mating type) have

deletions of the *cbf1, ade3* and *lys2* genes. These strains also contain an *ade2-1* mutation, which, in the presence of an otherwise intact adenine biosynthetic pathway, results in the accumulation of red pigment (JONES and FINK 1981). The plasmid YCpCBFAL, which contains the wild-type *CBF1, ADE3* and *LYS2* genes, was transformed into these strains to make YNN534 and YNN535. Because Ade3p lies upstream of Ade2p in the adenine biosynthetic pathway, cells containing YCpCBFAL (which confers wild-type Ade3p function) are red, whereas cells lacking the plasmid are white. Since *CBF1* is a nonessential gene, cells that lose YCpCBFAL are viable. As a consequence wild-type colonies are composed of red sectors (consisting of cells containing YCpCBFAL) and white sectors (consisting of cells from which this plasmid has been lost). However, in mutants that depend on Cbf1p for viability, cells that lose YCpCBFAL are unable to grow. *CBF1*-dependent mutants, therefore, form colonies that are red with no white sectors.

Strains YNN534 and YNN535 were mutagenized and screened for red nonsectoring colonies. Three hundred thirty-seven mutants that appeared to require YCpCBFAL by this criterion were then subjected to a secondary screen in which they were assessed for their ability to grow on media containing α-aminoadipate. α-aminoadipate selects against cells with wild-type Lys2p function (encoded on the indicator plasmid, YCpCBFAL). Therefore only those cells from which YCpCBFAL has been lost are able to grow on this medium. One hundred thirty-one of the 337 nonsectoring mutants failed to grow on α-aminoadipate-containing medium, indicating that these mutants required some sequence within YCpCBFAL for ongoing cell division.

To determine whether it was the *CBF1* gene in YCpCBFAL required by these mutants for viability, two plasmids unrelated to YCpCBFAL were individually transformed into each candidate mutant. These two plasmids, pYCBFUS and pUN55, differ only in that pYCBFUS carries *CBF1*, whereas pUN55 does not. In 48 of the remaining 131 candidates, only pYCBFUS restored both the ability to form white sectors and the ability to grow on medium containing α-aminoadipate. The presence of a second *CBF1* gene in these mutants therefore relieves selective pressure for retaining YCpCBFAL, strongly suggesting that these mutants depend on Cbf1p expression for viability.

Since Cbf1p functions in both transcriptional activation and in chromosome segregation, an additional analysis was performed to assist in identifying mutants that depend on *CBF1* primarily for its function in chromosome segregation. The 48 *CBF1*-dependent mutants were tested for their ability to grow on media containing the microtubule-disrupting compound thiabendazole. Failure to grow in the presence of low concentrations of related benzimidazole compounds is a feature shared by many mutants defective in chromosome segregation (CAI and DAVIS 1990; STEARNS *et al.* 1990; BROWN *et al.* 1993). Twelve of the 48 *CBF1*-dependent mutants displayed an increased sensitivity to thiabendazole relative to wild-type cells.

TABLE 2

Capacity of *cbf1* separation of function alleles to support growth in the *cdp1* mutant

Relevent genotype	Doubling time (hr)
CDP1 CBF1	1.89
CDP1 cbf1-9	2.29
CDP1 cbf1-16	3.04
cdp1-1 CBF1	2.74
cdp1-1 cbf1-9	6.01
cdp1-1 cbf1-16	34.20

Strains YNN531 (*CDP1 cbf1Δ*) and YNN537 (*cdp1-1 cbf1Δ*) were transformed with plasmids containing *CBF1, cbf1-9* or *cbf1-16*. YCpCBFAL was evicted from YNN537 transformants by selection on α-aminoadipate. Doubling times were determined at 30° by measuring the optical density of liquid cultures at 600 nm.

Each of the 12 *CBF1*-dependent, thiabendazole-hypersensitive mutants was backcrossed to wild-type cells. In four of these mutants, the *CBF1*-dependence and thiabendazole hypersensitivity cosegregated as defects in single genes upon sporulation of the diploids. Each of the mutations was recessive and belonged to a separate complementation group. The four complementation groups were designated *CDP1, CDP2, CDP3* and *CDP4*. The single allele of *CDP1* isolated, *cdp1-1*, failed to grow at 37° and was analyzed in greater detail.

The *cdp1* mutant depends on CBF1 for its function in chromosome segregation: To further explore the nature of the dependence of the *cdp1* mutant on *CBF1* for ongoing cell division, two mutant alleles of *cbf1* were examined for their ability to support growth of the *cdp1-1* mutant. *cbf1-9* and *cbf1-16* contain single amino acid substitutions that preferentially impair the ability of Cbf1p to function in transcriptional activation and chromosome segregation, respectively (FOREMAN and DAVIS 1993). Cells containing *cbf1-9* are largely competent for the chromosome segregation function of Cbf1p, but are methionine auxotrophs, indicating that they have a severe defect in Cbf1p-mediated transcriptional activation. In contrast, cells containing *cbf1-16* are markedly compromised in chromosome segregation, but phenotypically wild type for transcriptional activation. Each of these alleles was substituted for a wild-type copy of *CBF1* in wild-type and *cdp1-1* mutant cells. Table 2 shows that the *cbf1-9, cbf1-16* and *cdp1-1* alleles each have modest effects on the rate of growth of cells on rich medium at 30°. In cells bearing both *cbf1-9* and *cdp1-1*, the doubling time is increased to approximately twice that of cells carrying the *cdp1-1* mutation alone and almost three times that of cells containing *cbf1-9* alone. Interestingly, in cells bearing both

cbf1-16 and *cdp1-1* the doubling time is more than 12 times slower than *cdp1-1* cells and 11 times slower than cells containing *cbf1-16* alone. The fact that cells containing *cbf1-16* grow dramatically slower than cells containing *cbf1-9* in the *cdp1-1* background (but only slightly slower in a wild-type background) suggests that the *cdp1* mutant depends on *CBF1* primarily for its function in chromosome segregation, and supports the notion that the *cdp1-1* mutant may be defective in some aspect of chromosome segregation. Furthermore, these results formally demonstrate that the *cdp1-1* mutant depends on expression of Cbf1p, rather than merely the presence of the *CBF1* gene, for viability.

Cloning and sequence analysis of the *CDP1* gene: The *CDP1* gene was cloned by complementing the dependence of the *cdp1-1* mutant on *CBF1*. A *URA3*-marked, low copy number yeast genomic plasmid library with an average insert size of 4 kb (RAMER *et al.* 1992) was transformed into YNN537. Approximately 20,000 transformants were replica plated to medium containing α-aminoadipate to select against cells containing the *CBF1*-bearing plasmid YCpCBFAL. Six transformants were obtained that grew robustly on this medium, indicating that they no longer required YCpCBFAL for growth. Three of these maintained methionine prototrophy following eviction of YCpCBFAL, suggesting that they had received a wild-type copy of *CBF1* from the library. The remaining three were methionine auxotrophs. To assess whether growth on α-aminoadipate required sequences the transformants had received from the library, they were plated on medium containing 5-FOA, which selects against cells containing the *URA3* marker. All six clones failed to grow on this medium indicating that following eviction of YCpCBFAL, they required the library plasmid for growth. Library plasmids were isolated from two met^- and one met^+ transformant. Restriction mapping of the plasmid that conferred methionine prototrophy confirmed that the insert contained the *CBF1* gene. Transformation of YNN537 with each of the remaining two plasmids, derived from the methionine auxotrophs, rescued the *CBF1*-dependence, thiabendazole hypersensitivity and temperature sensitivity of this strain. These plasmids had inserts of ~3.4 and ~3.7 kb that overlapped over the length of the smaller clone.

The larger plasmid insert was sequenced entirely. It contained a 3413-bp open reading frame (ORF) (Figure 1) spanning almost the entire insert. A nearly identical sequence derived from the left arm of chromosome *XV* was recently identified by the yeast genome sequencing project (CASSAMAYOR *et al.* 1995). The two sequences differ at amino acids 197, 461, 674, 786, 903, 907, 926, 927, 956, 957, 959 and 987. These differences may arise from sequence variations between the different strains used in these studies. The 1077-amino acid protein encoded by this ORF has a predicted molecular weight of 125 kD. The most similar sequence in the databases is a hypothetical 1174-amino acid human protein (GenBank D63875) that is 27% identical over its entire length. A recently reported mouse protein, $p150^{TSP}$, displays a similar level of identity and is 99% identical to the human sequence (MALEK *et al.* 1996). In addition, the predicted Cdp1p sequence is 19% identical to a *C. elegans* ORF (SULSTON *et al.* 1992) (PIR S28279) that is 31% identical to $p150^{TSP}$. Cdp1p may, therefore, belong to an evolutionarily conserved family of proteins.

To demonstrate that the ORF recovered by complementing the *cdp1-1* mutation represents the *CDP1* gene, a *TRP1* marker was integrated at the locus of the cloned gene by homologous recombination. In the resulting strain (YNN539), the *CDP1* gene is partially duplicated without disruption of the wild-type gene. YNN539 was mated to YNN537 (*cdp1-1*) and tetrads were dissected. In all 23 tetrads analyzed, the *cdp1* temperature-sensitive phenotype and the *TRP1* marker segregated away from one another, indicating that the cloned DNA is tightly linked to the *CDP1* gene (<4.5 cM).

The *cdp1* gene is essential at 37° and is required for sporulation: To further explore the role of Cdp1p in cell division, the *CDP1* gene was deleted by one step gene replacement (ROTHSTEIN 1983). Replacement of amino acids 31–974 with the *TRP1* gene revealed that cells lacking Cdp1p grew very poorly at 30°, and like cells containing *cdp1-1*, were inviable at 37°. Both of these defects were rescued by a *CDP1*-containing plasmid. Figure 2A shows a time course of growth arrest for *cdp1-1* and *cdp1*Δ cells at 37°. At the restrictive temperature, the behavior of cells containing these alleles is indistinguishable. Growth arrests slowly over the course of two to three generations and the distribution of cells through the cell cycle after 3 and 8 hr at the restrictive temperature is very similar to that observed in wild-type cells (data not shown). As shown in Figure 2B, *cdp1* cells lose viability concomitant with the cessation of division. In addition, diploid cells homozygous for either *cdp1-1* or *cdp1*Δ fail to sporulate.

To determine whether cells lacking Cdp1p also depend on Cbf1p for viability, *cdp1*Δ cells (strain YNN543) were crossed with *cbf1*Δ cells (YNN531), in which the *CBF1* gene has been replaced with the *LEU2* gene. The resulting LEU^+, TRP^+ diploids were sporulated and tetrads were dissected. Sixteen tetrads were analyzed and although colonies were observed that were either LEU^+ (*cbf1*Δ) or TRP^+ (*cdp1*Δ), no colonies were observed that were both LEU^+ and TRP^+. Therefore, like cells containing the *cdp1-1* allele, *cdp1*Δ cells require Cbf1p for growth.

***cdp1* mutants are differentially sensitive to benzimidazole compounds:** Like many mutants defective in chromosome segregation, the *cdp1-1* mutant was unable to grow on a media containing a low concentration of the benzimidazole compound thiabendazole. Both thiabendazole and the closely related benzimidazole

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                                    ↓ 35                                    70
MTNAMKVEGY PSMEWPTSLD IPLKASEELV GIDLETDLPD DPTDLKTLLV EENSEKEHWL TIALAYCNHG

                                   105                                     140
KTNEGIKLIE MALDVFQNSE RASLHTFLTW AHLNLAKGQS LSVETKEHEL TQAELNLKDA IGFDPTWIGN

                                   175                                     210
MLATVELYYQ RGHYDKALET SDLFVKSIHA EDHRSGRQSK PNCLFLLLRA KLLYQKKNYM ASLKIFQELL

                                   245                                     280
VINPVLQPDP RIGIGLCFWQ LKDSKMAIKS WQRALQLNPK NTSASILVLL GEFRESFTNS TNDKTFKEAF

                                   315                                     350
TKALSDLNNI FSENQHNPVL LTLLQTYYYF KGDYQTVLDI YHHRILKMSP MIAKIVLSES SFWCGRAHYA

                                   385                                     420
LGDYRKSFIM FQESLKKNED NLLAKLGLGQ TQIKNNLLEE SIITFENLYK TNESLQELNY ILGMLYAGKA

                                   455                                     490
FDAKTAKNTS AKEQSNLNEK ALKYLERYLK LTLATKNQLV VSRAYLVISQ LYELQNQYKT SLDYLSKALE

                                   525                                     560
EMEFIKKEIP LEVLNNLACY HFINGDFIKA DDLFKQAKAK VSDKDESVNI TLEYNIARTN EKNDCEKSES

                                   595                                     630
IYSQVTSLHP AYIAARIRNL YLKFAQSKIE DSDMSTEMNK LLDLNKSDLE IRSFYGWYLK NSKERKNNEK

                                   665                                     700
STTHNKETLV KYNSHDAYAL ISLANLYVTI ARDGKKSRNP KEQEKSKHSY LKAIQLYQKV LQVDPFNIFA

                                   735                                     770
AQGLAIIFAE SKRLGPALEI LRKVRDSLDN EDVQLNLAHC YLEMREYGKA IENYELVLKK FDNEKTRPHI

                                   805                                     840
LNLLGRAWYA RAIKERSVNF YQKALENAKT ALDLFVKESS KSKFIHSVKF NIALLHFQIA ETLRRSNPKF

                                   875                                     910
RTVQQIKDSL EGLKEGLELF RELNDLKEFN MIPKEELEQR IQLGETTMKS ALERSLNEQE EFEKEQSAKI

                                   945                                ↓    980
DEARKILEEN ELKEQERMKQ EEEARRLKLE KQAEEYRKLQ DEAQKLIQER EAMAISEHNV KDDSDLSDKD

                                  1015                                    1050
NEYDEEKPRQ KRKRSTKTKN SGESKRRKAA KKTLSDSDED DDDVVKKPSH NKGKKSQLSN EFIEDSDEEE

                             1077
AQMSGSEQNK NDDNDENNDN DDNDGLF
```

FIGURE 1.—Predicted amino acid sequence of Cdp1p. The endpoints of the region deleted in strains YNN543, YNN544 and YNN545 are indicated by vertical arrows. The full nucleotide sequence of *CDP1* is available from GenBank under accession number U31217.

compound benomyl (the active breakdown product of which is carbendazim) bind to tubulin heterodimers and inhibit microtubule polymerization *in vivo* and *in vitro* (HOEBEKE *et al.* 1976; DAVIDSE and FLACH 1977, 1978; FRIEDMAN and PLATZER 1978) reviewed in DAVIDSE (1986). To determine the relative effects of these compounds on the *cdp1* mutants, serial dilutions of wild-type and mutant cells were spotted onto solid media containing various concentrations of benomyl or thiabendazole. Unexpectedly, although *cdp1-1* and *cdp1*Δ cells were considerably more sensitive than wild-type cells to thiabendazole in the medium, they grew significantly better than wild-type cells on benomyl-containing medium. Figure 3 shows that the *cdp1* mutant cells are unable to grow on medium containing 80 μg/ml thiabendazole, whereas the growth of wild-type cells is not eliminated until the concentration reaches 120 μg/ml. In contrast, the growth of wild-type cells is significantly inhibited at 10 μg/ml benomyl, whereas 15 μg/ml is required to observe a significant effect on the growth of the *cdp1* mutant cells.

***cdp1* mutants exhibit defects in nuclear division and chromosome segregation:** The nuclear morphology of *cdp1* mutants was examined. Wild-type, *cdp1-1* and *cdp1*Δ cells were grown logarithmically at 30° and then shifted to 37° for 3 hr. Cells were fixed and the nuclei

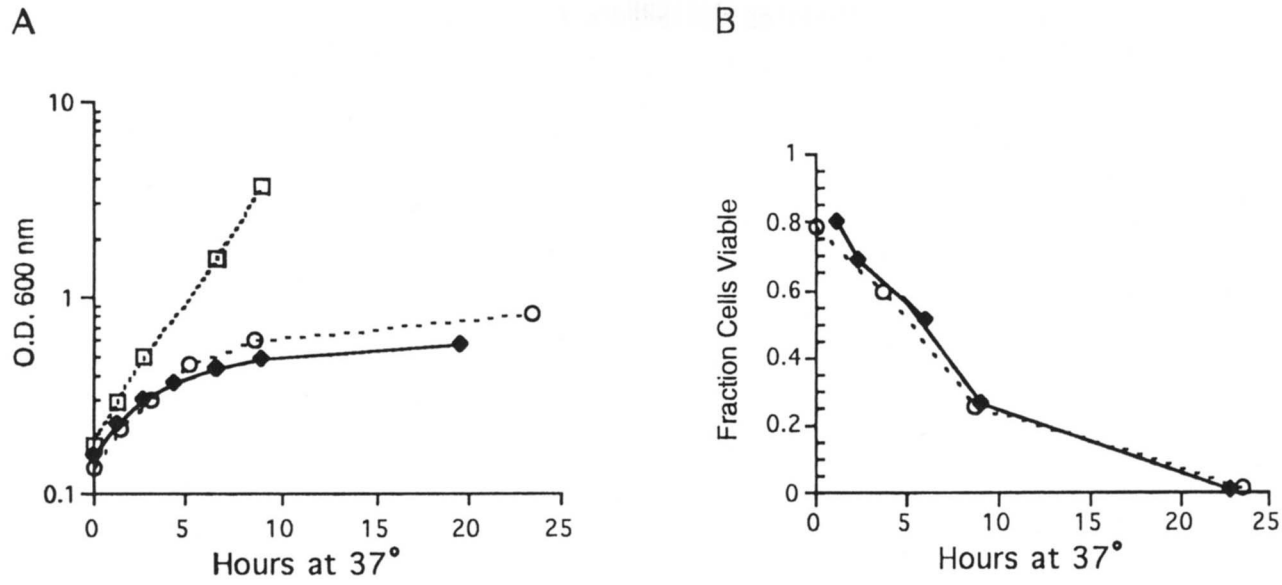

FIGURE 2.—Growth curve and viability of *cdp1* mutant cells at 37°. YCRY3 (wild-type, □), YNN540 (*cdp1-1*, ○) and YNN544 (*cdp1Δ*, ●) cells were grown logarithmically at 30° for several hours and shifted to 37°. (A) Optical density of cultures at various times following the temperature shift. Identical growth curves are obtained if cells are counted rather than measuring their number by optical density. (B) Viability of cells was determined by removing aliquots of cells at the indicated times, counting on a haemocytometer and plating onto YPD plates. The fraction of cells that formed colonies on the plates is indicated.

were observed by DAPI staining. Nuclear staining patterns were scored as either anucleate, having two nuclei per cell, or having three or more nuclei per cell. Figure 4 shows the results of this analysis. Less than 1% of unbudded or budded wild-type cells were binucleate and no cells were observed that had more than two nuclei. In contrast, *cdp1* mutant cells displayed abnormal nuclear configurations at both the permissive and restrictive temperatures. At 37°, ~35% of *cdp1Δ* cells contained two or more nuclei, and ~9% were anucleate. Multinucleate and anucleate cells were observed in cells containing the *cdp1-1* allele at frequencies considerably lower than cells containing the null allele at 30°. At 37°, the frequency of *cdp1-1* cells displaying abnormal nuclear configurations increased dramatically, and was between one-third and one-half of the frequency of *cdp1Δ* cells with similar configurations. The DAPI staining pattern of *cdp1* mutant cells was more diffuse than in wild-type cells. The diffuse DAPI staining was particularly pronounced at 37°. As a result, it was difficult to establish with certainty in some cases how many nuclei were present and whether some cells truly lacked a nucleus. Only cells in which the nuclear configuration was unambiguous (>90%) were scored.

To determine whether the *cdp1* mutants exhibited a chromosome segregation defect, the chromosome loss frequency was determined in wild-type, *cdp1-1* and *cdp1Δ* cells at the permissive temperature. Diploid strains were constructed that contained a marked ~135-kb linear fragment of chromosome III. Table 3 shows the loss frequency for this marked chromosome fragment in wild-type and *cdp1* mutant cells. At 30°, the *cdp1-1* mutant lost the chromosome fragment at a frequency ~10- to 35-fold higher than wild-type cells. In *cdp1Δ*

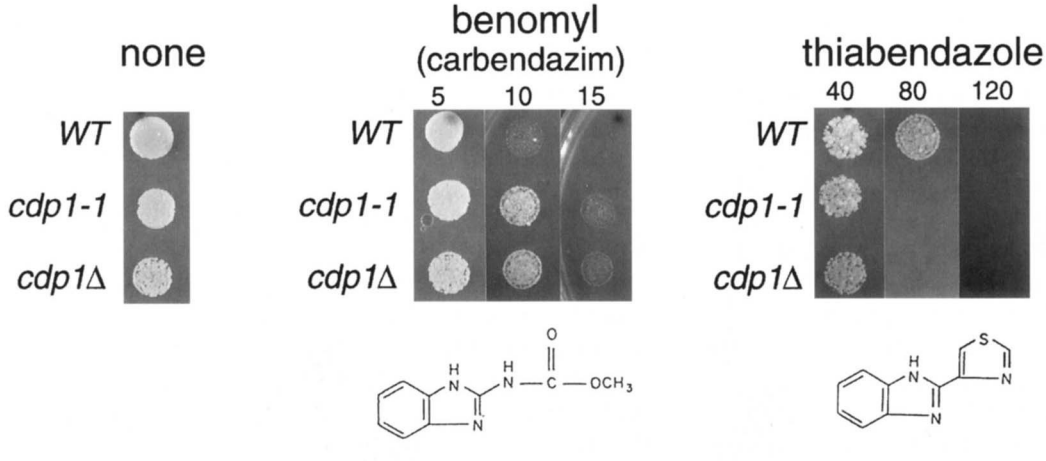

FIGURE 3.—Differential sensitivity of *cdp1* mutant cells to microtubule-disrupting compounds. Approximately 5000 logarithmically grown YCRY2 (wild-type), YNN536 (*cdp1-1*) and YNN543 (*cdp1Δ*) cells were spotted onto plates containing the indicated compounds. The numbers above each column reflect the concentration of the compound in μg/ml. Cells were allowed to grow at ~26° on YPD for 3 days, on thiabendazole for 8 days and on benomyl for 4 days.

TABLE 3

Chromosome loss in *cdp1* mutants

Strain	*CDP1* genotype	Loss frequency CFIII
YNN541	*CDP1*	$1.0 \pm 0.4 \times 10^{-4}$
YNN542	*cdp1-1*	$1.8 \pm 0.4 \times 10^{-3}$
YNN545	*cdp1Δ*	$1.1 \pm 0.3 \times 10^{-2}$

cells, the loss rate was ~60- to 230-fold higher than in wild-type cells. Both the *cdp1-1* and *cdp1Δ* mutants therefore exhibit more severe chromosome loss defects than the *cbf1Δ* mutant (BAKER and MASISON 1990; CAI and DAVIS 1990; FOREMAN and DAVIS 1993).

***cdp1* mutants exhibit abnormal microtubule morphology:** The differential sensitivity of the *cdp1* mutants to microtubule-disrupting drugs suggested the possibility that Cdp1p might play a role in tubulin function. To further explore this possibility, microtubules were observed in intact fixed cells by indirect immunofluorescence staining using anti-tubulin antibodies. Figure 5A shows the pattern of staining observed in wild-type cells grown at 37°. The top panels show a cytoplasmic microtubule array characteristic of unbudded cells and the bottom panels show cells in successive stages of mitosis.

Figure 5B shows the microtubule-staining pattern of *cdp1-1* cells grown logarithmically for several generations and shifted to 37° for 3 hr. At all stages of the cell cycle, >80% of these cells were larger and displayed dramatically longer arrays of cytoplasmic microtubules than were observed in wild-type cells. In addition, whereas wild-type cells rarely show more than three cytoplasmic MT bundles large enough to see by this method, *cdp1* cells typically had at least four bundles emanating from each spindle pole. The relative abundance of MT bundles was particularly apparent in unbudded cells (compare top panels Figure 5, A and B). At 30°, *cdp1-1* cells were similar in appearance to wild-type cells, with 2–3% of cells containing long cytoplasmic MT arrays. *cdp1Δ* cells displayed longer and more numerous cytoplasmic MT bundles than wild-type cells at both 30° and 37° (data not shown).

In wild-type cells, the spindle projects through the bud neck with one pole in the mother cell and the other in the bud. In *cdp1* cells grown at the 37°, we occasionally (<5%) observed spindles that were contained entirely within one cell (presumably the mother). As a result, both daughter nuclei are contained within a single cell (Figure 5C). This phenomenon is likely the mechanism by which binucleate and mutinucleate cells arise.

DISCUSSION

This paper describes a novel yeast gene, *CDP1*, that appears to play a general role in governing MT dynamics. Cells defective in Cdp1p function exhibit abnormal microtubule morphology typified by unusually long and numerous cytoplasmic MT bundles. Cytoplasmic MTs function in migration of the nucleus into the bud neck before chromosome segregation and in orienting the mitotic spindle (JACOBS *et al.* 1988; PALMER *et al.* 1992; SULLIVAN and HUFFAKER 1992). The frequent occurrence of cells with improperly oriented spindles as well as binucleate and multinucleate cells suggests that these processes are defective in *cdp1* mutants. In addition, the fact that these mutants lose chromosomes at a substantially elevated frequency suggests that nuclear MTs may also be altered in the *cdp1* mutant.

The differential sensitivity of *cdp1* mutant cells to the related microtubule-disrupting compounds thiabendazole and benomyl suggests the possibility that Cdp1p closely interacts with MTs or tubulin. These benzimidazole compounds bind to tubulin heterodimers and inhibit microtubule polymerization *in vivo* and *in vitro* (HOEBEKE *et al.* 1976; DAVIDSE and FLACH 1977, 1978; FRIEDMAN and PLATZER 1978) (reviewed in DAVIDSE 1986). A single amino acid substitution in the β-tubulin gene of *Aspergillus nidulans* causes differential sensitivity to MT disrupting drugs (JUNG and OAKLEY 1990). However, mutations in other genes have not previously been shown to have this effect. Studies of the binding of these compounds to tubulins derived from a variety of species have shown that the binding affinity apparently solely determines whether or not a benzimidazole has antifungal activity (DAVIDSE 1986; DAVIDSE and FLACH 1977). Therefore, one implication of our data might be that in *cdp1* mutant cells the tubulin binding affinity of thiabendazole is increased and the binding affinity of benomyl is decreased. Such an effect might be observed if Cdp1p normally interacts with tubulin directly or indirectly in a manner that alters the structure or

Strain	*CDP1* genotype	Temperature	% anucleate cells	% cells with 2 nuclei	% cells with ≥3 nuclei	Cells Counted
YCRY2	*CDP1*	30°	1.9	0.4	0	403
YCRY2	*CDP1*	37°	2.0	0.6	0	325
YNN536	*cdp1-1*	30°	4.4	2.2	0.4	265
YNN536	*cdp1-1*	37°	5.7	6.8	5	438
YNN543	*cdp1Δ*	30°	10.2	14.2	9.6	154
YNN543	*cdp1Δ*	37°	9.2	20.1	14.7	129

FIGURE 4.—Nuclear DNA in *cdp1* mutants. DAPI-stained cells were scored for abnormal nuclear morphology. Cells growing logarithmically at 30° or shifted to 37° for 3 hr were fixed and stained with DAPI. Cells were scored for nuclear morphology and aberrant cells containing zero, two, or three or more nuclei per mother cell are tabulated. Nuclei contained in presumptive daughter cells (always zero or one) are not included.

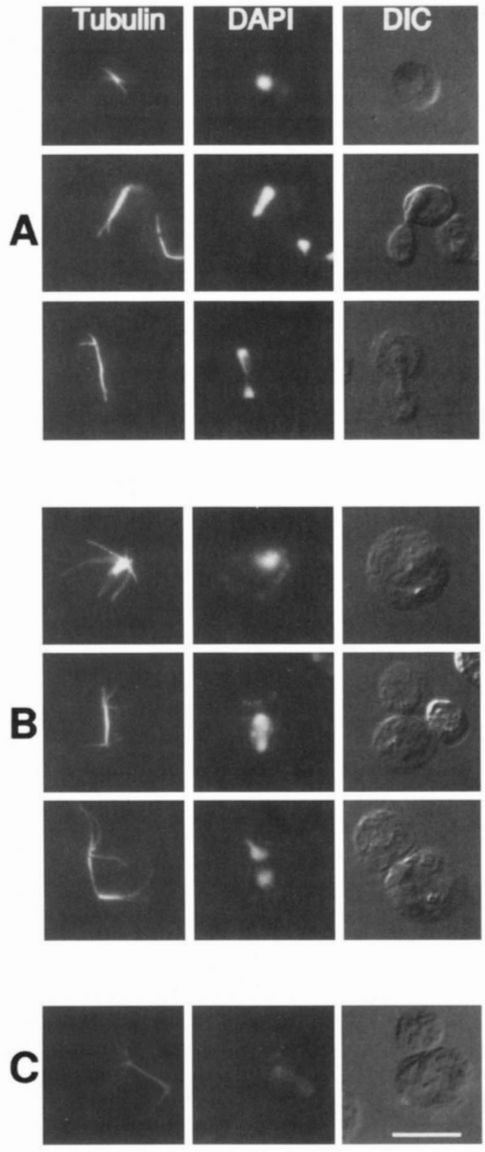

FIGURE 5.—Microtubule staining pattern of wild-type and *cdp1* mutant cells. The microtubule morphology of wild-type and *cdp1* mutant cells was determined by indirect immunofluorescence (left panels). Nuclei were visualized by DAPI staining (center panels). The DIC images of the corresponding fields are shown in the right panels. (A) YCRY3 (wild-type) cells at progressive stages of the cell cycle. (B) YNN540 (*cdp1-1*) cells at approximately equivalent stages of the cell cycle. (C) A YNN540 (*cdp1-1*) cell in which the spindle is contained entirely within the mother cell. Bar, 10 μm.

accessibility of the benzimidazole binding site. Further studies will be required to test this notion as well as to determine whether Cdp1p interacts directly or indirectly with MTs or tubulin.

In cells lacking Cdp1p, the *CBF1* gene product is essential for viability. The basis for the synthetic lethality between *cbf1* and *cdp1* mutations is unclear. Cbf1p binds to an 8-bp sequence located both within centromeres (CDEI) and upstream of several genes involved in methionine biosynthesis, where it plays a role in transcriptional activation. We have demonstrated, using separation of function alleles of *cbf1*, that *cdp1* mutants depend on Cbf1p primarily for its function in chromosome segregation. It is possible that both Cdp1p and Cbf1p interact directly with MTs at the centromere. Alternatively, the cumulative effects of impaired MT function (either nuclear or cytoplasmic), due to a mutation in *cdp1* and impaired kinetochore function due to a *cbf1* mutation, might result in an inability to sustain ongoing cell division. For example, one possibility is that in the absence of Cdp1p, MT turnover might be sufficiently altered so that the number of encounters between nascent MTs and the kinetochore is lowered. In this case failure to capture a MT in a few encounters (perhaps as a result of defective cbf1p function) or failure to maintain attachment could cause chromosome loss. If Cbf1p were involved in improving the efficiency of capturing or maintaining attachment to a nascent kinetochore MT (perhaps by altering centromere or kinetochore function), the cumulative effects of the two mutations might prove lethal. In this regard, it should be noted that neither the *cdp1-1* nor the *cbf1*Δ mutation causes more than a modest effect on cell growth in rich media making it unlikely that the synthetic lethality caused by the combined mutations is nonspecific. Numerous other models could also explain the relationship between *CBF1* and *CDP1* and further efforts to characterize the genetic interaction are currently underway.

Comparison of the predicted sequence of Cdp1p with other proteins in the databases revealed a mouse protein, $p150^{TSP}$, that is notably similar in sequence. $p150^{TSP}$ has been shown to localize to the nucleus in mouse cells and binds to peptides containing SH2 domains (MALEK *et al.* 1996). Two other anonymous ORFs from human and *C. elegans* also display considerable similarity to both Cdp1p and $p150^{TSP}$. $p150^{TSP}$ and these ORFs contain putative tetratricopeptide repeat (TPR) sequences arranged in tandem (MALEK *et al.* 1996). The TPR motif is a 34-amino acid motif that may mediate interactions with other TPR-containing proteins (SIKORSKI *et al.* 1990, 1991, 1993). For example, the Cdc27p TPR repeats are important in the interaction between Cdc27p and Cdc23p, which form part of a complex that promotes anaphase in yeast (LAMB *et al.* 1994). In the mouse protein, $p150^{TSP}$, the domain containing the TPR mediates self-association. Although $p150^{TSP}$ and Cdp1p sequences display a fairly high degree of identity within the putative TPR repeats, the predicted Cdp1p sequence contains few of the signature amino acids found in TPR repeats. Further studies will be required to determine if these regions mediate interactions with other TPR-containing proteins in yeast.

Several other yeast proteins that may also play roles in MT function have been identified by various approaches, [*e.g.*, Cdc20p (HARTWELL *et al.* 1973; HARTWELL and SMITH 1985; SETHI *et al.* 1991); Cin1p, Cin2p,

Cin4p (HOYT *et al.* 1990; STEARNS *et al.* 1990); Bik1p (BERLIN *et al.* 1990); Ase1p (PELLMAN *et al.* 1995); Kar9p (KURIHARA *et al.* 1994)]. In some instances, these proteins have been shown to localize to the spindle apparatus (BERLIN *et al.* 1990; BARNES *et al.* 1992). Like the *CDP1* gene, the *CIN1, CIN2, CIN4,* and *BIK1* genes are nonessential (BERLIN *et al.* 1990; HOYT *et al.* 1990; STEARNS *et al.* 1990). However, in contrast to *cdp1* mutant cells, which have unusually long and numerous bundles of MTs, cells individually lacking these gene products display diminished cytoplasmic MT arrays when grown at restrictive temperatures (BERLIN *et al.* 1990; HOYT *et al.* 1990). In addition, overexpression of Bik1p results in a transient increase in the length of cytoplasmic MTs (BERLIN *et al.* 1990). It will be of interest to investigate the relationship between these gene products and Cdp1p.

Mutations in three other genes (*CDP2, CDP3,* and *CDP4*) were identified in this study that cause dependence on *CBF1* for viability and hypersensitivity to thiabendazole. The products of these genes may constitute additional proteins involved in MT dynamics. Since single alleles of these genes were identified, the genetic screen employed to identify the *CDP* genes is unlikely to be saturated. Therefore, additional new genes may be identified by this method. Analysis of these genes is likely to provide new insights into the function of MTs, their structural composition and the regulation of their assembly.

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