Gene, 90 (1990) 93–98
Elsevier

GENE 03565

Isolation and sequence analysis of *CDC43*, a gene involved in the control of cell polarity in *Saccharomyces cerevisiae*

(Recombinant DNA; yeast; cell-division cycle; bud emergence; helix-turn-helix; DNA-binding proteins)

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Received by J. Marmur: 16 December 1989
Accepted: 21 February 1990

SUMMARY

The *Saccharomyces cerevisiae CDC43* gene product is involved in establishing cell polarity during the cell-division cycle. When grown at restrictive temperatures, temperature-sensitive *cdc43* mutants are unable to form buds and display delocalized cell-surface deposition [Adams et al., J. Cell Biol. (1990) in press]. We have isolated a *cdc43*-complementing plasmid from a yeast genomic-DNA library and localized the *CDC43* gene, by subcloning and transposon-mutagenesis experiments, to a 1.2-kb region of DNA that contained only one significant ATG-initiated open reading frame of 213 codons. The putative *CDC43* gene product contains a possible nuclear-localization signal sequence, a cysteine-rich domain and a histidine-rich domain, and a region that is similar in structure to α-helix-turn-α-helix structural domains present in some prokaryotic and eukaryotic DNA-binding proteins.

INTRODUCTION

The *S. cerevisiae CDC24*, *CDC42*, and *CDC43* gene products are involved in the development of cell polarity and the localization of secretion and cell-surface deposition during the yeast cell cycle (Hartwell et al., 1974; Sloat et al., 1981; Adams et al., 1990; Johnson and Pringle, 1990). Yeast strains containing lethal ts mutations in these genes are unable to bud when grown at restrictive temperature, but their nuclear cycles continue along with an increase in cell mass and volume, resulting in greatly enlarged, multinucleate, unbudded cells. The cytoplasmic actin networks appear disorganized (Adams and Pringle, 1984), and chitin and other cell-surface materials appear to be deposited randomly or uniformly throughout the enlarging cell walls. The abnormal positioning of budding sites associated with some *cdc24* mutants and with overexpression of the *CDC42* gene product suggests that these gene products are involved in the initial selection and organization of the budding site.

There is genetic evidence to suggest that the *CDC24*, *CDC42*, and *CDC43* gene products interact within the cell. Strains containing ts mutations in both *CDC42* and *CDC43* are inviable when grown at their normal permissive temperature of 23°C (Adams et al., 1990). In addition, the *CDC42* gene, when present on a multicopy plasmid, is able to suppress a *cdc24* ts mutation (Bender and Pringle, 1989).

The identification of a *cdc24* mutant among a set of Ca^{2+}-sensitive mutants (Ohya et al., 1986) and the finding

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Abbreviations: aa, amino acid(s); bp, base pair(s); *CDC*, gene(s) controlling cell-division cycle; kb, 1000 bp; mTn, minitransposon; ORF, open reading frame; *ori*, origin of DNA replication; PolIk, Klenow (large) fragment of *E. coli* DNA polymerase I; *S.*, *Saccharomyces*; SDS, sodium dodecyl sulfate; SSPE, 0.18 M NaCl/0.01 M Na·phosphate/0.001 M EDTA pH 7.4; Tn, transposon; ts (Ts), temperature-sensitive.

that the predicted *CDC24* gene product contains two putative Ca^{2+}-binding domains (Miyamoto et al., 1987) suggest a possible interaction of this gene product with Ca^{2+}. In contrast, *CDC42* is a member of the *rho* family (Madaule et al., 1987) of *ras*-related genes; its product is predicted both to bind and hydrolyze GTP and to undergo C-terminal modification leading to membrane association (Johnson and Pringle, 1990).

In the hope of gaining further insight into the mechanisms of polarity establishment and budding in yeast, we have attempted to clone and sequence the *CDC43* gene. Although no strong conclusions can be drawn from the predicted aa sequence of the *CDC43* gene product, the results raise the possibilities that this gene product may be localized to the nucleus and perhaps involved in the regulation of gene expression.

MATERIALS AND METHODS

(a) Reagents

Enzymes, M13 dideoxy-sequencing kits, and other reagents were obtained from standard commercial sources and used according to the suppliers' specifications. [α-^{35}S]dATP and [α-^{32}P]dATP were obtained from Amersham (Arlington Heights, IL).

(b) Media, growth conditions, strains and plasmids

Conditions for the growth and maintenance of bacterial and yeast strains have been described (Maniatis et al., 1982; Lillie and Pringle, 1980; Sherman et al., 1986). The permissive and restrictive temperatures for growth of ts mutants were 21°C and 36°C, respectively. *Escherichia coli* strain HB101 was routinely used as a plasmid host. The *S. cerevisiae* strains used were TD4, *MAT***a** *ura3 his4 leu2 trp1 gal2*, and TD1, *MAT*α *ura3 his4 trp1 gal2* (both provided by G.R. Fink); JPT198BD4-4A, *MAT***a** *cdc43*-2

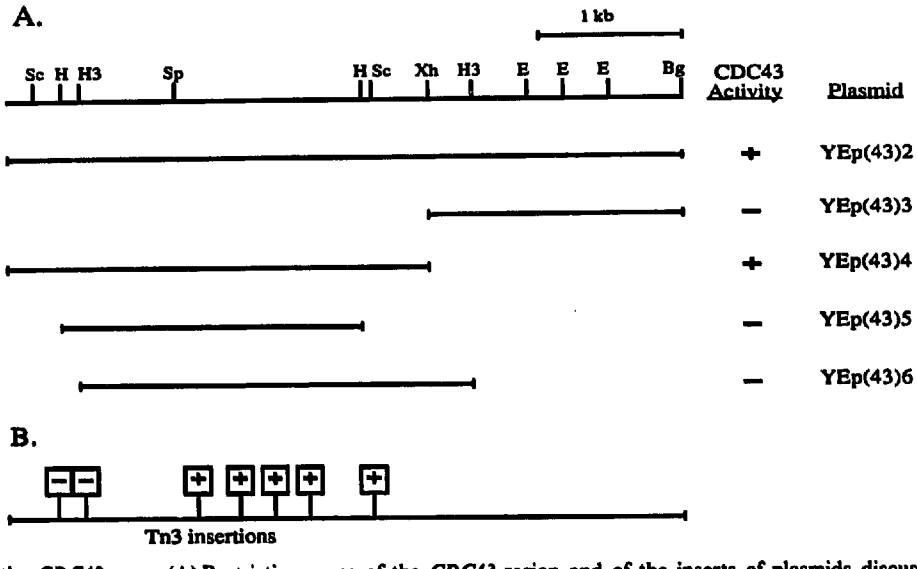

Fig. 1. Delimitation of the *CDC43* gene. (A) Restriction maps of the *CDC43* region and of the inserts of plasmids discussed in RESULTS AND DISCUSSION, sections a, b, c. Standard procedures were used for recombinant-DNA manipulations (Maniatis et al., 1982), *E. coli* and yeast transformations (Maniatis et al., 1982; Sherman et al., 1986), and plasmid isolation from *E. coli* (Birnboim and Doly, 1979) and yeast (Sherman et al., 1986). YEp(43)2 was constructed by deleting an ≃4-kb *Bgl*II-*Sal*I fragment (the *Sal*I site was in the YEp24 vector) from YEp(43)1 (see RESULTS AND DISCUSSION, section a) by digesting with *Bgl*II and *Sal*I, blunting with PolIk, and religating to regenerate a *Bgl*II site. YEp(43)3 was constructed by deleting the 3-kb *Xho*I-*Nhe*I fragment from YEp(43)2 by digestion with *Xho*I + *Nhe*I (the *Nhe*I site was in the YEp24 vector), blunting with PolIk, and religating. YEp(43)4 was constructed by deleting the 1.8-kb *Xho*I-*Bgl*II fragment from YEp(43)2 by digestion with *Xho*I + *Bgl*II, blunting with PolIk, and religating. YEp(43)5 was constructed by inserting the 2.2-kb *Hpa*I fragment from YEp(43)2 into *Pvu*II-digested YEp24. YEp(43)6 was constructed by inserting the 2.7-kb *Hin*dIII fragment from YEp(43)2 into *Hin*dIII-digested YEp103. The *cdc43*-complementing activity of each plasmid was determined by streaking plasmid-containing CJ198-2B cells onto YEPD plates at 36°C; a plus symbol indicates essentially uniform growth at 36°C; a minus symbol indicates no growth at 36°C. Bg, *Bgl*II; E, *Eco*RI; H, *Hpa*I; H3, *Hin*dIII; Sc, *Sca*I; Sp, *Spe*I; Xh, *Xho*I. All sites are shown for each enzyme. (B) Sites of mTn*3*(*URA3*) insertions. The mTn*3*(*URA3*) (Seifert et al., 1986) was used for insertional inactivation of the *CDC43* gene. A 2.2-kb *Eco*RI fragment from YEp24 containing the 2μ plasmid *ori* was inserted into the unique *Eco*RI site of plasmid pHSS6 (Seifert et al., 1986) to generate plasmid pHSS6 + 2μ. A 4.8-kb *Sma*I + *Bgl*II fragment from YEp(43)2 (see part A; the *Sma*I site is in the YEp24 vector) was inserted into *Sma*I + *Bam*HI-cleaved pHSS6 + 2μ to generate plasmid pHSS6 + 2μ(43). After co-transformation of pHSS6 + 2μ(43) and an mTn*3*(*URA3*)-containing plasmid into the appropriate *E. coli* strain (Seifert et al., 1986), cells that contained an mTn*3*(*URA3*) inserted into pHSS6 + 2μ(43) were selected. The locations and orientations of the insertions were then determined relative to the *Xho*I and *Eco*RI sites of pHSS6 + 2μ(43) by restriction-enzyme analysis (data not shown; see RESULTS AND DISCUSSION, section b). Sites of mTn*3*(*URA3*) insertions that did (–) or did not (+) inactivate *cdc43*-complementing activity are shown; the scale is the same as in part A.

(Adams et al., 1990; a segregant from the fourth backcross of JPT198 to C276-4A and C276-4B); and CJ198-2B, *MATα cdc43-2 ura3 trp1* (constructed by crossing JPT198BD4-4A to TD1). Plasmids pBR322 and YEp24 have been described elsewhere (Maniatis et al., 1982; Botstein et al., 1979). The yeast–*E. coli* shuttle plasmid YEp103 contains the *URA3* selectable yeast marker and the 2μ plasmid *ori* (S. Lillie, personal communication). The yeast genomic-DNA library in plasmid YEp24 (provided by D. Botstein) contains fragments produced by partial *Sau*3A digestion of DNA from *S. cerevisiae* strain DBY939 (Carlson and Botstein, 1982).

RESULTS AND DISCUSSION

(a) Isolation and identification of *CDC43*

A plasmid that complemented the *cdc43*-2 ts mutation in strain CJ198-2B was isolated from a yeast genomic-DNA

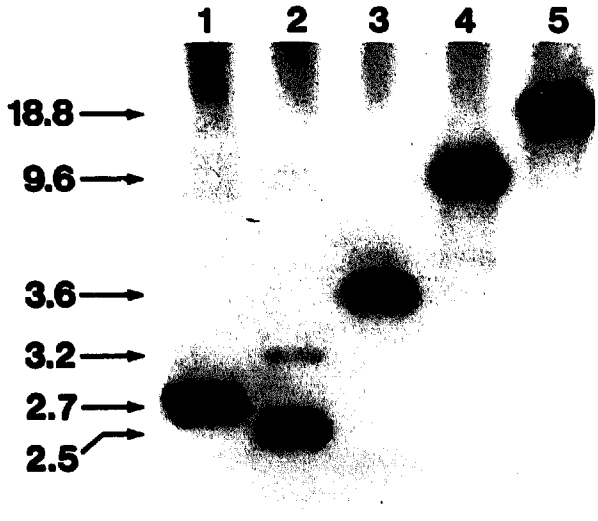

Fig. 2. DNA-DNA blot-hybridization analysis of chromosomal DNA from parental and transformed strains. Total yeast DNA was isolated essentially as described previously (Bloom and Carbon, 1982). DNA blot hybridizations were performed using 1% agarose gels and nitrocellulose paper (Maniatis et al., 1982). The DNA-DNA hybridizations were performed at 65°C for ≃16 h in a solution containing 5 × SSPE buffer (Maniatis et al., 1982) and 1% SDS. After digestion with the indicated restriction enzymes, DNA fragments were separated and hybridized to a radioactively labeled probe. The probe was single-stranded DNA prepared by primer extension in the presence of [α-^{32}P]dATP on a M13mp19 template containing DNA between the *Spe*I and *Xho*I sites of YEp(43)2 (Fig. 1A). The sizes of the fragments are indicated in kb on left margin. Total DNA from strain TD4 (lanes 1–4) and from the same strain after integration of the 9.3-kb plasmid containing *cdc43*-complementing sequences and the *URA3* gene (RESULTS AND DISCUSSION, section a, lane 5) was digested with *Hin*dIII (lane 1), *Sca*I (lane 2), *Spe*I + *Bgl*II (lane 3), and *Bgl*II (lanes 4 and 5). As the integrated vector sequence contains no *Bgl*II sites, the replacement of the original 9.6-kb *Bgl*II fragment (lane 4) with an 18.8-kb *Bgl*II fragment in the transformant (lane 5) indicates that the integration of the 9.3-kb plasmid had occurred at the chromosomal site homologous to the *cdc43*-complementing DNA.

library in the *URA3*-containing plasmid YEp24. From a primary Ura$^+$Ts$^+$ yeast transformant, a plasmid [designated YEp(43)1] was recovered into *E. coli* that could retransform CJ198-2B to Ura$^+$ Ts$^+$. The Ura$^+$ and Ts$^+$ phenotypes of these transformants co-segregated after growth on nonselective media (data not shown), indicating that the complementation of *cdc43*-2 was indeed due to the autonomously replicating recombinant plasmid. Plasmid YEp(43)1 contained an ≃8-kb insert (data not shown). Digestion and religation of YEp(43)1, as described in the legend to Fig. 1, yielded YEp(43)2, which was used in all subsequent experiments. A DNA-DNA blot-hybridization experiment using total yeast DNA and an appropriate probe revealed only the fragments expected if the cloned DNA was derived without rearrangement from contiguous chromosomal DNA that was single copy in the haploid genome (Fig. 2, lanes 1–4).

To test the identity of the cloned DNA, we integrated a plasmid containing the *cdc43*-complementing sequences and the yeast selectable marker *URA3* into a *CDC43* yeast strain, and then determined the meiotic linkage between the integrated *URA3* gene and a *cdc43* mutation. A 5-kb *Eco*RI fragment (one of the *Eco*RI sites was in the YEp24 vector) containing the *cdc43*-complementing region and the *URA3* gene was subcloned from YEp(43)2 into the *Eco*RI site of pBR322 (which cannot replicate autonomously in yeast). The resulting plasmid was linearized within the insert at the unique *Xho*I site and transformed into strain TD4, selecting for Ura$^+$. Two stable Ura$^+$ transformants were shown by DNA-DNA blot hybridization to have the plasmid integrated at the chromosomal site homologous to the *cdc43*-complementing DNA (Fig. 2, lane 5; only one transformant is shown). These transformants were crossed to the *cdc43*-2 strain CJ198-2B. Of 22 four-spore tetrads, 22 were parental ditypes (2 Ura$^+$ Ts$^+$: 2 Ura$^-$Ts$^-$), showing tight linkage between the *cdc43* mutation and the integrated *URA3* gene. Thus, integration had been directed to the *CDC43* locus, suggesting that the *cdc43*-complementing activity is due to the bona fide *CDC43* gene.

(b) Localization of *CDC43* on the complementing DNA

To localize the *CDC43* gene, subclones were constructed as described in Fig. 1A, legend. Complementation of the *cdc43*-2 mutation by plasmid YEp(43)4 but not by plasmids YEp(43)3, YEp(43)5, and YEp(43)6 indicated that *CDC43* lies within the 3-kb region to the left of the *Xho*I site, overlaps the left-hand *Hin*dIII site and one or both of the *Hpa*I sites. *CDC43* was also localized by determining the sites at which transposon insertion could inactivate *cdc43*-complementing activity. Transposon-containing plasmids were collected and the sites of insertion were determined by restriction analyses (Fig. 1B, legend). These plasmids were then tested for *cdc43*-complementing activity in strain

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                                    GA TCTTCTTTTT TACCTGTTTT ATTCACTTTT TTTTTTTTTT      -211
CTGAGTTGTT GCGCCTTGGA AGAATGGAAA AGCAATAGTT TCAGTGACTA TAGTATACAA TCAAAACACC      -141
CGCCAGTTCA AAACTATTAC TTGGTCACTA ACCGCCAAGT CATCTCTAGC AATTTAAATT TCAATTTTAA       -71
GAAAACCTTT ATTTATCCAA CGTGAACAAG TACTTTCAAG CAGTTCTGCC CACCGCTATA TCGTGGAAAA        -1

ATG TGT CAA GCT ACC AAT GGC CCG AGT AGA GTT GTG ACT AAA AAG CAT AGG AAA TTT TTC      60
Met Cys Gln Ala Thr Asn Gly Pro Ser Arg Val Val Thr Lys Lys His Arg Lys Phe Phe

GAA AGA CAT CTA CAG TTG CTT CCC TCT TCA CAT CAG GGA CAT GAC GTG AAC AGA ATG GCG     120
Glu Arg His Leu Gln Leu Leu Pro Ser Ser His Gln Gly His Asp Val Asn Arg Met Ala

ATA ATA TTC TAC TCA ATC TCA GGA CTC TCT ATA TTT GAT GTT AAC GTT TCT GCG AAG TAC     180
Ile Ile Phe Tyr Ser Ile Ser Gly Leu Ser Ile Phe Asp Val Asn Val Ser Ala Lys Tyr

GGC GAT CAT CTT GGC TGG ATG CGC AAA CAT TAT ATC AAA ACA GTG CTG GAT GAT ACA GAA     240
Gly Asp His Leu Gly Trp Met Arg Lys His Tyr Ile Lys Thr Val Leu Asp Asp Thr Glu

AAT ACT GTG ATA TCT GGA TTT GTT GGA AGC TTA GTC ATG AAT ATC CCT CAC GCA ACA ACG     300
Asn Thr Val Ile Ser Gly Phe Val Gly Ser Leu Val Met Asn Ile Pro His Ala Thr Thr

ATT AAT CTA CCA AAT ACT CTC TTT GCA TTG TTG TCC ATG ATT ATG CTG AGA GAT TAC GAG     360
Ile Asn Leu Pro Asn Thr Leu Phe Ala Leu Leu Ser Met Ile Met Leu Arg Asp Tyr Glu

TAT TTT GAG ACT ATA CTA GAC AAA AGA AGC CTG GCG AGA TTT GTT TCT AAG TGC CAA CGA     420
Tyr Phe Glu Thr Ile Leu Asp Lys Arg Ser Leu Ala Arg Phe Val Ser Lys Cys Gln Arg

CCT GAC CGT GGC TCG TTT GTA TCT TGT TTA GAC TAT AAG ACA AAT TGT GGA TCT TCG GTT     480
Pro Asp Arg Gly Ser Phe Val Ser Cys Leu Asp Tyr Lys Thr Asn Cys Gly Ser Ser Val

GAT TCA GAC GAT TTA AGG TTT TGC TAC ATC GCA GTT GCC ATT CTG TAC ATA TGC GGA TGC     540
Asp Ser Asp Asp Leu Arg Phe Cys Tyr Ile Ala Val Ala Ile Leu Tyr Ile Cys Gly Cys

CGA TCC AAA GAA GAC TTT GAT GAA TAC ATT GAT ACT GAG AAG TTG CTT GGC TAT ATA ATG     600
Arg Ser Lys Glu Asp Phe Asp Glu Tyr Ile Asp Thr Glu Lys Leu Leu Gly Tyr Ile Met

TCG CAA CAA TGC TAC AAC GGA GCT TTC GGT GCC CAC CAA TGA                            642
Ser Gln Gln Cys Tyr Asn Gly Ala Phe Gly Ala His Gln End

ACCACACTCA GGGTACACAT CTTGTGCGCT GTCTACCTTA GCTTTACTTC TCTAGTTTGG AAAAGCTATC      712
AGACAAGTTT AAGAAGACAC CATAACCTGG CTATTACATA GGCAAGTATC AAGCCATGGA TGTATGAAAT      782
TTGAAAGCGA ATTGAATGCC AGCTATGATC AATCTGATGA TGGCGGTTTC CAGGGAAGGG AGAACAAGTT      852
CGCTGATACG TGTTACGCAT TTTGGTGCTT AAATTCACTA CACTTACTAA CAAAGGATTG GAAAATGCTA      922
TGCCAAACTG AACTAGT                                                                939
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Fig. 3. Nucleotide sequence of the *CDC43* region and predicted aa sequence of the *CDC43* product. M13 dideoxy sequencing (Sanger et al., 1977) was performed essentially as described in the Sequenase™ sequencing manual of the United States Biochemicals Corp. (Cleveland, OH), using [α-^{35}S]dATP and the vectors M13mp18 and M13mp19. Sequencing primers were provided by E.I. DuPont de Nemours & Co., Inc. (Wilmington, DE) and Dr. John Burke (Department of Microbiology and Molecular Genetics, University of Vermont, VA). Sequences were analyzed using the University of Wisconsin Genetics Computer Group (Madison, WI) sequence analysis programs. The aa sequence similarities were determined using the Pustell sequence analysis programs (IBI, New Haven, CT). The nt sequence is numbered relative to the A of the putative start codon. The *Sca*I, *Hpa*I, and *Hin*dIII sites (indicated with solid lines) are located at nt −42 to −37, + 160 to + 165, and + 267 to + 272, respectively. Possible TATA promoter sequences (indicated with dashed lines) are present at nt −121 to −126 and −76 to −71. In addition, a 14 bp stretch of poly(dA-dT) is present at nt −224 to −211. Similar stretches of poly(dA-dT) have been implicated in the constitutive expression of certain promoters (Struhl, 1986). The underlined aa sequence is similar to the α-helix-turn-α-helix structural motif (RESULTS AND DISCUSSION, section c). GenBank accession number is M31114.

CJ198-2B. The results (Fig. 1B) suggested that the *CDC43* gene lies within a 1.2-kb region lying to the left of the *Spe*I site.

(c) Analysis of *CDC43* nt sequence and deduced aa sequence

The 3-kb region to the left of the *Xho*I site (Fig. 1A) was inserted into *Sma*I + *Sal*I-digested M13mp18 and M13mp19 using the unique *Sma*I and *Xho*I sites in YEp(43)2 (Fig. 1A; the *Sma*I site was in the YEp24 vector). These phages were then used in dideoxy-sequencing reactions. Both strands of the *cdc43*-complementing region to the left of the *Spe*I site (Fig. 1A) were completely sequenced (Fig. 3). The sequence revealed the presence of only one significant, ATG-initiated ORF that spanned both of the left-hand *Hin*dIII and left-hand *Hpa*I sites (Fig. 1A). Thus, this 213-codon ORF presumably encodes the *CDC43* gene product. The absence of a TACTAAC consensus splicing sequence (Langford et al., 1984) suggests a lack of introns.

The predicted *CDC43* product is a 213-aa polypeptide with an M_r of 24266 and a net charge of + 1 (Fig. 3). Comparison of the predicted aa sequence with the National Biomedical Research Foundation protein database and the GenBank DNA/protein database revealed no significant similarities to any known proteins. However, there are several features of the predicted aa sequence that are potentially informative. First, a sequence similar to known or suspected nuclear-localization signal sequences is present at aa 13–18 (Fig. 4). This basic aa sequence is very similar to sequences that have been shown experimentally to be involved in the localization of proteins to the nucleus (Silver

CDC43 (*S. cerevisiae*)	Lys	Lys	His	Arg	Lys^{18}
Large T antigen (SV40)	Lys	Lys	Lys	Arg	Lys^{127}
GAL4 (*S. cerevisiae*)	Lys	Lys	Leu	Lys	Cys^{21}
Histone 2B (*S. cerevisiae*)	Lys	Lys	Arg	Ser	Lys^{36}

Fig. 4. A possible nuclear-localization signal sequence in *CDC43*. Similarities between aa 13–18 of the predicted aa sequence of the *CDC43* gene product (see Fig. 3) and known or inferred nuclear-localization signal sequences of the SV40 large T antigen, the *S. cerevisiae GAL4* gene product, and the *S. cerevisiae* histone 2B protein are shown (Silver and Goodman, 1989). The positions of these sequences in their respective proteins are indicated by the residue number for the last aa.

and Goodson, 1989). Second, separate His-rich and Cys-rich aa domains with the sequences His-Xxx_6-His-Xxx_7-His-Xxx_2-His and Cys-Xxx_{10}-Cys-Xxx_6-Cys-Xxx_{11}-Cys-Xxx_9-Cys-Xxx-Cys (Xxx is any aa) are present at aa 16–34 and 138–180, respectively (Fig. 3). These sequences show no strong resemblances to the Cys_2His_2 or Cys_x zinc-finger domains implicated in the DNA binding of several eukaryotic regulatory proteins (Berg, 1988). While this does not necessarily preclude the possibility that these regions are involved in DNA binding, it is more likely that they are involved in some other aspect of protein function such as an interaction with metal ions (Szczypka and Thiele, 1989) or disulfide bridge formation. Third, a region of the predicted aa sequence (aa 55–76, Fig. 3) is similar to the α-helix-turn-α-helix structural motif found or inferred in a wide variety of prokaryotic and eukaryotic DNA-binding proteins (Brennan and Matthews, 1989). The *CDC43* sequence contains a Gly^{65} residue along with hydrophilic residues at positions 57, 59, 62, 63, 68, 69, 70, 73, and 74, and hydrophobic residues at positions 58, 61, 64, 72, 75, and 76. This sequence would have the proper alignment of aa residues that could allow the formation of a helix-turn-helix structure with nonpolar residues facing the hydrophobic core and exposed polar residues that could interact with DNA (Pabo and Sauer, 1984). Taken together, these inferred structural features raise the possibilities that the *CDC43* gene product is localized to the nucleus, interacts with metal ions, and binds to DNA.

(d) Conclusions

The *CDC43* gene product is involved in the morphogenetic steps of the yeast cell cycle. To begin exploring *CDC43* function at the molecular level, we have isolated and sequenced this gene. Although the inferred aa sequence of the *CDC43* product allows no strong conclusions, it does provide some interesting and unexpected clues to possible functions of this protein.

(1) The presence of a possible nuclear-localization signal raises the possibility that the *CDC43* gene product performs its cell-cycle function within the nucleus. Testing of this possibility will depend on the use of *CDC43*-specific antibodies in immunofluorescence localization studies.

(2) The presence of an aa domain within *CDC43* that is similar to the α-helix-turn-α-helix structural motif found in some DNA-binding proteins raises the possibility that *CDC43* could interact with DNA in some manner. While it is clear that transcriptional regulation plays a part in the control of some *CDC* genes in yeast (Peterson et al., 1985; White et al., 1987), there is no evidence as yet for transcriptional regulation of *CDC43* or any of the other genes whose products are involved in the control of cellular polarity. Thus, evaluation of the significance of this possible structural domain must await the results of studies of the possible transcriptional regulation of these genes.

The 'synthetic lethality' of *cdc43 cdc42* double mutants suggests that these genes or their products interact within the cell. If the *CDC43* gene product is in fact localized to the nucleus and interacts with DNA, it could be speculated that *CDC43* is involved in the transcriptional regulation of *CDC42*. It is possible that the presence of a mutant *CDC43* gene product could lead to a change in the expression of *CDC42* in a *cdc43 cdc42* double mutant, thereby leading to a new lethal phenotype in these cells. This speculation should be testable by analyzing *CDC42*-specific mRNA levels in *cdc43* mutants.

ACKNOWLEDGEMENTS

We are indebted to John Pringle, in whose laboratory some of this work was performed, and we thank him and members of his laboratory for helpful discussions and comments on this paper. We also thank John Burke for providing oligodeoxyribonucleotide primers; Peter Miller, Gerard Bouffard, Brian Foley, and Chris Fenno for assistance in running computer programs; and David Pederson and John Burke for helpful discussions. This research was supported by National Science Foundation-VT EPSCOR grant R11-8610679 (University of Vermont, D.I.J.), grants from the American Cancer Society (Vermont Division) and the University of Vermont College of Medicine (D.I.J.), National Institutes of Health (NIH) grant GM 31006 (J.R. Pringle), and NIH postdoctoral fellowships GM 10913 (D.I.J.) and GM 09727 (C.W.J.).

REFERENCES

Adams, A.E.M. and Pringle, J.R.: Relationship of actin and tubulin distribution to bud growth in wild-type and morphogenetic-mutant *Saccharomyces cerevisiae*. J. Cell Biol. 98 (1984) 934–945.

Adams, A.E.M., Johnson, D.I., Longnecker, R.M., Sloat, B.F. and Pringle, J.R.: *CDC42* and *CDC43*, two additional genes involved in budding and the establishment of cell polarity in the yeast *Saccharomyces cerevisiae*. J. Cell Biol. (1990) in press.

Bender, A. and Pringle, J.R.: Multicopy suppression of the *cdc24* budding defect in yeast by *CDC42* and three newly identified genes including

the *ras*-related *RSR1* gene. Proc. Natl. Acad. Sci. USA 86 (1989) 9976–9980.

Berg, J.M.: Proposed structure for the zinc-binding domains from transcription factor IIIA and related proteins. Proc. Natl. Acad. Sci. USA 85 (1988) 99–102.

Birnboim, H.C. and Doly, J.: A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. 7 (1979) 1513–1523.

Bloom, K.S. and Carbon, J.: Yeast centromere DNA is in a unique and highly ordered structure in chromosomes and small circular minichromosomes. Cell 29 (1982) 305–317.

Botstein, D., Falco, S.C., Stewart, S.E., Brennan, M., Scherer, S., Stinchcomb, D.T., Struhl, K. and Davis, R.W.: Sterile host yeasts (SHY): a eukaryotic system of biological containment for recombinant DNA experiments. Gene 8 (1979) 17–24.

Brennan, R.G. and Matthews, B.W.: Structural basis of DNA-protein recognition. Trends Biochem. Sci. 14 (1989) 286–290.

Carlson, M. and Botstein, D.: Two differentially regulated mRNAs with different 5′ ends encode secreted and intracellular forms of yeast invertase. Cell 28 (1982) 145–154.

Hartwell, L.H., Culotti, J., Pringle, J.R. and Reid, B.J.: Genetic control of the cell division cycle in yeast. Science 183 (1974) 46–57.

Johnson, D.I. and Pringle, J.R.: Molecular characterization of *CDC42*, a *Saccharomyces cerevisiae* gene involved in the development of cell polarity. J. Cell Biol. (1990) in press.

Langford, C.J., Klinz, F.-J., Donath, C. and Gallwitz, D.: Point mutations identify the conserved, intron-contained TACTAAC box as an essential splicing sequence in yeast. Cell 36 (1984) 645–653.

Lillie, S.H. and Pringle, J.R.: Reserve carbohydrate metabolism in *Saccharomyces cerevisiae*: responses to nutrient limitation. J. Bacteriol. 143 (1980) 1384–1394.

Madaule, P., Axel, R. and Myers, A.M.: Characterization of two members of the *rho* gene family from the yeast *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA 84 (1987) 779–783.

Maniatis, T., Fritsch, E.F. and Sambrook, J.: Molecular Cloning. A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982.

Miyamoto, S., Ohya, Y., Ohsumi, Y. and Anraku, Y.: Nucleotide sequence of the *CLS4* (*CDC24*) gene of *Saccharomyces cerevisiae*. Gene 54 (1987) 125–132.

Ohya, Y., Miyamoto, S., Ohsumi, Y. and Anraku, Y.: Calcium-sensitive *cls4* mutant of *Saccharomyces cerevisiae* with a defect in bud formation. J. Bacteriol. 165 (1986) 28–33.

Pabo, C.O. and Sauer, R.T.: Protein-DNA recognition. Annu. Rev. Biochem. 53 (1984) 293–321.

Peterson, T.A., Prakash, L., Prakash, S., Osley, M.A. and Reed, S.I.: Regulation of *CDC9*, the *Saccharomyces cerevisiae* gene that encodes DNA ligase. Mol. Cell. Biol. 5 (1985) 226–235.

Sanger, F., Nicklen, S. and Coulson, A.R.: DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA 74 (1977) 5463–5467.

Seifert, H.S., Chen, E.Y., So, M. and Heffron, F.: Shuttle mutagenesis: a method of transposon mutagenesis for *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA 83 (1986) 735–739.

Sherman, F., Fink, G.R. and Hicks, J.B.: Methods in Yeast Genetics. A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1986.

Silver, P. and Goodson, H.: Nuclear protein transport. CRC Crit. Rev. Biochem. Mol. Biol. 24 (1989) 419–435.

Sloat, B.F., Adams, A. and Pringle, J.R.: Roles of the *CDC24* gene product in cellular morphogenesis during the *Saccharomyces cerevisiae* cell cycle. J. Cell Biol. 89 (1981) 395–405.

Struhl, K.: Yeast promoters. In Reznikoff, W. and Gold, L. (Eds.), Maximizing Gene Expression. Butterworth, Boston, MA, 1986, pp. 35–78.

Szczypka, M.S. and Thiele, D.J.: A cysteine-rich nuclear protein activates yeast metallothionein gene transcription. Mol. Cell. Biol. 9 (1989) 421–429.

White, J.H.M., Green, S.R., Barker, D.G., Dumas, L.B. and Johnston, L.H.: The *CDC8* transcript is cell cycle regulated in yeast and is expressed coordinately with *CDC9* and *CDC21* at a point preceding histone transcription. Exp. Cell Res. 171 (1987) 223–231.