Isolation and sequence analysis of \textit{CDC43}, a gene involved in the control of cell polarity in \textit{Saccharomyces cerevisiae}

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

Douglas I. Johnson**b, Jeanne M. O'Brien* and Charles W. Jacobs***

*Department of Microbiology and Molecular Genetics, University of Vermont, Burlington, VT 05405 (U.S.A.) and **Department of Biology, The University of Michigan, Ann Arbor, MI 48109 (U.S.A.)

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

SUMMARY

The \textit{Saccharomyces cerevisiae} \textit{CDC43} gene product is involved in establishing cell polarity during the cell-division cycle. When grown at restrictive temperatures, temperature-sensitive \textit{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 \textit{cdc43}-complementing plasmid from a yeast genomic-DNA library and localized the \textit{CDC43} gene, by subeloning 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 \textit{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 \textit{w}-helix-turn-\textit{w}-helix structural domains present in some prokaryotic and eukaryotic DNA-binding proteins.

INTRODUCTION

The \textit{S. cerevisiae} \textit{CDC24}, \textit{CDC42}, and \textit{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; Sloot 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 \textit{cdc24} mutants and with overexpression of the \textit{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 \textit{CDC24}, \textit{CDC42}, and \textit{CDC43} gene products interact within the cell. Strains containing ts mutations in both \textit{CDC42} and \textit{CDC43} are inviable when grown at their normal permissive temperature of 23°C (Adams et al., 1990). In addition, the \textit{CDC42} gene, when present on a multicopy plasmid, is able to suppress a \textit{cdc24} ts mutation (Bender and Pringle, 1989).

The identification of a \textit{cdc24} mutant among a set of Ca^{2+}-sensitive mutants (Ohya et al., 1986) and the finding
that the predicted CDC24 gene product contains two putative Ca²⁺-binding domains (Miyamoto et al., 1987) suggest a possible interaction of this gene product with Ca²⁺. 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-³⁵S]dATP and [α-³²P]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; Lilley 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, MATa ura3 his4 leu2 trp1 gal2, and TD1, MATa ura3 his4 trp1 gal2 (both provided by G.R. Fink); JPT198BD4-4A, MATa 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, e. 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 8-kb BglII-SalI fragment (the SalI site was in the YEp24 vector) from YEp(43)1 (see RESULTS AND DISCUSSION, section a) by digesting with BglII and SalI, blunting with PolIII, and religating to regenerate a BglII site. YEp(43)3 was constructed by deleting the 3-kb XhoI-Nhel fragment from YEp(43)2 by digestion with XhoI + Nhel (the Nhel site was in the YEp24 vector), blunting with PolIII, and religating. YEp(43)4 was constructed by deleting the 1.5-kb XhoI-BglII fragment from YEp(43)2 by digestion with XhoI + BglII, blunting with PolIII, and religating. YEp(43)5 was constructed by inserting the 2.2-kb HpaI fragment from YEp(43)2 into HpaI-digested YEp24. YEp(43)6 was constructed by inserting the 2.7-kb HindIII fragment from YEp(43)2 into HindIII-digested Yepl03. The cdc43-complementing activity of each plasmid was determined by streaking plasmid-containing CJI98-2B 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, BglII; E, EcoRI; H, HpaI; H3, HphIII; Sc, ScaI; Sp, SpeI; Xh, XhoI. All sites are shown for each enzyme. (B) Sites of mTn3(URA3) insertions. The mTn3(URA3) (Seifert et al., 1986) was used for insertional inactivation of the CDC43 gene. A 2.2-kb EcoRI fragment from YEp24 containing the 2μ plasmid ori was inserted into the unique EcoRI site of plasmid pHSS6 (Seifert et al., 1986) to generate plasmid pHSS6 + 2μ. A 4.8-kb SmaI + BglII fragment from YEp(43)2 (see part A; the SmaI site is in the YEp24 vector) was inserted into SmaI + BamHI-cleaved pHSS6 + 2μ to generate plasmid pHSS6 + 2μ(43). After co-transformation of pHSS6 + 2μ(43) and an mTn3(URA3)-containing plasmid into the appropriate E. coli strain (Seifert et al., 1986), cells that contained an mTn3(URA3) inserted into pHSS6 + 2μ(43) were selected. The locations and orientations of the insertions were then determined relative to the XhoI and EcoRI sites of pHSS6 + 2μ(43) by restriction-enzyme analysis (data not shown; see RESULTS AND DISCUSSION, section b). Sites of mTn3(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, MATA cdc43-2 ura3 trpl (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 Sau3A 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 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 re-transform 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 EcoRI fragment (one of the EcoRI sites was in the YEp24 vector) containing the cdc43-complementing region and the URA3 gene was subcloned from YEp(43)2 into the EcoRI site of pBR322 (which cannot replicate autonomously in yeast). The resulting plasmid was linearized within the insert at the unique XhoI 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 XhoI site, overlaps the left-hand HindIII site and one or both of the HpaI 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

---

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 x 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 [-32P]dATP on a M13mp19 template containing DNA between the SpeI and XhoI 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 HindIII (lane 1), ScaI (lane 2), SpeI + BglII (lane 3), and BglII (lanes 4 and 5). As the integrated vector sequence contains no BglII sites, the replacement of the original 9.6-kb BglII fragment (lane 4) with an 18.8-kb BglII 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.
of the left-hand only one significant, ATG-initiated ORF that spanned both to the left of the sequenced (Fig. 3). The sequence revealed the presence of reactions. Both strands of the phages were then used in dideoxy-sequencing YEp(43)2 (Fig. 1A; the site.

The results (Fig. 1B) suggested that the CDC43 gene lies within a 1.2-kb region lying to the left of the CJ 198-2B. The results (Fig. 1B) suggested that the CDC43 gene lies within a 1.2-kb region lying to the left of the SpeI site.

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

The 3-kb region to the left of the XhoI site (Fig. 1A) was inserted into Smal + SalI-digested M13mp18 and M13mp19 using the unique Smal and XhoI sites in YEp(43)2 (Fig. 1A; the Smal 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 SpeI 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 HindIII and left-hand HpaI 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 Mr 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 essentially 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 essentially informative.
(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 z-helix-turn-z-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


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