A Ser/Thr-rich Multicopy Suppressor of a cdc24 Bud Emergence Defect

ALAN BENDER* AND JOHN R. PRINGLE†

Department of Biology, The University of Michigan, Ann Arbor, MI 48109-1048, U.S.A.

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MSB2 was identified previously as a multicopy suppressor of a temperature-sensitive mutation in CDC24, a gene required for polarity establishment and bud formation in Saccharomyces cerevisiae. The inferred MSB2 product contains 1306 amino acids, 42% of which are Ser or Thr. Its Ser+Thr-richness and hydrophobicity profile suggest that Msb2p may be an integral membrane protein containing a long, periplasmic, N-terminal domain and a short, cytoplasmic, C-terminal domain. Cells that lack MSB2 display no obvious mutant phenotypes. MSB2 is located between the centromere and KSS1 on the right arm of chromosome VII. Although physical mapping suggests that MSB2 and LEU1 (on the left arm of chromosome VII) are approximately 40 kb apart, the genetic map distance observed between leu1 and an msb2::URA3 marker was only 2.3 cM.

**KEY WORDS** — Saccharomyces cerevisiae; cell cycle; bud emergence; chromosome VII; recombination frequency.

INTRODUCTION

The reorganization of cytoskeletal elements is among the earliest known events associated with the development of a bud in Saccharomyces cerevisiae (Drubin, 1991; Chant and Pringle, 1991). About 15 min before bud emergence, actin and the neck-filament proteins, which are both required for proper bud development, become concentrated at a single site, from which the bud will subsequently emerge (Kim et al., 1991; Ford and Pringle, 1991). The CDC24 gene is required for the assembly of these cytoskeletal structures and for the subsequent formation of the bud (Hartwell et al., 1974; Sloat et al., 1981; Adams and Pringle, 1984; Kim et al., manuscript in preparation). CDC24 is also required for proper bud-site selection; cells that contain a temperature-sensitive (Ts−) cdc24-4 mutation display a random pattern of bud-site selection during growth at the permissive temperature (Sloat et al., 1981).

To investigate the role of CDC24 in bud-site selection and bud formation, and to identify other genes involved in these processes, we previously sought interacting genes by screening a high-copy-number yeast genomic-DNA library for genes whose overexpression could suppress the lethality caused by a Ts− cdc24 mutation. This screen identified the known gene CDC42 and three previously unknown genes, RSR1, MSB1, and MSB2 (Bender and Pringle, 1989). The ability of these genes to act as multicopy suppressors of cdc24 does not, by itself, prove that they normally function in bud formation. However, independent lines of evidence support the conclusion that CDC42, RSR1, and MSB1 do in fact play such roles: CDC42 is essential for bud formation (Adams et al., 1990; Johnson and Pringle, 1990), RSR1 is required for proper bud-site selection (Bender and Pringle, 1989; Chant and Herskowitz, 1991), and MSB1 interacts with three other genes that are themselves required...
Table 1. Yeast strains used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source</th>
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<tbody>
<tr>
<td>Y147</td>
<td>MATa cdc24-4 ura3 leu2 his3</td>
<td>Bender and Pringle, 1989</td>
</tr>
<tr>
<td>Y246</td>
<td>MATa/MATa ura3/ura3 leu2/leu2 his3/HIS3</td>
<td>Bender and Pringle, 1989</td>
</tr>
<tr>
<td>Y377</td>
<td>MATa/MATa msh2::URA3/MSB2 ura3/ura3 leu2/his3</td>
<td>&quot;msb2::URA3 derivative (see text) of Y246</td>
</tr>
<tr>
<td>Y393</td>
<td>MATa msh2::URA3 ura3 leu2 his3</td>
<td>Segregant from Y377</td>
</tr>
<tr>
<td>2209</td>
<td>MATa msh1::his3 ura3 leu2 his4 trp1 can1</td>
<td>A. Mitchell</td>
</tr>
<tr>
<td>Y540</td>
<td>MATa ura3 his3</td>
<td>Segregant from the cross of Y393 × 2209</td>
</tr>
<tr>
<td>Y548</td>
<td>MATa ura3 his3</td>
<td>W. Courchesne</td>
</tr>
<tr>
<td>Y550</td>
<td>MATa ura3 his3</td>
<td>Segregant from the cross of BC168 × Y540</td>
</tr>
<tr>
<td>Y551</td>
<td>MATa ura3 his3</td>
<td>Segregant from the cross of Y125 × XS144-S22</td>
</tr>
<tr>
<td>Y125</td>
<td>MATa ura3 his3</td>
<td>Yeast Genetics Stock Center, Berkeley, CA 94720</td>
</tr>
<tr>
<td>XS144-S22</td>
<td>MATa ura3 his3</td>
<td>Segregant from the cross of Y125 × XS144-S22</td>
</tr>
<tr>
<td>Y555</td>
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<td>Segregant from the cross of Y548 × Y550</td>
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<tr>
<td>Y551</td>
<td>MATa ura3 his3</td>
<td>Segregant from the cross of Y125 × XS144-S22</td>
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</table>

*Segregant from the cross of C276-4B (Wilkinson and Pringle, 1974) × SY1229 (Bender and Sprague, 1989).

for proper bud formation (Bender and Pringle, 1989, 1991). In the study reported here, we undertook a molecular characterization of MSB2 in order to evaluate whether it, too, is involved in bud formation.

MATERIALS AND METHODS

Strains, plasmids, and media

The S. cerevisiae strains used in this study are listed in Table 1. YEp24 (Botstein et al., 1979) and pSL113 (Bender and Pringle, 1989) are high-copy-number plasmids containing the 2-μm-plasmid origin of replication and either the URA3 (YEp24) or the LEU2 (pSL113) selectable marker. Standard rich (YPD) and synthetic complete (SC) media were used (Sherman et al., 1986). Other media were SC-U (SC lacking uracil), SC-L (SC lacking leucine), SC-H (SC lacking histidine), SC-U+ Sorb (SC-U containing 1 μm-sorbitol), and SC-L+ Sorb (SC-L containing 1 μm-sorbitol). X-gal indicator plates were made by spreading 0.1 ml of X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside) from a 20 mg/ml stock made in N,N-dimethylformamide onto plates containing SC plus 0.1 μM-KH₂PO₄, adjusted to pH 7.0 with KOH.

Recombinant-DNA procedures

DNA was sequenced using the dideoxy chain-termination method (Sanger et al., 1977). All restriction sites used for subcloning were crossed (although in some cases by only 10 bp), and both strands were sequenced except in three short regions of the MSB2 open reading frame (see Figure 1). For DNA-DNA blot hybridization, DNA fragments were separated by electrophoresis and blotted to nitrocellulose (Maniatis et al., 1982). The filter was hybridized to a 32P-labeled DNA probe for 20 h at 42°C in a solution containing 50% formamide, 5X SSC, 50 mM-sodium phosphate (pH 6.5), 200 μg/ml sonicated salmon sperm DNA, 0.1% sodium dodecyl sulfate, and 5X Denhardt’s reagent (Maniatis et al., 1982). The filter was then washed at 65°C for 4 h in 2X SSC, 0.1% sodium dodecyl sulfate.

Multicopy suppression

Fragments of DNA containing all or part of MSB2 were subcloned into plasmid YEp24 or pSL113. The ability of the resulting subclones to effect multicopy suppression of cdc24 was tested in strain Y147 at 36°C on SC-U+Sorb or SC-L+Sorb, as described previously (Bender and Pringle, 1989).
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Ability to suppress cdc24

<table>
<thead>
<tr>
<th>E</th>
<th>Sm</th>
<th>H</th>
<th>Xh</th>
<th>B</th>
<th>E</th>
<th>Sp</th>
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<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Figure 1. Localization of MSB2. The lines indicate segments of DNA tested for the ability to suppress a cdc24 mutation when expressed from high-copy plasmids. The small arrows indicate the regions of each strand that were sequenced; the large arrows indicate open reading frames. Restriction sites shown are E (EcoRI), H (HindIII), Sm (SmaI), Xh (XhoI), B (BamHI), and Sp (SphI).

RESULTS

Plasmid pPB207 was isolated from a YEp24 genomic-DNA library (Carlson and Botstein, 1982) on the basis of its ability to rescue the lethality caused by a Ts- cdc24 mutation (Bender and Pringle, 1989); it contained a 6.1 kb insert of yeast DNA. By subcloning and testing for multicopy suppression of cdc24, the suppressing gene (named MSB2) was localized to a 5.2 kb segment running from just left of the left-hand EcoRI site to the SphI site (Figure 1). Surprisingly, although sequences at both ends of this segment were required for suppression, a central 2.1 kb XhoI-BamHI segment was found to be dispensable (Figure 1). The DNA sequence of the 5.1 kb EcoRI-SphI segment was determined (Figures 1 and 2). It contains overlapping and oppositely-oriented open reading frames of 227 and 1306 codons (Figure 1). The subcloning analysis clearly indicates that MSB2 corresponds to the larger of these open reading frames (Figure 1). Consistent with this interpretation, the subclone lacking the 2.1 kb XhoI-BamHI segment is predicted to preserve the continuity of the upstream and downstream portions of the larger open reading frame, creating a fused open reading frame of 706 codons.

The most striking feature of the predicted Msb2p amino acid sequence is its richness in Ser plus Thr (29% Ser and 13% Thr), with the density of Ser + Thr remaining relatively constant throughout most of the sequence. Another noteworthy feature of the MSB2 coding region is the presence of a 51 bp motif that is repeated, almost identically, seven times in tandem (Figure 3). The MSB2 DNA sequence and inferred Msb2p amino acid sequence were searched against the GenBank (release 68, ktp=4) and SWISS-PROT (release 18, ktp=1) data bases using the FASTA sequence comparison programs (Pearson and Lipman, 1988). No significant similarities were found. A hydrophathy analysis using the Kyte and Doolittle (1982) program suggests that Msb2p may contain one or two membrane-spanning domains, one at the N-terminus, which might serve as a signal sequence, and one approximately 100 amino acids from the C-terminus, which might serve as a membrane-anchoring domain (Figure 4).

To investigate the role of MSB2, we disrupted it by gene replacement in a diploid using a fragment of DNA in which the 3.7 kb Smal-EcoRI segment (Figure 1; positions 753-4470 in Figure 2) of MSB2 was replaced with a 1.8 kb Smal-EcoRI fragment that contained the URA3 gene and some M13 vector sequences. In this construction, only 19 N-terminal and 48 C-terminal codons of the MSB2 open reading frame remain. Two Ura+ transformants were subjected to tetrad analysis. In 20 tetrads analysed (ten from each transformant), all but two spores germinated and grew well, and Ura+: Ura- segregated 2:2 in all 18 tetrads that contained four viable spores. DNA-DNA blot hybridization confirmed the success of the construction (Figure 5).

The msb2::URA3 segregants were viable and grew as well as the MSB2 segregants at all temperatures tested (ranging from 12°C to 39°C). The msb2::URA3 cells also displayed normal morphology (including normal chitin ring formation; data not shown) and responded normally to pheromone (as judged by cell-cycle arrest and shmoo formation; data not shown). Furthermore, homozygous msb2::URA3/msb2::URA3 diploids sporulated as efficiently as wild-type cells and gave rise to tetrads that produced four viable spores. In summary, no obvious defect resulting from deletion of MSB2 was observed.

By hybridization to a library of overlapping yeast chromosomal DNA clones, MSB2 was physically mapped to the right arm of chromosome VII, near the centromere and on the opposite side from LEU1 (L. Riles and M. Olson, personal communication). KSS1 and RME1 are located in this vicinity (Mortimer et al., 1989; Courchesne et al., 1990; Rine et al., 1981). Tetrad analysis suggested that MSB2
is centromere proximal to KSSI, which in turn is centromere proximal to RME1 (Table 2). Surprisingly, although the physical mapping data indicate that LEU1 and MSB2 are approximately 40 kb apart (1 kb typically corresponds to approximately 0.4 cM; Petes et al., 1991), the genetic map distance observed was only 2.3 cM (Table 2). Suppression of recombination by the centromere may account at least in part for this result (Petes et al., 1991); the replacement of MSB2 by URA3 could conceivably also contribute to the apparent inhibition of recombination in this region. A combination of the physical and genetic mapping data suggest that the order of markers is LEU1-CEN7-MSB2-KSSI-RME1.

DISCUSSION

Of the four known multicopy suppressors of cdc24, MSB2 was identified least frequently (twice) and had the weakest suppression activity (Bender and Pringle, 1989); only a small fraction of cdc24 cells containing MSB2 on a high-copy-number plasmid can reproduce at restrictive temperature, and even these cells appear sick and proliferate only slowly (unpublished data). Deletion of MSB2 has provided little insight into the role of this gene, as the deletion cells displayed no obvious mutant phenotypes. A similar result was obtained with MSB1, another multicopy suppressor of cdc24 (Bender and Pringle, 1991). The absence of MSB2 only a small fraction of cells carrying mutations in certain other genes involved in bud formation (Bender and Pringle, 1989); Leu2-95 cells displayed no obvious mutant phenotypes. In contrast, MSB1 suppresses Leu2 lethality when present on a high-copy-number plasmid (unpublished data). Deletion of MSB1 and MSB2 in combination of the two genes was not viable and each gene is functionally redundant with one or more other genes, or both. It is worth noting that although MSB1 is not normally required for viability, it becomes essential in cells carrying mutations in certain other genes involved in bud formation (Bender and Pringle, 1991).
Figure 2. Nucleotide sequence of MSB2 and the predicted amino acid sequence of Msb2p. Restriction sites are indicated by underlining. These sequences have been deposited with GenBank, Accession No. M77354.

search for similar mutants in which MSB2 is essential for viability, or the use of low-stringency DNA–DNA hybridizations, may reveal other genes with which MSB2 is functionally redundant.

Although the inferred sequence of Msb2p is not similar to that of any other known protein, several features of the sequence provide clues to where Msb2p might function. The most striking feature of the sequence is its high content (42%) of Ser+Thr. However, even among yeast proteins, this feature is not unique. In particular, several cell-surface proteins are known to be rich in Ser+Thr: chitinase (required for digestion of chitin during cell separation) is 60% Ser+Thr over a domain of 157 amino acids (Kuranda and Robbins, 1991); Stalp (extracellular glucoamylase) is 60% Ser+Thr over a domain of 238 amino acids (Yamashita et al., 1985); Kre1p (involved in β-glucan formation) is 40% Ser+Thr over its entire length of 313 amino acids (Boone et al., 1990); Fus1p (involved in cell fusion) is 46% Ser+Thr over its 71 N-terminal amino acids (McCaffrey et al., 1987); α-agglutinin (required for sexual agglutination) is 37% Ser+Thr over its C-terminal 352 amino acids (Lipke et al., 1989); and the core subunit of α-agglutinin is 55% Ser+Thr over its entire length of 725 amino acids (Boone et al., 1991). The seven 51-bp repeats (see text and Figure 3) are indicated by underlining. These sequences have been deposited with GenBank, Accession No. M77354.
Ser+Thr in Msb2p suggests that it, too, is probably O-glycosylated. Consistent with this model is the observation that the N-terminus of Msb2p contains a hydrophobic sequence that may be a signal sequence for import into the secretory pathway.

Most of the known Ser+Thr-rich proteins in yeast contain repeated sequences; for example, there are two copies of a 15-mer in Kre1p and 26 copies of a 7-mer in Stalp. Msb2p also contains a repeated sequence: seven copies of a 17-mer. The repeats in the different proteins are not similar to each other except for being rich in Ser+Thr. The repeats presumably arose from unequal crossing-over events and perhaps are tolerated because the consequence of such events is simply to increase the length of fairly homogeneous Ser+Thr-rich regions.

A clue to how Msb2p might function comes from the distribution of its Ser and Thr residues. There appear to be two classes of Ser+Thr-rich proteins in yeast: those that contain discrete domains rich in Ser+Thr residues and those that are rich in Ser+Thr across most or all of their lengths. Chitinase, Stalp, and α-agglutinin all are members of the first class and possess enzymatic or recognition activities that are separable from their Ser+Thr-rich domains. Kre1p and the core subunit of α-agglutinin, like Msb2p, are members of the second class. Although no enzymatic or recognition activity for Kre1p has yet been reported, the α-agglutinin-binding activity of α-agglutinin is known to reside in a separate subunit. Given that the Ser+Thr-richness in Msb2p extends across nearly its entire length, we predict that Msb2p does not itself contain an enzymatic or recognition activity, but that it might instead be part of a heteromeric protein whose other subunit(s) has (have) an enzymatic or recognition activity.

The multicopy suppression of cdc24 by MSB2 could reflect a direct interaction between Cdc24p and Msb2p that stabilizes a thermolabile form of the former. Another possibility is that Msb2p might be able to substitute partially for the function of Cdc24p. In this regard, it is worth noting that the inferred sequence of Cdc24p contains two regions of approximately 100 amino acids apiece that are approximately 40% Ser+Thr (Miyamoto et al., 1987). It is also possible that Msb2p does not normally play a specific role in bud formation but instead plays some more general function in the construction or maintenance of the cell wall. If this is the case, then the overexpression of MSB2 might increase the strength of the cell wall and only indirectly suppress mutations in CDC24 by allowing the mutant cells to undergo more rounds of cell division before lysing than they otherwise could.

The final feature of the Msb2p sequence that bears on its possible location and function is the presence of a predicted membrane anchor followed by a cytoplasmic tail. If Msb2p does in fact span the plasma membrane, it would be the first protein implicated in bud formation whose sequence suggests this property. Interestingly, Fuslp, the only other O-glycosylated transmembrane protein of which we are aware, is implicated in the localized reorganization of the cell wall during conjugation (Truehart et al., 1987; Truehart and Fink, 1989; McCaffrey et al., 1987). An intriguing possibility is that the presumed cytoplasmic portion of Msb2p interacts with proteins (such as Cdc24p) that are involved in organizing the cytoskeleton during bud formation while the presumed periplasmic portion of Msb2p interacts with proteins that are involved in reorganizing the cell wall during bud initiation.

ACKNOWLEDGEMENTS

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Figure 4. Hydropathy plot of Msb2p. The mean hydropathic indices (Kyte and Doolittle, 1982) of successive segments of 9 amino acids are plotted against the amino acid positions of these segments. Values exceeding +2 represent highly hydrophobic, potential membrane-spanning regions.

Table 2. Linkage data for MSB2

<table>
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<th>Marker pair</th>
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<td>24</td>
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Linkage data are from a cross between Y551 and Y555. Msb2::URA3 deletion allele contains M13 vector sequences and so can hybridize with the probe. The measured sizes of the resulting fragments are indicated and correspond to the expected sizes.

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REFERENCES


