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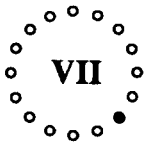

Yeast Sequencing Reports

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

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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

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Table 1. Yeast strains used in this study

Strain	Genotype	Source
Y147	*MAT***a** *cdc24-4 ura3 leu2 his3*	Bender and Pringle, 1989
Y246	*MAT*α/*MAT***a** *ura3/ura3 leu2/leu2 his3/HIS3*	Bender and Pringle, 1989
Y377	*MAT*α/*MAT***a** *msb2::URA3/MSB2 ura3/ura3 leu2/leu2 his3/HIS3*	*msb2::URA3* derivative (see text) of Y246
Y393	*MAT*α *msb2::URA3 ura3 leu2 his3*	Segregant from Y377
2209	*MAT***a** *rme1::lacZ ura3 leu2 his4 trp1 can1*	A. Mitchell
Y540	*MAT***a** *msb2::URA3 rme1::lacZ ura3 leu2 his3 his4 trp1*	Segregant from the cross of Y393 × 2209
BC168	*MAT*α *kss1::HIS3 ura3 leu2 his3 trp1*	W. Courchesne
Y548	*MAT*α *msb2::URA3 kss1::HIS3 rme1::lacZ ura3 leu2 his3 trp1*	Segregant from the cross of BC168 × Y540
Y125	*MAT***a** *ura3 his3*	This study[a]
XS144-S22	*MAT*α *leu1 trp5 ade5 aro2 met13 lys5 gal1 gal2 cyh2*	Yeast Genetics Stock Center, Berkeley, CA 94720
Y550	*MAT***a** *ura3 leu1 his3 trp5 ade5 aro2 met13*	Segregant from the cross of Y125 × XS144-S22
Y555	*MAT***a** *msb2::URA3 kss1::HIS3 rme1::lacZ ura3 his3 ade5*	Segregant from the cross of Y548 × Y550
Y551	*MAT*α *ura3 leu1 his3 trp5 aro2*	Segregant from the cross of Y125 × XS144-S22

[a]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 M-sorbitol), and SC-L + Sorb (SC-L containing 1 M-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 M-KH_2PO_4, 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 ^{32}P-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).

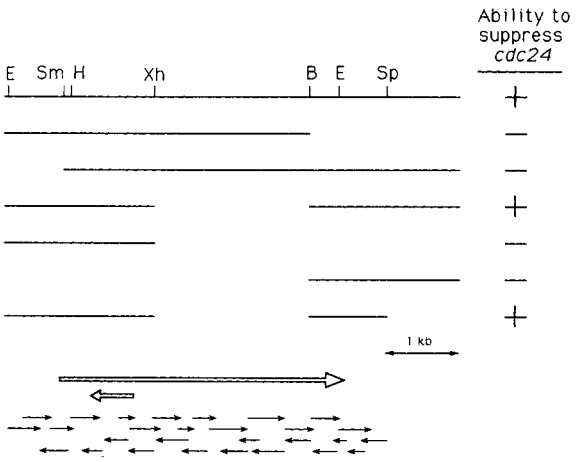

Figure 1. Localization of *MSB2*. The lines indicate segments of DNA tested for the ability to suppress a *cdc24* mutation when expressed from high-copy plasmids. The small arrows indicate the regions of each strand that were sequenced; the large arrows indicate open reading frames. Restriction sites shown are E (*Eco*RI), H (*Hin*dIII), Sm (*Sma*I), Xh (*Xho*I), B (*Bam*HI), and Sp (*Sph*I).

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 *Eco*RI site to the *Sph*I site (Figure 1). Surprisingly, although sequences at both ends of this segment were required for suppression, a central 2·1 kb *Xho*I-*Bam*HI segment was found to be dispensable (Figure 1). The DNA sequence of the 5·1 kb *Eco*RI-*Sph*I 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 *Xho*I-*Bam*HI 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, ktup = 4) and SWISS-PROT (release 18, ktup = 1) data bases using the FASTA sequence comparison programs (Pearson and Lipman, 1988). No significant similarities were found. A hydropathy 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 *Sma*I-*Eco*RI segment (Figure 1; positions 753–4470 in Figure 2) of *MSB2* was replaced with a 1·8 kb *Sma*I-*Eco*RI 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 segregants. 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*

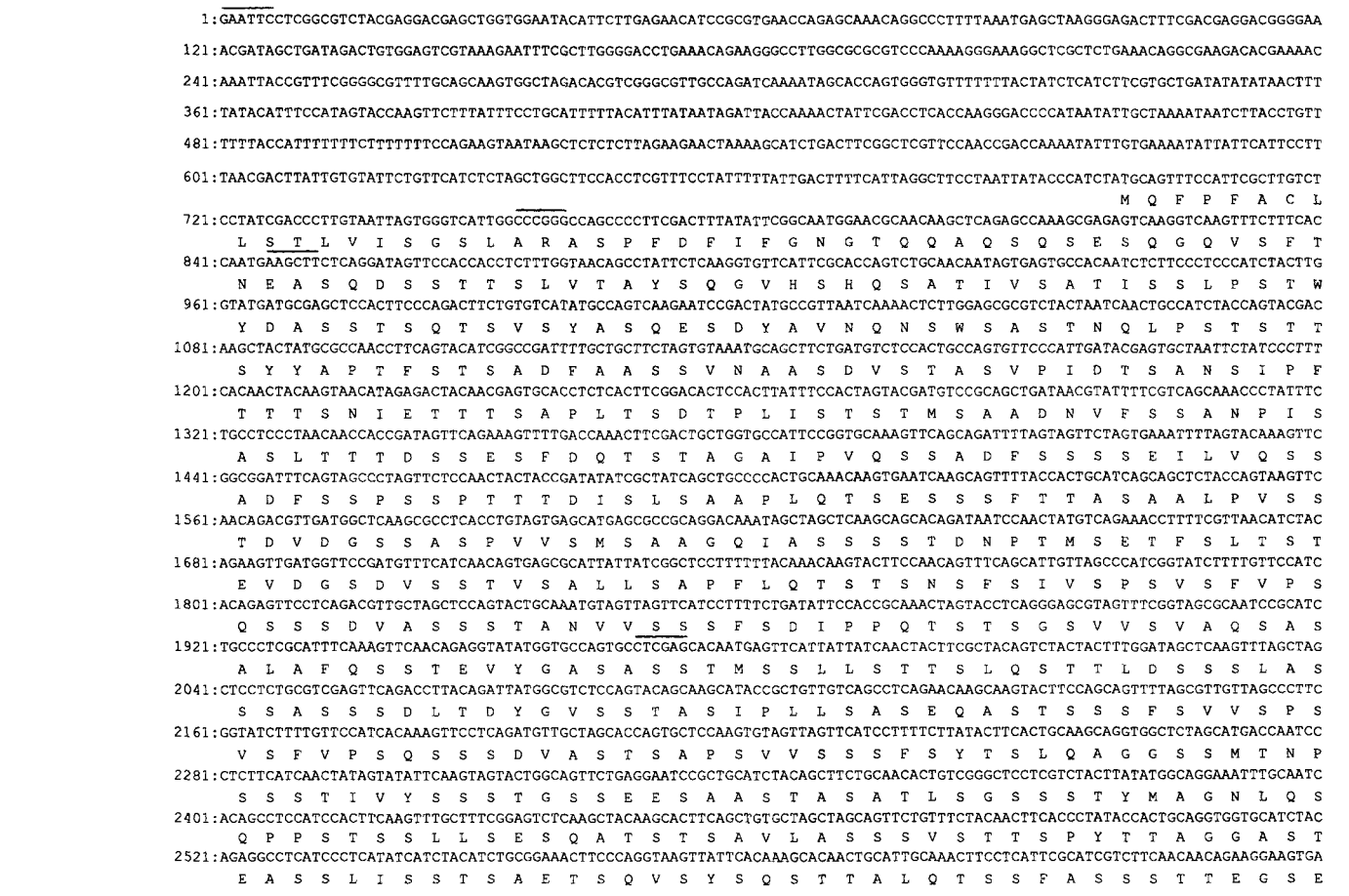

Figure 2.1.

is centromere proximal to *KSS1*, 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-KSS1-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 mutant phenotypes for *msb1*, *msb2*, and *msb1 msb2* (unpublished data) strains suggests that *MSB1* and *MSB2* are not important for bud formation, that 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). A

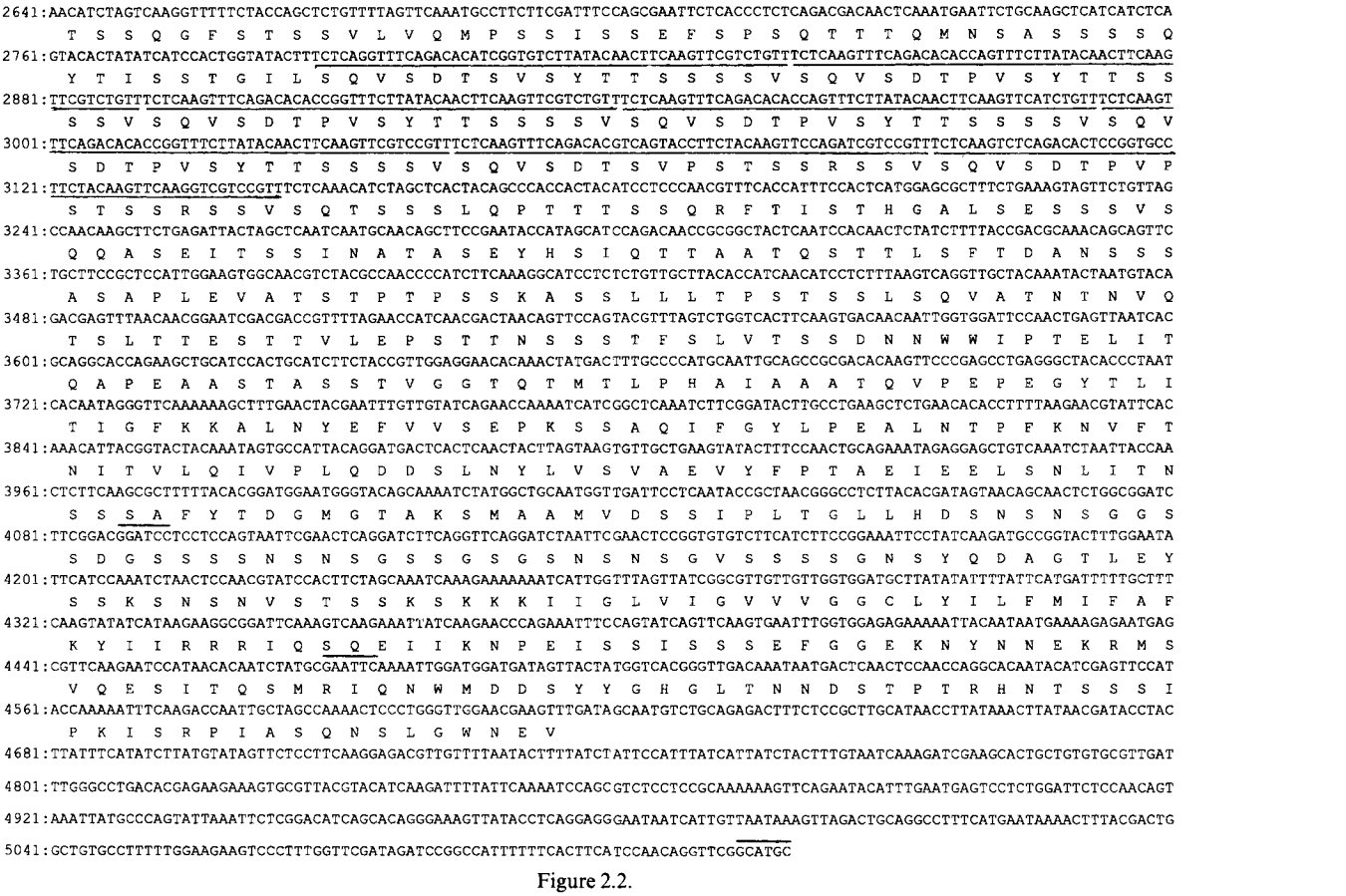

Figure 2.2.

Figure 2. Nucleotide sequence of *MSB2* and the predicted amino acid sequence of Msb2p. Restriction sites are indicated by overlining: *Eco*RI (1), *Sma*I (753), *Hin*dIII (846), *Xho*I (1966), *Bam*HI (4088), *Eco*RI (4470), and *Sph*I (5114). The seven 51-bp repeats (see text and Figure 3) 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); Sta1p (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; Trueheart *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 (Roy *et al.*, 1991). Chitinase, Kre1p, Fus1p, α-agglutinin, and the core subunit of α-agglutinin have all been shown to be O-glycosylated on Ser/Thr residues (Kuranda and Robbins, 1991; Boone *et al.*, 1990; Truehart and Fink, 1989; Terrance *et al.*, 1987; N. Wagner and P. Lipke, personal communication). In addition, an O-glycosylated cell-wall mannoprotein fraction has been purified and shown to contain 45% Ser+Thr (Frevert and Ballou, 1985). The high content of

A.

```
      TCTCAAGTTTCAGACACACCGGTTTCTTATACAACTTCAAGTTCGTCTGTT
2789  -----G----------T----G--------------------------
2840  -------------------A------------------------------
2891  ---------------------------------------------------
2942  -------------------A----------------------A------
2993  ---------------------------------------------C---
3044  ----------------GT-A--AC---C-----G---C--A-----C---
3095  --------C--------T-----GC---C-----G------G-----C---
```

B.

```
      S Q V S D T P V S Y T T S S S S V
696   - - - - - - S - - - - - - - - - -
713   - - - - - - - - - - - - - - - - -
730   - - - - - - - - - - - - - - - - -
747   - - - - - - - - - - - - - - - - -
764   - - - - - - - - - - - - - - - - -
781   - - - - - - S - P S - S - R - - -
798   - - - - - - - - P S - S - R - - -
```

Figure 3. Nucleotide repeats within *MSB2* (A) and amino acid repeats within Msb2p (B). Consensus sequences are indicated at the top of each panel; dashes indicate positions that are identical with the consensus sequences. The locations of the nucleotide repeats are indicated relative to the coordinates in Figure 2. The locations of the amino acid repeats are indicated relative to the presumptive initiation Met (Figure 2).

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 Krel p and 26 copies of a 7-mer in Sta1p. 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, Sta1p, 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. Krel p and the core subunit of α-agglutinin, like Msb2p, are members of the second class. Although no enzymatic or recognition activity for Krel p 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, Fus1p, 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; Trueheart 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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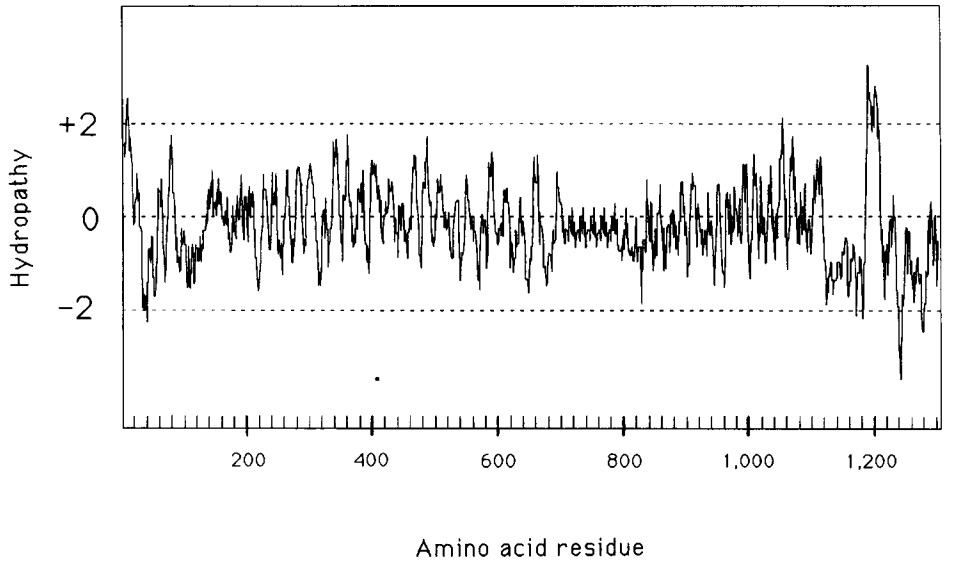

Figure 4. Hydropathy plot of Msb2p. The mean hydropathic indices (Kyte and Doolittle, 1982) of successive segments of 9 amino acids are plotted against the amino acid positions of these segments. Values exceeding +2 represent highly hydrophobic, potential membrane-spanning regions.

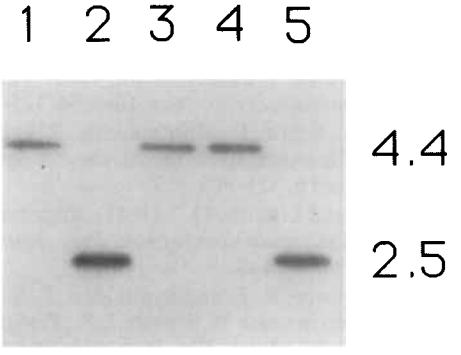

Figure 5. DNA–DNA blot hybridization analysis of strains with and without the *msb2::URA3* deletion. Lane 1 is the starting diploid strain Y246. Lanes 2 and 5 are Ura$^+$ segregants, and lanes 3 and 4 are Ura$^-$ segregants, from a complete tetrad from the transformed diploid strain Y377. Genomic DNA was digested with *Sma*I and *Sph*I (Figure 1; positions 753–5114 in Figure 2) and hybridized with an M13 DNA probe that contained the 1·1 kb *Hin*dIII-*Xho*I (Figure 1; positions 846–1966 in Figure 2) fragment of *MSB2*. The *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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Table 2. Linkage data for *MSB2*

Marker pair		No. of tetrads PD	T	NPD	Map distance
leu1	*msb2*	123	6	0	2·3
leu1	*kss1*	104	23	1	11
leu1	*rme1*	98	29	1	14
msb2	*kss1*	112	18	1	9·2
msb2	*rme1*	106	24	1	11
kss1	*rme1*	124	6	0	2·3

Linkage data are from a cross between Y551 and Y555. *msb2* was *msb2::URA3*, scored as Ura$^+$, *kss1* was *kss1::HIS3*, scored as His$^+$, and *rme1* was *rme1::lacZ*, scored as Blue$^+$ on X-gal indicator plates. Map distances were calculated using Perkins's formula [$X_p = 50(T+6N)/(P+T+N)$, where P, N, and T are the numbers of PD (parental ditype), NPD (non-parental ditype), and T (tetratype) tetrads, respectively].

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