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Genetics

Complete nucleotide sequence of a gene conferring polymyxin B resistance on yeast: Similarity of the predicted polypeptide to protein kinases

(membranes/receptors/phosphorylation)

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ABSTRACT **Polymyxin B is an antibiotic that kills sensitive cells by disrupting their membranes. We have cloned a wild-type yeast gene that, when present on a high-copy-number plasmid, renders the cells resistant to the drug. The nucleotide sequence of this gene is presented. A single open reading frame within the sequence has the potential to encode a polypeptide (molecular mass of 77.5 kDa) that shows strong homologies to polypeptides of the protein kinase family. The gene, *PBS2*, located on chromosome X, is not allelic to the previously described *PBS1* gene (where PBS signifies polymyxin B sensitivity). Although *pbs1* mutations confer resistance to high levels of polymyxin B, double mutants, *pbs1 pbs2*, are not resistant to the drug, indicating that *PBS2* is essential for *pbs1* activity. Models based on the proposed protein kinase activity of the *PBS2* gene product are presented to explain the interaction between *PBS1* and *PBS2* gene products involved in conferring polymyxin B resistance on yeast cells.**

Previous work from this laboratory demonstrated that the cells of *Saccharomyces cerevisiae* are killed by large doses (>0.5 mM) of polymyxin B, a polyamine peptide antibiotic (1). At low sublethal levels (<0.1 mM), polymyxin B renders cells permeable to various drugs, and it [and its nontoxic derivative, polymyxin B nonapeptide] completely abolishes mating (refs. 1 and 2; unpublished data). The latter is caused by interference with sexual agglutination (2). In addition, polymyxin B enhances the effect of mating pheromone MFα on *MATa STE2*$^+$ cells but not on *MATa ste2* cells, indicating a degree of specificity of interaction with the MFα receptor (ref 2; unpublished data). These results suggest a complicated pattern of interactions between this antibiotic and the cells of *S. cerevisiae*.

Mutants resistant to high levels of polymyxin B (>1 mM) have been isolated (1). The *pbs* mutations are recessive, and all mutants tested thus far belong to a single complementation group. The *PBS1* gene has been mapped to the right arm of chromosome XV, about 10 centimorgans (cM) from the *ADE2* locus, proximal to the centromere (1).

The present communication describes the cloning, sequencing, and characterization of a yeast gene that confers resistance to polymyxin B when present on a high-copy-number plasmid. We shall refer to this gene as *PBS2* because the present study shows that it is not allelic to *PBS1* (see below). The examination of the sequence of the putative polypeptide encoded by the cloned DNA reveals regions of striking homology to a number of protein kinases from viral, mammalian, and yeast sources. We also show that the activity of the *PBS2* gene is essential for the expression of polymyxin B resistance in *pbs1* mutants and propose a model for the interaction between the *PBS1* and *PBS2* genes.†

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MATERIALS AND METHODS

Yeast strains used in this work are listed in Table 1. The solid and liquid media, standard growth conditions, and genetic techniques have been described (4, 5). Yeast transformations were done by the method of Ito *et al.* (6). Procedures for plasmid isolation from *Escherichia coli*, restriction fragment analysis, subcloning, gel electrophoresis of DNA and RNA, and nucleic acid hybridizations have been described in detail (4). Total yeast RNA was prepared as described by Rose and Botstein (7) and analyzed by electrophoresis on formaldehyde/agarose gels (4). Total yeast DNA was obtained by the method of Nasmyth and Reed (8), extracted with phenol, and dissolved in Tris/EDTA buffer. The yeast DNA library in YEp24 (9) was prepared from DBY939 (*PBS1*$^+$ *PBS2*$^+$) and was kindly provided by D. Botstein (10). Polymyxin B was purchased from Sigma. Resistance to the drug was tested as described (1). DNA sequencing procedures were those of Sanger *et al.* (11) with modifications (12). More than 95% of the sequence was obtained by subcloning random sonication fragments (13) into M13mp18 or by the ordered deletion method of Dale *et al.* (14). The remaining portions of the sequence were determined by using internal primers (15).

RESULTS

Selection of Clones Resistant to Polymyxin B (PBS^r). The rationale of Rine *et al.* (16) was used in screening the yeast DNA library in YEp24 vector (10). The method relies on an increased dosage of a wild-type gene product to overcome the inhibitory effects of the drug. Yeast strain YNN27 was transformed with the DNA library to uracil prototrophy (Ura$^+$). Approximately 13,000 Ura$^+$ transformants were examined for resistance to the antibiotic (PBS^r) and 64 Ura$^+$ PBS^r clones were identified. Linkage between the Ura$^+$ phenotype and polymyxin B resistance was demonstrated by subjecting these clones to several cycles of growth to saturation under nonselective conditions. This regimen allowed the cells to segregate the plasmid and thus to become Ura$^-$. Only those isolates that simultaneously became Ura$^-$ and PBS sensitive (PBS^s) were retained. Six independent clones (Ura$^+$ PBS^r) were obtained in this manner.

Characterization of Cloned DNA. The structure of one plasmid isolate named R5H is shown in Fig. 1. The plasmid consists of the YEp24 vector moiety and a 7.8-kilobase (kb) insert. The plasmid DNA can be subdivided into five *Cla* I fragments. The *Cla* I fragments 1 through 4 were subcloned

Abbreviations: PBS, polymyxin B sensitivity; PBS^r, polymyxin B resistant; Ura$^+$, uracil prototrophy; cM, centimorgan.

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†The sequence reported in this paper is being deposited in the EMBL/GenBank data base (Bolt, Beranek, and Newman Laboratories, Cambridge, MA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J02946).

Table 1. Yeast strains

Strain	Relevant genotype	Source or ref.
YNN27	*MATα trp1-289 ura3-52*	R. W. Davis*
DBY703	*MATα trp1-289 his3-1 ura3-52 [cir°]*	D. Botstein†
DBY939	*MATα suc2-215am ade2-110*	D. Botstein†
KFY1-1	*MATα leu2-3,112 his5 ura3-52 [cir°]*	J. Broach‡
KFY1-4	*MATa leu2-3,112 trp1 his3 ura3-52 [cir°]*	J. Broach‡
GBH4	*MATa his4-519 leu2-3,112 can1 pbs1-12*	1
GBH14	*MATα trp1-289 can1 pbs1-12*	1
GBH17	*MATa trp1-289 ura3-52 can1 pbs1-12*	1
GBH21	*MATα trp1-289 ura3-52 pbs1-273*	From YNN27 (2)
GBD4	Diploid from cross GBH4 × YNN27	1
GBD7	Diploid from cross GBH4 × GBH14	This work
GBD13	Diploid from cross KFY1-1 × KFY1-4	This work
GBD16	Diploid from cross DBY939 × GBH17	This work
GBD18	Diploid from cross KFY1-4 × YNN27	This work
K396-11A	*MATa spo11 ura3 ade1 his1 leu1 lys7 met3 trp5 [cir+]*	3

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separately into YEp24 and used to transform YNN27; however, none of the fragments could by itself confer resistance (not shown). Examination of RNA blot-hybridization patterns (Fig. 1) with a DNA probe prepared from *Cla* I fragment 3 revealed that this probe hybridized to two large RNAs (2.7 and 3.6 kb). The transcripts are present at low levels in untransformed yeast cells. Deletion analysis determined that a 3.2-kb *Dra* I–*Sac* I fragment (encoding the 2.7-kb RNA) is sufficient for the resistance phenotype (not shown).

Sequence of *PBS2* Gene. The nucleotide sequence of the gene and the predicted polypeptide sequence are shown in Fig. 2. The single open reading frame (positions 1–2130) has the potential to code for a protein of 710 amino acids and molecular mass of 77.5 kDa. The predicted protein is strongly hydrophilic, with only a few hydrophobic pockets (e.g., the proline–leucine string at positions 94–99). Regions of a rather unusual amino acid composition within the deduced polypeptide include two threonine + serine sequences (amino acids 36–41 and 280–287), and a sequence rich in serine, glycine, and asparagine (positions 301–316). The polypeptide terminates in a short, strongly hydrophobic string of amino acids, reminiscent of yeast RAS1 and RAS2 proteins (18).

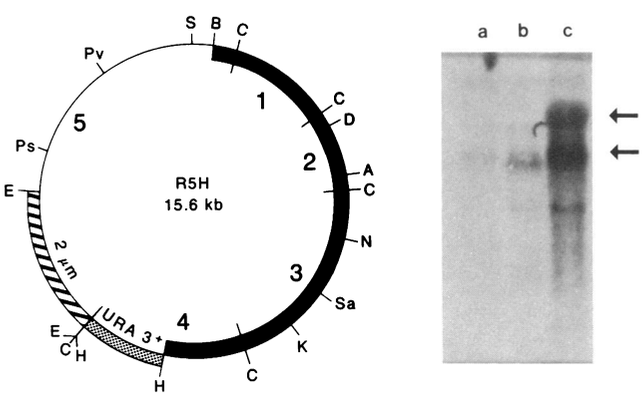

FIG. 1. Polymyxin B-resistance plasmid R5H. (*Left*) Essential features of R5H, the solid bar representing the cloned DNA. The numbers refer to *Cla* I fragments discussed in the text. The sizes of the *Cla* I fragments are as follows: 1, 1.45 kb; 2, 1.2 kb; 3, 4.15 kb; and 4, 2.1 kb. Fragment 5 (6.7 kb) consists of YEp24 sequences (except for the *URA3*+ gene, which is part of fragment 4). The restriction sites are indicated as follows: A, *Aat* II; B, *Bam*HI; C, *Cla* I; D, *Dra* I; E, *Eco*RI; H, *Hin*dIII; K, *Kpn* I; N, *Nar* I; Ps, *Pst* I; Pv, *Pvu* II; S, *Sal* I; and Sa, *Sac* I. In one experiment, the unique *Kpn* I site was replaced by a *Sal* I site to produce plasmid R5H.Sal. Note the absence of *Pvu* II sites in the cloned DNA (also see Fig. 5). (*Right*) Blot hybridization of total RNA from YNN27 [untransformed (lane a), transformed with YEp24 (lane b), and transformed with R5H (lane c)] probed with labeled *Cla* I fragment 3. The arrows show the two most abundant RNA species hybridizing to this probe. The lower species (2.7 kb) also hybridizes to *Cla* I fragment 2, and the upper RNA (3.6 kb) also hybridizes to *Cla* I fragment 4. The resistance gene is contained within the *Dra* I–*Sac* I segment of the cloned yeast DNA.

Amino Acid Sequence Homologies. Studies on the action of polymyxin B on mammalian cells have shown that this antibiotic is a strong inhibitor of the phospholipid/Ca^{2+}-dependent protein kinase C (19–21). This fact prompted us to compare the predicted sequence of PBS2 protein with several recently described protein kinases (22–25). The PBS2 protein indeed appears to be related to polypeptides of the protein kinase family (Fig. 3), including the oncogene protein-tyrosine kinases and serine/threonine-protein kinases (27–29). The regions of relatedness include (in order) the sequences known to be essential for ATP binding [Gly-Xaa-Gly-Xaa-Xaa-Gly-$(Xaa)_{14}$-Ala-Xaa-Lys-] and for phosphoreceptor activity [Arg-Asp-Val-$(Xaa)_{17}$-Asp-Phe-Gly-$(Xaa)_{20}$-Ala-Pro-Glu-$(Xaa)_{16}$-Asp-Xaa-Trp-Xaa-Xaa-Gly-] (26, 30) as well as other conserved regions shown in Fig. 3. Notable is the match between *PBS2* and *STE7* sequences (100 perfect matches and 25 additional highly conserved substitutions within the aligned domains of 252 and 263 amino acids, respectively).

Genetic Analysis. The cloned *PBS2* gene confers polymyxin B resistance when present in high copy number. To determine the importance of copy number and the relationship with the previously described *PBS1* gene (1), the cloned *PBS2* DNA (a 6-kb *Bam*HI–*Sal* I fragment from R5H.Sal; see Fig. 1) was ligated into a centromeric plasmid, YCp50 (9), to yield YCp50.PBS2. This plasmid is maintained at 1 or 2 copies per cell and does not confer polymyxin B resistance on wild-type cells (Fig. 4). A *pbs1* mutant strain GBH21 was transformed with YCp50.PBS2, and the Ura^+ transformants were tested for resistance. Since the *pbs1* mutation is recessive, the wild-type *PBS2* gene on YCp50 plasmid should, if it were allelic, complement the mutation and restore the sensitive phenotype. However, as Fig. 4 shows, the strain remained resistant to the drug. Therefore, *PBS2* gene is not functionally allelic with *PBS1*. Note that strain DBY939 from which *PBS2* DNA was obtained is sensitive to polymyxin B (Fig. 4), so it is unlikely that a suppressor of *PBS1*+ would have been cloned by our procedure.

A gene-disruption experiment was carried out to determine whether the *PBS2* gene is essential for viability. A 1.17-kb *Aat* II–*Nar* I fragment (nucleotides 114–1285 in Fig. 2)

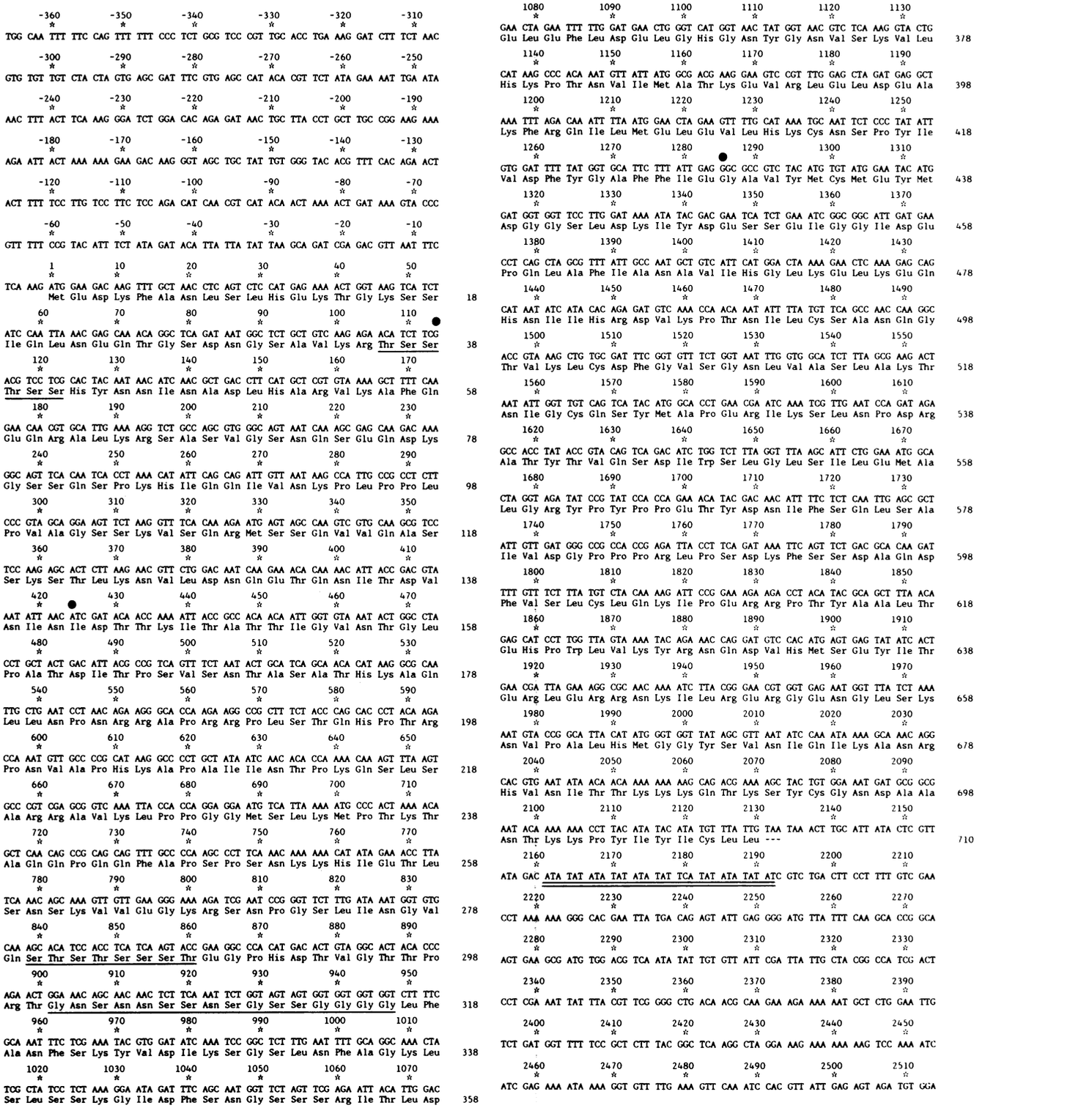

FIG. 2. Nucleotide sequence of the *PBS2* gene and the predicted amino acid sequence of the product. Nucleotides are numbered above the sequence; amino acids are numbered on the right side of the sequence. Regions rich in serine and threonine, and the Gly-Asn-Ser stretch (amino acids 301–316) are underlined. A potential stem–loop sequence at the 3′ untranslated region is doubly underlined. A possible transcription termination sequence (TAG/TATGA/TTT) of the kind observed by Zaret and Sherman (17) is found between nucleotides 2157 and 2262. The black dots at nucleotides 114, 424, and 1285 indicate the locations of *Aat* II, *Cla* I, and *Nar* I sites, respectively, used in the subcloning of an internal fragment of *PBS2* and subsequent gene disruption experiments (see the text).

spanning the *Cla* I site between fragments 2 and 3 (see Fig. 1) was subcloned into an integrating plasmid, YIp5 (9), digested with *Aat* II and *Nar* I (plasmid YIp5-AN117; 5.4 kb). The DNA was cut with *Cla* I, and a diploid GBD13 was transformed to uracil prototrophy. The diploid was sporulated, and 10 tetrads were dissected. All tetrads yielded four viable spores (2 Ura^+: 2 Ura^-), showing that *PBS2* is not essential for viability. The site of integration was outside of the *ura3* locus (31). Identical results were obtained when a haploid strain YNN27 was transformed with YIp5-AN117. Southern blot analysis of the URA^+ transformants showed that the *PBS2* gene was disrupted by integration of a single copy of the plasmid (Fig. 5) and that the gene was not duplicated elsewhere in the genome.

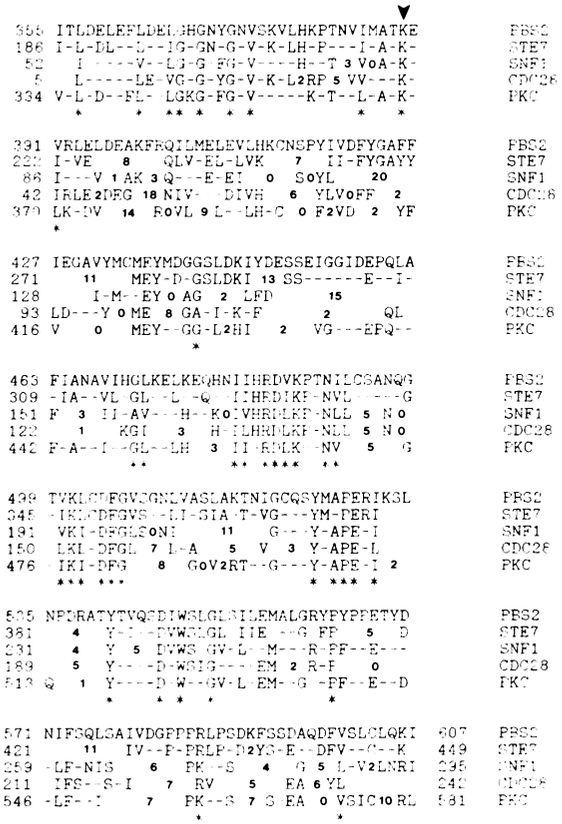

FIG. 3. Homologies between the predicted sequence of PBS2 protein and some other members of the protein kinase family; the single-letter amino acid code is used. The sequences were aligned by eye. The following equivalences were assumed for the purpose of the present alignment: I=L=V, F=Y=W, G=A, K=H=R, E=D, and Q=N. Dashes indicate identical spacing but different amino acids, while small numbers within open gaps show distances between indicated amino acids in the corresponding sequences. Asterisks show positions that are either identical or highly conserved in all five sequences. An arrowhead points to the conserved lysine residue that is postulated to form a part of the catalytic site of kinases (26). The sequences compared to *PBS2* were taken from references 25 (*STE7*), 23 (*SNF1*), 22 (*CDC28*), and 24 (*PKC*). The *STE7* gene product has not been shown yet to possess protein kinase activity, but it is included here for its exceptionally high degree of homology to *PBS2*.

When the *PBS2* gene in *pbs1* strain GBH21 was disrupted by integration of plasmid YIp5-AN117 (Fig. 5), the resulting double mutant was also viable and grew well on standard media. However, the disruption resulted in the disappearance of resistance (Fig. 6), even though GBH21 is normally resistant to polymyxin B concentration as high as 1.5 mM.

We used the 2-μm plasmid method of Falco and Botstein (32) to assign the *PBS2* gene to a chromosome. A plasmid, YEp24-4.15, carrying *Cla* I fragment 3 (see Fig. 1) was linearized with *Kpn* I and used to transform strain DBY703 [cir^0]. The strain with integrated plasmid was then crossed to a mapping strain K396-11A[cir$^+$] (3). The presence of 2-μm DNA in the resulting diploid induced instability of the chromosome with the integrated YEp24-4.15 and unmasked a *met3* mutation, thus locating the *PBS2* gene on chromosome X. This assignment was confirmed by hybridization of *Cla* I fragment 3 to the chromosome X band resolved by orthogonal field-alternating gel electrophoresis (ref. 33; data not shown). Three-factor crosses demonstrated linkage of *PBS2* to *INO1* and *ARG3* genes (this work; John Cannon, University of Pennsylvania, personal communication). Among 108 tetrads tested for *ARG3-PBS2* linkage, 55 were parental ditype, 51 were tetratype, and 2 were nonparental ditype. For the *PBS2-INO1* pair, we observed 69 parental ditype and 39 tetratype asci; for the *INO1-ARG3* pair, we found 31 parental ditype, 73 tetratype, and 4 nonparental ditype asci. From these results, the calculated map distances are 18.0 cM for *INO1-PBS2*, 29.1 cM for *PBS2-ARG3*, and 44.9 cM for *INO1-ARG3*, and the order of the genes is *INO1-PBS2-ARG3* as determined from the double crossover (lowest frequency) class of recombinant spores and from the distances calculated above.

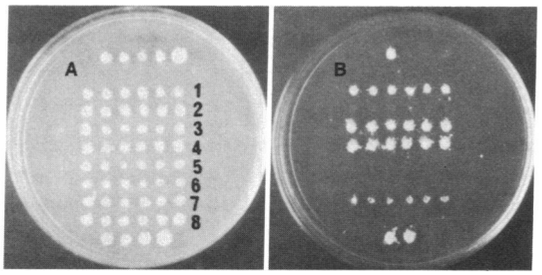

FIG. 4. Effect of the copy number of the *PBS2* gene on the expression of resistance to polymyxin B. Yeast strains were transformed to Ura$^+$ with YCp50 (top and bottom rows) or with R5H (odd-numbered rows) or YCp50.PBS2 (even-numbered rows). The control plate (*A*) was printed onto a yeast extract-peptone-glucose plate containing 1.0 mM polymyxin B (*B*) and incubated 48 hr at 30°C. Rows: top (unnumbered), from left to right: YNN27, GBH21, GBD13, GBD18, and GBD16; 1 and 2, YNN27; 3 and 4, GBH21; 5 and 6, GBD13; 7 and 8, GBD18; bottom (unnumbered), from left to right: DBY939, GBH17, GBD7, and GBD4. Note that some "wild-type" strains are more sensitive to polymyxin B than others (e.g., compare GBD13, row 5, and GBD18, row 7). Even though the cells are transformed with the same plasmid R5H, GBD13 remains sensitive while GBD18 acquires resistance. The reason for this variability is unknown.

DISCUSSION

We have identified a yeast gene that confers PBS^r phenotype when propagated on a high-copy-number plasmid. The *PBS2*

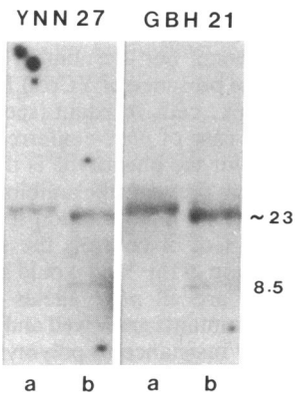

FIG. 5. Southern blot hybridization analysis of *PBS2* gene and *pbs2* disruption. Total DNA was isolated from yeast strains YNN27 (*PBS1*$^+$) and GBH21 (*pbs1-273*), digested with *Pvu* II, and fractionated on 0.8% agarose gel. DNA was transferred onto nitrocellulose membrane and hybridized [2× SSPE (1× = 0.15 M NaCl/10 mM sodium phosphate, pH 7.4/2 mM EDTA, pH 7.4) at 37°C for 20 hr] to a probe prepared from the *Cla* I fragment 3 (see Fig. 1) subcloned into pBR322 deleted for the *Hin*dIII–*Pvu* II DNA. The membrane was washed three times at room temperature with 0.8× SSPE containing 0.1% NaDodSO$_4$ and then incubated in the same solution for 30 min at 65°C. Lanes: a, DNA without disruption; b, DNA from transformants obtained by integration of YIp5-AN117 plasmid. Since there are no *Pvu* II sites within the *PBS2* gene (see Fig. 1) and only one in the YIp5 moiety, cutting the chromosomal DNA with *Pvu* II generates one hybridizing fragment in the absence of integration and two smaller fragments when a single plasmid integration has occurred. Numbers in the margin show the approximate sizes (kb) of the fragments generated by *Pvu* II digestion of the disrupted DNA.

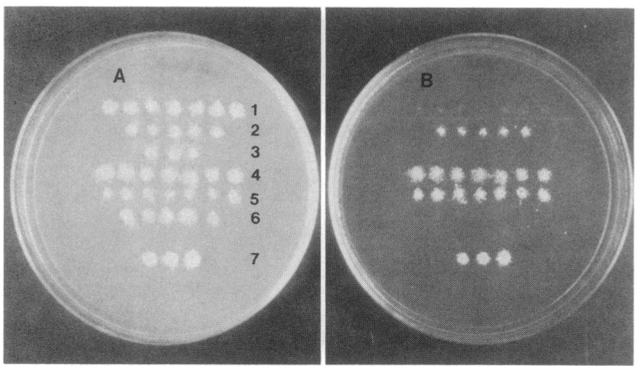

FIG. 6. Effect of *PBS2* gene disruption on polymyxin B resistance. Strains YNN27 (rows 1–3) and GBH21 (rows 4–7) were transformed to Ura$^+$ with the following plasmids: YEp24-BN445 (rows 1 and 4), R5H (rows 2 and 5), YIp5-AN117 linearized with *Cla* I (rows 3 and 6), and YEp24 (row 7). YEp24-BN445 carries a fragment from R5H that extends from the unique *Bam*HI site just upstream of the cloned yeast DNA to the *Nar* I site at nucleotide position 1285 (see Figs. 1 and 2). The control plate (*A*) was printed onto a yeast extract-peptone-glucose plate containing 0.9 mM polymyxin B (*B*) and incubated 24 hr at 30°C. See the text for further details.

gene is located on chromosome X and is not allelic to the previously described *PBS1* gene (1), nor does its high dosage appear to make *pbs1* mutants more resistant to the drug (not shown). Gene disruption experiments and Southern blot analysis show that *PBS2* exists in a single copy in the yeast genome and is not essential for viability. However, the inactivation of the *PBS2* gene in a *pbs1* background leads to a complete loss of resistance to polymyxin B. This result demonstrates that *PBS2* is necessary for the expression of *pbs1* activity.

How do cells become resistant to polymyxin B? The resistance observed when cells are transformed with R5H multicopy plasmid is most likely due to titration of the inhibitor by the excess of *PBS2* gene product. It is not known how many copies of *PBS2*$^+$ gene have to be present for the cell to acquire resistance, but the number must be greater than three because the presence of YCp50.PBS2 in a diploid is not sufficient to make cells resistant (see Fig. 4, GBD18, rows 7 and 8). In the case of *pbs1*-conferred resistance, the simplest explanation for the phenotype is that a mutation in a membrane component prevents the antibiotic from reaching its site(s) of action. (This interpretation implies that *PBS1* gene product is a part of or controls the synthesis of that component.) A mutation of this kind would be expected to be recessive, as indeed are all *pbs1* alleles tested thus far. However, since *pbs1* mutants grow well and have no obvious phenotype except for resistance to polymyxin B, the mutation could not have caused a dramatic functional or structural change in the membrane.

The deduced amino acid sequence (Figs. 2 and 3) strongly suggests a protein kinase function for the putative *PBS2* polypeptide, and deletions of portions of the single open reading frame from plasmid R5H abolish the *PBS2*-mediated resistance (Fig. 6). Therefore, although biochemical characterization of the *PBS2* gene product is still lacking, it seems reasonable to assume that it is a protein, most likely a protein kinase. Consequently, one model that can be suggested is that the PBS2 kinase could be involved in phosphorylation of a membrane component (a receptor?) that is affected by polymyxin B and that is modified by the *pbs1* mutation. In wild-type cells, polymyxin B may prevent efficient phosphorylation and thus cause inhibition of growth. The drug also may cross the membrane and inhibit the kinase directly. In contrast, the *pbs1* mutant cells may have undergone modification in the receptor so as to exclude polymyxin B because of a much increased affinity for phosphate groups donated by the kinase and because of the resulting conformational change in the receptor. When *PBS2* gene is disrupted, no phosphorylation of the receptor can occur, polymyxin B is able to bind, and inhibition of growth is seen. Alternatively, one could postulate that physical binding of the *PBS2* kinase to the *pbs1* receptor precedes (or follows) phosphorylation and is essential for resistance. Again, in the absence of the kinase protein, the resistance would be lost. The definitive test of these models awaits the isolation and characterization of the postulated protein kinase and the cloning and characterization of the *PBS1* gene. Whether *PBS2* gene encodes a yeast equivalent of protein kinase C, the kinase specifically inhibited by polymyxin B in mammalian cells (19–21), also remains to be seen.

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1. Boguslawski, G. (1985) *Mol. Gen. Genet.* **199,** 401–405.
2. Boguslawski, G. (1986) *Antimicrob. Agents Chemother.* **29,** 330–332.
3. Klapholz, S. & Esposito, R. E. (1982) *Genetics* **100,** 387–412.
4. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
5. Sherman, F., Fink, G. R. & Hicks, J. B. (1983) *Methods in Yeast Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
6. Ito, H., Fukuda, Y., Murata, K. & Kimura, A. (1983) *J. Bacteriol.* **153,** 163–168.
7. Rose, M. & Botstein, D. (1983) *J. Mol. Biol.* **170,** 883–904.
8. Nasmyth, K. A. & Reed, S. I. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 2119–2123.
9. Parent, S. A., Fenimore, C. M. & Bostian, K. A. (1985) *Yeast* **1,** 83–138.
10. Carlson, M. & Botstein, D. (1983) *Cell* **28,** 145–154.
11. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
12. Biggin, M. D., Gibson, T. J. & Hong, G. F. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 3963–3965.
13. Bankier, A. T. & Barrell, B. G. (1983) *Techniques in Nucleic Acid Biochemistry* (Elsevier, Limerick, Ireland), Vol. B508, pp. 1–33.
14. Dale, R. M. K., McClure, B. A. & Houchins, J. P. (1985) *Plasmid* **13,** 31–40.
15. Pescador, R. S. & Urdea, M. S. (1984) *DNA* **3,** 339–343.
16. Rine, J., Hansen, W., Hardeman, E. & Davis, R. W. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 6750–6754.
17. Zaret, K. S. & Sherman, F. (1982) *Cell* **28,** 563–573.
18. Powers, S., Michaelis, S., Broek, D., Santa Anna-A., S., Field, J., Herskowitz, I. & Wigler, M. (1986) *Cell* **47,** 413–422.
19. Wrenn, R. W. & Wooten, M. W. (1984) *Life Sci.* **35,** 267–276.
20. Nel, A. E., Wooten, M. W., Goldschmidt-Clermont, P. J., Miller, P. J., Stevenson, H. C. & Galbraith, R. M. (1985) *Biochem. Biophys. Res. Commun.* **128,** 1364–1372.
21. Stutchfield, J., Jones, P. M. & Howell, S. L. (1986) *Biochem. Biophys. Res. Commun.* **136,** 1001–1006.
22. Lorincz, A. T. & Reed, S. I. (1984) *Nature (London)* **307,** 183–185.
23. Celenza, J. L. & Carlson, M. (1986) *Science* **233,** 1175–1180.
24. Parker, P. J., Coussens, L., Totty, N., Rhee, L., Young, S., Chen, E., Stabel, S., Waterfield, M. D. & Ulrich, A. (1986) *Science* **233,** 853–859.
25. Teague, M. A., Chaleff, D. T. & Errede, B. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 7371–7375.
26. Kamps, M. P., Taylor, S. S. & Sefton, B. M. (1984) *Nature (London)* **310,** 589–592.
27. Hunter, T. & Cooper, J. A. (1985) *Annu. Rev. Biochem.* **54,** 897–930.
28. van Beveren, C. & Verma, I. M. (1986) *Curr. Topics Microbiol. Immunol.* **123,** 73–98.
29. Knopf, J. L., Lee, M.-H., Sultzman, L. A., Kriz, R. W., Loomis, C. R., Hewick, R. M. & Bell, R. M. (1986) *Cell* **48,** 491–502.
30. Groffen, J., Heisterkamp, N., Reynolds, F. H. & Stephenson, J. R. (1983) *Nature (London)* **304,** 167–169.
31. Winston, F., Chumley, F. & Fink, G. R. (1983) *Methods Enzymol.* **101,** 211–228.
32. Falco, S. C. & Botstein, D. (1983) *Genetics* **105,** 857–872.
33. Carle, G. F. & Olson, M. V. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 3756–3760.