A yeast gene (*BEM1*) necessary for cell polarization whose product contains two SH3 domains

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CELL polarization requires that a cellular axis or cell-surface site be chosen and that the cytoskeleton be organized with respect to it. Details of the link between the cytoskeleton and the chosen axis or site are not clear[1]. Cells of the yeast *Saccharomyces cerevisiae* exhibit cell polarization in two phases of their life cycle, during vegetative growth and during mating, which reflects responses to intracellular and extracellular signals, respectively. Here we describe the isolation of two mutants defective specifically in cell polarization in response to peptide mating pheromones. The mutants carry special alleles (denoted *bem1-s*) of the *BEM1* gene required for cell polarization during vegetative growth[2,3]. Unlike other *bem1* mutants, the *bem1-s* mutants are normal for vegetative growth. Complete deletion of *BEM1* leads to the defect in polarization of vegetative cells seen in *bem1* mutants[2,3]. The predicted sequence of the *BEM1* protein (Bem1p) reveals two copies of a domain (denoted SH3) that is found in many proteins associated with the cortical cytoskeleton and which may mediate binding to actin or some other component of the cell cortex[4,5]. The sequence of Bem1p and the properties of mutants defective in this protein indicate that it may link the cytoskeleton to morphogenetic determinants on the cell surface.

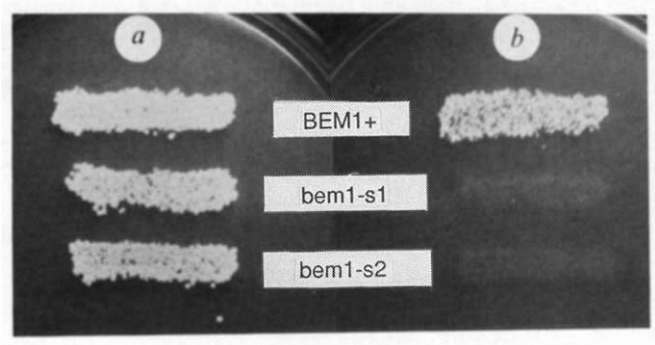

FIG. 2 Mating defect of *bem1-s* mutant cells. *a*, Mating to *BEM1*$^+\alpha$ strain IH1793. *b*, mating to *bem1-s1* α strain JC107. The **a** strains are *BEM1*$^+$, JC2-1B; *bem1-s1*, JC-G11; *bem1-s2*, JC-F5.
METHODS. Patches of **a** strains isogenic except at the *BEM1* locus were replica-plated to lawns of wild-type or mutant α strains on YEPD (permissive) medium and incubated at 30 °C for roughly 5 h to allow mating. These plates were then replica-plated to minimal (restrictive) medium on which only diploid cells were able to grow and incubated for a further 36 h. *MAT***a** *bem1-s* and *MAT*α *bem1-s* strains had similar mating defects.

The *bem1-s* mutants were identified in a screen designed to detect nonmating mutants specifically defective in localizing cell-surface growth towards a mating partner (J.C. and I.H., manuscript in preparation; see Fig. 1 legend). In contrast to

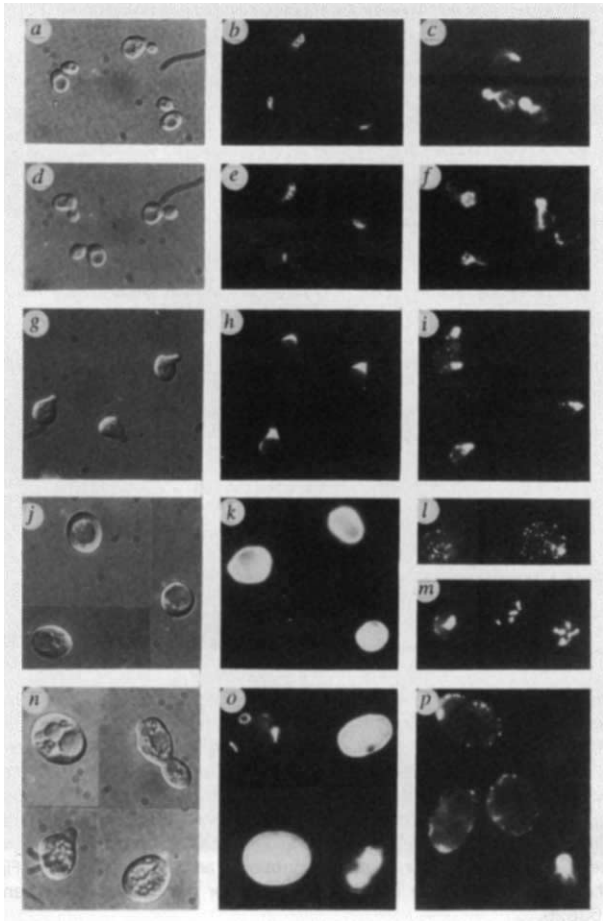

FIG. 1 Morphology (left column), chitin distribution (middle column), and actin distribution (right column) in wild-type and *bem1* mutant cells of **a** mating type. *a–c*, Wild-type strain KO2-5A; *d–f*, *bem1-s1* mutant strain JC-G11; *g–i*, wild-type strain JC2-1B treated with α-factor; *j–l* *bem1-s1* mutant strain JC-G11 treated with α-factor; *m*, *bem1-s2* mutant strain JC-F5 treated with α-factor; *n–p* *bem1*-deletion strain KO2-5C. Final magnifications are the same in all panels. Representative cells are shown.
METHODS. Mutants were isolated in a two-step screen. First, mutants were isolated that were unable to mate with strains that themselves have reduced mating ability (defective in *FAR1* (ref. 7) or in *FUS1* and *FUS2* (ref. 16)). These mutants were then examined for their morphological response to mating pheromone, which in wild-type cells induces the formation of shmoos (*g*). For viewing under the microscope, yeast strains were grown in YEPD medium to early log phase at 30 °C, except for the *bem1*-deletion strain, which was grown at 25 °C. Where appropriate, pheromone (α-factor; Sigma) was added to a final concentration of 10^{-6} M, and the cells were grown for a further 2–3 h. To observe morphology, live cells were viewed by differential interference contrast microscopy. Cells were fixed and stained with phalloidin to visualize actin or with Calcofluor to visualize chitin[6]. Identical results were obtained when an anti-actin antibody was used to visualize actin (data not shown). The strain deleted for *BEM1* contains the *LEU2* gene in place of the entire *BEM1* coding sequence and was constructed as follows. A fragment containing *BEM1* and several hundred base pairs of flanking DNA was cloned into a pUC vector. A portion of this plasmid was amplified by the polymerase chain reaction using divergent primers that hybridized just upstream of the *BEM1* start codon and just downstream of the stop codon. The resulting linear fragment was cleaved at a site introduced by the primers and circularized, yielding a plasmid that contained just the *BEM1* flanking regions joined by a restriction site. A selectable marker (*LEU2*) was then introduced into this site. A linear fragment containing the marker gene surrounded by the *BEM1* flanking regions was used to replace the chromosomal *BEM1* gene by the one-step gene replacement method.[17] Southern blotting confirmed that gene replacement had occurred by homologous recombination (data not shown).

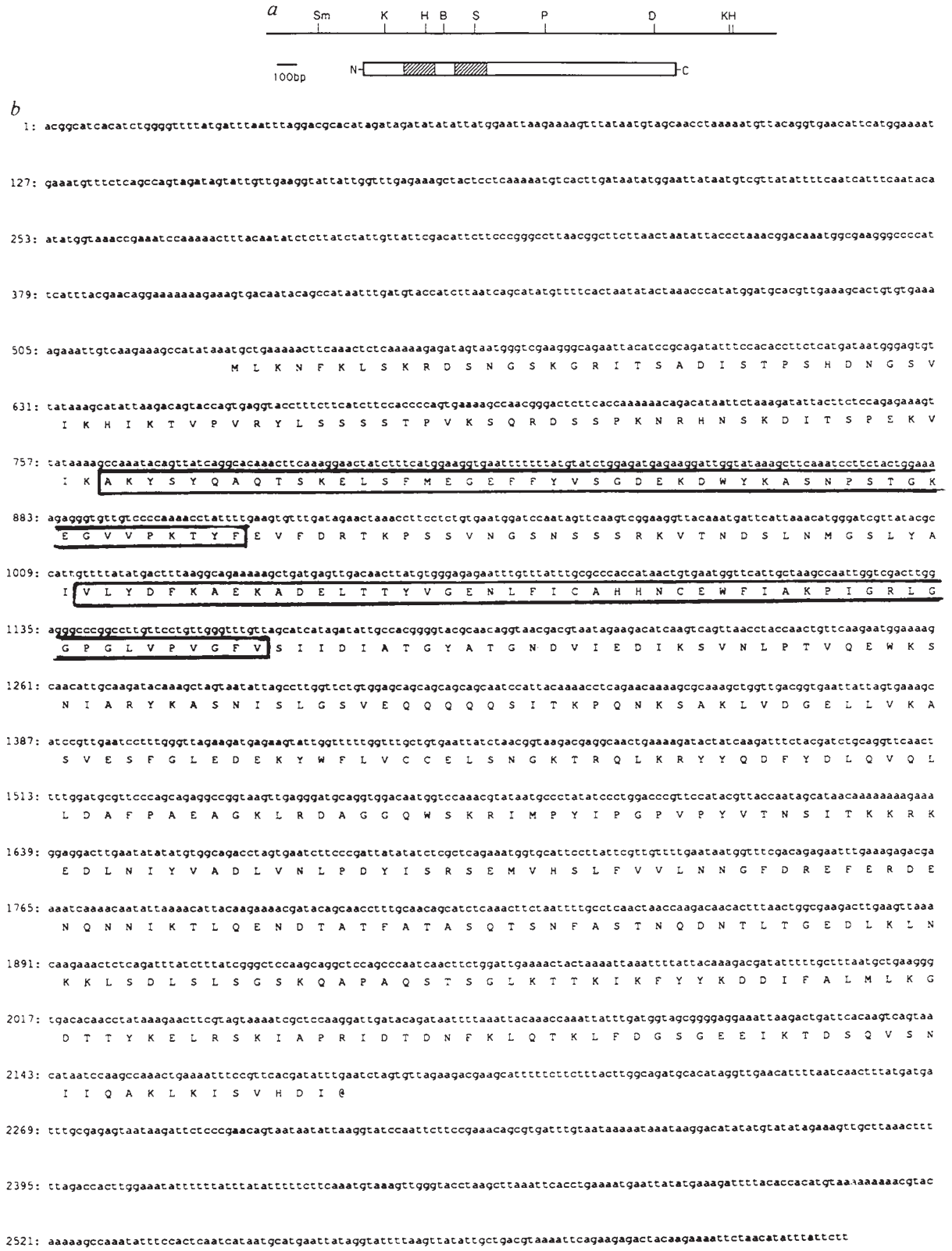

FIG. 3 *BEM1* map and sequence. *a*, Restriction map of sequenced region and location of the open reading frame. Hatched boxes indicate the positions of the SH3 domains. Restriction sites are as follows: B, *Bam*HI; D, *Dra*I; H, *Hin*dIII; K, *Kpn*I; P, *Pst*I; S, *Sal*I; Sm, *Sma*I. *b*, Nucleotide and predicted amino-acid sequences of *BEM1*. The SH3 domains are boxed.

METHODS. *BEM1* was cloned using a genomic DNA library in a low-copy number vector (YCp50)[18] by complementation of the mating defect of a *bem1-s1* strain. The complementing gene was confirmed to be the wild-type allele of the *bem1-s* mutation and not a suppressor by segregation analysis using the original *bem1-s* mutation and a mutation constructed *in vitro* in the cloned gene which was integrated by homologous recombination (data not shown). The dideoxy chain termination method was used for sequencing M13 clones generated using restriction sites or a series of *Exo*III-generated deletions in pBluescript SK^+ and KS^+. The nucleotide sequences of both strands were determined independently in both laboratories and agreed exactly. The predicted protein sequence was compared with the PIR/Dayhoff database using the FASTA program[19]; significant homologies were detected only for proteins containing an SH3 domain (see Fig. 4). The EMBL Data Library accession number for the nucleotide sequence is X63826.

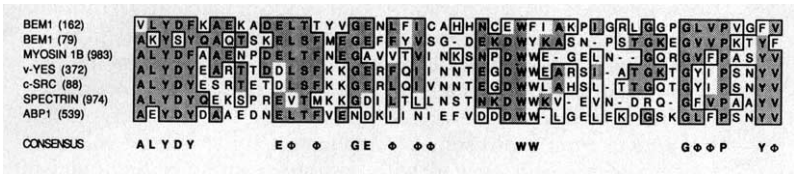

FIG. 4 Amino-acid alignment of homologous regions of Bem1p and representative SH3 domain-containing proteins. The amino acid of the first residue of each sequence is given in parentheses. Φ, Hydrophobic residues. Boxed residues, conservation with either BEM1 domain; residues that are identical are boxed and shaded. References to amino-acid sequences: *Acanthamoeba* myosin-IB[20], v-yes[21], c-src[22], α-spectrin[23], yeast ABP1[5], consensus[4].

wild-type strains, which form polarized cells called shmoos when treated with mating pheromone (Fig. 1*g*), two mutants were found that enlarged in a uniform manner (the Shmoo$^-$ phenotype; Fig. 1*j*). The mutations in these strains, designated *bem1-s1* and *bem1-s2,* affect the same gene by segregation and complementation tests (data not shown). These mutants had a weak mating defect when mated to wild-type cells and a strong mating defect when mated with other *bem1-s* mutants (Fig. 2). The *bem1-s* mutants grew normally at all temperatures on rich and minimal media. Their morphologies and budding patterns were similar to those of wild-type cells (Fig. 1*a, b, d, e*).

To examine the polarization defect of the *bem1-s* mutants in more detail, we determined the distribution of cell-wall chitin by staining with Calcofluor and the distribution of actin by staining with rhodamine-phalloidin and by immunofluorescence using an actin-specific antibody[6]. Chitin is localized predominantly to the shmoo neck in wild-type cells exposed to pheromone (Fig. 1*h*). By contrast, *bem1-s* mutants deposited chitin throughout the enlarging cell surface (Fig. 1*k*). The actin organization in vegetatively growing *bem1-s* cells generally appeared normal: the actin patches were concentrated in the buds, and actin cables ran through the mother cells into the buds (compare Fig. 1*f* and *c*). Wild-type cells showed a similarly polarized actin distribution during shmoo formation (Fig. 1*i*). By contrast, the *bem1-s* cells had a delocalized actin distribution after addition of pheromone (Fig. 1*l*). Roughly one-third of *bem1-s2* mutant cells showed an additional, more aberrant actin distribution: 1–10 large clumps of actin per cell (Fig. 1*m*). The *bem1-s* mutations did not affect other aspects of pheromone response, notably cell-cycle arrest and induction of *FUS1-lacZ* (see ref. 7).

BEM1 was cloned by complementation of the mating defect of a *bem1-s1* strain (see Fig. 3 legend) and had been cloned independently by complementation of the vegetative growth defect of a *bem1-3* mutant[2]. The nucleotide sequences of both *BEM1* isolates were identical. The predicted *BEM1* protein contains 551 amino acids (Fig. 3) with a calculated relative molecular mass of 62,000. The amino-terminal half contains two regions similar to the 'SH3 domain' (Fig. 4), a motif of 50 amino-acid residues that has been found in a variety of disparate proteins, including non-receptor tyrosine kinases, myosin-I, phospholipase C, and the yeast actin-associated protein Abp1p (ref. 5). The function of the SH3 domain is unknown, but many of the proteins containing this motif associate with the cortical cytoskeleton or localize to the plasma membrane[4,5].

To investigate further the role of Bem1p during vegetative growth, a strain deleted for *BEM1* was constructed. The chromosomal *BEM1* coding sequence was replaced precisely by a fragment containing *LEU2* (see legend to Fig. 1). Cells deleted for *BEM1* were able to germinate but grew slowly at room temperature or at 30 °C and not at all at 14 °C or 37 °C. Cells grown at 25 °C or 30 °C displayed heterogeneous, odd morphologies (Fig. 1*n*): some were either triangular or shmoo-like; others were very large and rounded and usually unbudded. Many cells were multinucleate (as seen by 4′,6-diamidino-2-phenylindole staining; results not shown). Chitin deposition was delocalized (Fig. 1*o*) and the asymmetric actin distribution seen in wild-type cells was disrupted (Figs. 1*f, p*). Shift of a culture to 37 °C resulted in a population with heterogeneous terminal morphologies and an increased fraction of unbudded cells (70% versus 45% at lower temperatures). The phenotypes of the *bem1* null mutant are similar to those of *bem1-3* and *bem1-4* mutants identified previously[2].

Three observations thus indicate that Bem1p has a direct role in organizing the actin cytoskeleton. First, *bem1* null mutants have a disorganized actin cytoskeleton. Second, *bem1-s2* mutants produce distinctive clumps ('bars') of actin resembling those that accumulate in certain actin[8] or profilin[9] mutants. Third, Bem1p contains two SH3 motifs. We propose that Bem1p has two functional domains, one (containing the SH3 domains) concerned with binding actin or some other component of the cortical cytoskeleton, the second involved with recognizing the site for cell polarization during vegetative growth or mating. In vegetative cells, the morphological landmark may be a structure that is adjacent to a site of previous budding[10]. The *BUD* gene products may direct the positioning of Cdc24p, Cdc42p and Bem1p to this site[3,10,11]. During mating, a cell polarizes towards a signal from the mating partner that is probably a gradient of secreted pheromone[12]. This process requires, first, an override of the vegetative bud-site selection programme and, second, establishment of polarity towards the mating partner. Erasing the bud-site selection programme may be accomplished by pheromone-induced inactivation of one or more of the *BUD* gene products. The site-recognition domain of Bem1p would then be free to interact with the new site; *bem1-s* mutants might be unable to interact with this site (but capable of interacting with the site in vegetative cells). Several observations suggest that the morphogenetic marker in mating cells might be the pheromone receptor itself. First, cells lacking the carboxy-terminal segment of the pheromone receptor have the same polarization defect as *bem1-s* mutants[13]. Second, receptors cluster at the site of pheromone-induced morphogenesis[14]. They seem to have a role in cell polarization that is independent of their coupled G protein and associated signalling pathway[14]. Spa2p is also necessary for polarization in response to mating factors[15] and may interact with Bem1p or the morphogenetic determinant. By determining the location of Bem1p and molecules with which it interacts, we may begin to understand how the cytoskeleton recognizes signals on the cell surface to form a polarized cell. □

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