BIK1, a Protein Required for Microtubule Function during Mating and Mitosis in *Saccharomyces cerevisiae*, Colocalizes with Tubulin

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**Abstract.** BIK1 function is required for nuclear fusion, chromosome disjunction, and nuclear segregation during mitosis. The BIK1 protein colocalizes with tubulin to the spindle pole body and mitotic spindle. Synthetic lethality observed in double mutant strains containing a mutation in the BIK1 gene and in the gene for α- or β-tubulin is consistent with a physical interaction between BIK1 and tubulin. Furthermore, over- or underexpression of BIK1 causes aberrant microtubule assembly and function. bik1 null mutants are viable but contain very short or undetectable cytoplasmic microtubules. Spindle formation often occurs strictly within the mother cell, probably accounting for the many multinucleate and anucleate bik1 cells. Elevated levels of chromosome loss in bik1 cells are indicative of defective spindle function. Nuclear fusion is blocked in bik1 × bik1 zygotes, which have truncated cytoplasmic microtubules. Cells overexpressing BIK1 initially have abnormally short or nonexistent spindle microtubules and long cytoplasmic microtubules. Subsequently, cells lose all microtubule structures, coincident with the arrest of division. Based on these results, we propose that BIK1 is required stoichiometrically for the formation or stabilization of microtubules during mitosis and for spindle pole body fusion during conjugation.

Evidence obtained from biochemical experiments suggests that the key to the diverse functions of microtubules lies in the proteins associated with the microtubules. These proteins are thought to modulate the assembly and stability of microtubules and to mediate the interaction of microtubules with other cellular components. A number of proteins have been shown to coassemble with microtubules in vitro (reviewed by Olmsted, 1986) and associate with interphase and spindle microtubules (Vallee and Bloom, 1983; Bloom et al., 1984). Since most of the studies of microtubule-associated proteins (MAPs)1 have been conducted in systems not amenable to genetic analysis, the in vivo functions of these MAPs are still uncertain. For this reason, recent interest has focused on microtubule function in yeast, where genetic interactions between tubulin and MAPs can be established.

In yeast, microtubules polymerize from a microtubule-organizing center, called the spindle pole body, which is embedded in the nuclear envelope. Microtubules emanate from the spindle pole body into the nucleus to form the spindle and out of the nucleus to form cytoplasmic microtubules ar-

1. **Abbreviations used in this paper:** DAPI, 4'-6-diamino-2-phenylindole; MAP, microtubule-associated proteins; PI, protease inhibitors; SC, synthetic medium; SD, minimal medium; YPD, rich medium.

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Materials and Methods

Plasmids

The plasmids used in this work are listed in Table I. Ligations were performed in low-melting point agarose (Struhl, 1983) using 89 mM Tris-borate, 89 mM boric acid, and 10 mM EDTA, pH 8.0, as the electrophoresis buffer. Other DNA manipulations were performed as described by Maniatis et al. (1982). The plasmid pVB19 was propagated in Escherichia coli strain TG1 (Carter et al., 1985). E. coli strain LE392 (Borch et al., 1976) was used for all other transformations.

Strain Construction and Growth Conditions

Yeast strains used in this work are listed in Table II. Rich medium (YPD), minimal medium (SD), synthetic complete medium (SC), yeast cell culture, and tetrad analysis were as described by Sherman et al., (1986). All yeast transformations were carried out by the lithium acetate method of Ito et al., (1983). Transformations were carried out by the lithium acetate method of Ito et al., (1983).

Immunofluorescence Microscopy

Immunofluorescent staining of yeast cells was performed using a modification of the methods of Adams and Pringle (1984). Cells were fixed in 3.7% formaldehyde in 0.1 M KPO4, pH 6.5 for 90 rain at room temperature. Antibody incubations and washes were performed in 0.1 M KPO4, pH 7.5, 1.2 M sorbitol. Cells were applied to the wells of multiwell microscope slides (Cell-Line, Newfield, NJ) treated with 1% polyethylene-
Table II. Strains (continued)

| Strain                  | Genotype                                      |
|-------------------------|-----------------------------------------------|
| L3509                  | MATa ura3-52/ura3-52 leu2-3,112/leu2-3,112   |
|                         | +/his4-539/+ +/leu2-3,112 +/-ik61-518 tub2-402/+ |
| L4400                  | MATa ura3-52/ura3-52 leu2-3,112/leu2-3,112   |
|                         | his4-539/+ tub3::URA3/+ +/bik1-518            |
| L4401                  | MATa ura3-52/ura3-52 his4A200/has4200          |
|                         | leu2-3,112/+ +/lys2-801 cin4::HIS3/+ +/bik1-518 |
| L4402                  | MATa ura3-52/ura3-52 leu2-3,112/leu2-3,112   |
|                         | his4A200/+ +/bik-1/leu2/+ +/bik1-518          |
| S/B 503                | MATa his4l/+ +/trp1A1/trp1A1 ura3l/+ +cyh2l/+ |
|                         | +/+icanl/+                                  |
| S/B 506                | MATa bik1-l::TRP1/bik1-l::TRP1 his4l/+ +/trp1A1 |
|                         | trp1A1 ura3l/+ +/leu2-3,112/+ +/cyh2l/+ +/+icanl/+ |
|                        | (isogenic with S/B 503)                      |
| S/B 551                | L4320 transformed with pVB20                  |
|                        |                                              |
| VBY77                  | MATa ura3-52/ura3-52 leu2-3,112/leu2-3,112   |
|                         | bik1-1::TRP1/bik1-1::TRP1 trp1A1             |
|                         | (pDAD2)                                      |
| VBY79                  | MATa ura3-52/ura3-52 leu2-3,112/leu2-3,112   |
|                         | bik1-l::TRP1/bik1-l::TRP1 ura3-52/ura3-52    |
|                         | leu2-3,112/leu2-3,112                       |
|                         | trp1A1/trp1A1 ura3l/+ +/leu2-3,112/+ +/cyh2l/+ |
|                         | +/+icanl/+                                  |
| VBY82                  | MATa ura3-52/ura3-52 leu2-3,112/leu2-3,112   |
|                         | bik1-l::TRP1/bik1-l::TRP1 ura3-52/ura3-52    |
|                         | trp1A1/trp1A1 ura3l/+ +/leu2-3,112/+ +/cyh2l/+ |
|                         | +/+icanl/+                                  |
| JFY195                 | MATa ura3-52 trp1A1 lys2-801 cyh2 bik1-518    |
|                         | [hro]                                        |

Braces indicate that the strain harbors the autonomously replicating plasmid whose name is contained within the brackets. Brackets refer to the mitochondri-
al genotype. -: indicates integration of the gene indicated after the symbol at the locus imme-
mediately preceding the symbol.

References for tub and
alleles are:
- tub-1 (Stearns and Botstein, 1988);
- tub-104 and tub-105 (Thomas, 1985); tub-240, tub-240, tub-240, tub-240, tub-240.
- his3A200/his3A200 ade2/ade2 cyh2/cyh2 canl/canl
- ub3 (Schatz et al., 1986b); cin4::URA3
- biki-1::TRP1 his4l/+ +/trp1A1/trp1A1 ura3l/+ +/leu2-3,112/+ +/cyh2l/+ +/+icanl/+ (isogenic with S/B 503)

Preparation of Antibody to the BIK1 Gene Product

The E. coli strain TGI harboring pVB19 produced a fusion protein containing
the first 324 amino acids of the TrpE protein, 10 amino acids of the poly-
linker, and 240 amino acids of BIK1. trpE expression was induced with
indolacrylic acid (Koerner et al., 1990). An extract prepared from cells (8 ml
total) lysed directly in Laemmli (1970) sample buffer was boiled, sonicated,
and destained with several changes of water. The band corresponding to the fu-
sion protein, ~68 kD in size, was excised from the gel and the fusion protein
was electroeluted from the gel. Rabbits were injected intraperitoneally with
50 #g of the fusion protein mixed with an equal volume of Freund's incom-
plete adjuvant (Sigma Chemical Co.).

Blood was taken from the marginal ear vein by venipuncture.

Cells were grown overnight to saturation, diluted into fresh medium, and
grown to a density of 10^7 cells/ml. Cells lacking plasmids or containing
pVB20 or pRS314 were grown in SC medium or SC medium with uracil
or tryptophan omitted for plasmid maintenance (growth medium). Cells
were harvested by filtration on nitrocellulose filters (0.45 #m pore size; Na-

Synchronization of Cells

Cells were grown overnight to saturation, diluted into fresh medium, and
grown to a density of 10^7 cells/ml. Cells lacking plasmids or containing
pVB20 or pRS314 were grown in SC medium or SC medium with uracil
or tryptophan omitted for plasmid maintenance (growth medium). Cells
were harvested by filtration on nitrocellulose filters (0.45 #m pore size; Na-

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gene Co., Rochester, NY) and resuspended in the same medium, adjusted to pH 4.0, to which α-factor was added to 5 μM. Cells were incubated at 30°C for 2 h at which time >90% of the cells were unbudded. Cells were harvested by filtration on nitrocellulose filters, washed and resuspended in growth medium and incubated for various times at 14 or 30°C.

**Measurement of Chromosome Loss and Mitotic Recombination**

The assay for chromosome loss and mitotic recombination is based on that of Wood and Hartwell (1982). Five colonies of each strain grown at 30°C were inoculated individually into YPD medium at a density of 10⁶ cells/ml. Cultures were split and incubated at 30°C for 90 min or at 14°C for 24 h. Aliquots of each culture were then washed twice with water, diluted, and plated on YPD to measure the number of viable cells/ml and on SD containing 60 μg/ml canavanine and 20 mg/ml histidine. Colonies that grew on SD plus canavanine and histidine were replica-plated to SD and SD plus histidine. The frequency of mitotic recombination in the CEN/CAN1 interval equals the number of His⁺can⁺ cells/total viable cells and the frequency of loss of chromosome V equals the number of His⁻can⁻ cells/total cells.

**Analysis of Karyogamy**

Matings were performed by mixing together 10⁶ cells of each parent grown to log phase in YPD. Mixtures were pelleted, resuspended in 0.5 ml of YPD, spread on small YPD plates (60 × 15 cm), and incubated for various periods of time at 30°C. Mating mixtures were analyzed by fluorescence microscopy as described in a preceding section. To measure the formation of cytoductants, dilutions of the mating mixtures were plated on complete medium containing 3% glycerol, 0.1% glucose, and 10 μg/ml cycloheximide. Diploid formation and total cell counts were determined by plating dilutions of the mating mixtures on SD plus uracil and YPD, respectively.

**Overexpression of BIKI from the GAL1 Promoter**

Cells were grown to log phase in SC medium minus uracil containing 2% raffinose instead of glucose. Galactose was added to 2% and aliquots were removed at various times for Western blot analysis, for immunofluorescence, and to monitor cell density.

**Preparation of Cell Extracts and Immunoblotting**

Whole cell extracts from yeast were prepared by glass bead lysis directly in trichloroacetic acid (Ohashi et al., 1987). Samples were analyzed by electrophoresis on 10% SDS-polyacrylamide gels (Laemmli, 1970), which were blotted electrophoretically onto nitrocellulose filters. Filters were incubated for 30 min with shaking in blocking solution: PBS containing 0.5% Tween 20 (vol/vol), 0.5% sodium azide (wt/vol) and 5% nonfat dry milk (wt/vol). Filters were then transferred to fresh blocking solution containing anti-BIK1 antibody (diluted 1/200), 206.1 anti-β-tubulin antibody (diluted 1/1,000, incubated for 2 h at room temperature, and washed three times 10 min in PBS/Tween. Autoradiography was performed at −70°C using Kodak X-AR film. Western blots were quantitated according to the method of Suissa (1983).

**Computer Analysis of the BIKI Amino Acid Sequence**

Secondary structure analysis of BIKI was performed using the Garnier et al., (1978) and Chou and Fasman (1978) algorithms. The National Biomedical Research Foundation Protein Sequence Database was searched with the entire BIKI sequence using FASTA (Pearson and Lipman, 1988) and with the sequence CNX4CX3GHX4C using MATCH (George et al., 1986). The sequence of BIKI was compared directly to that of tau using FASTA (Pearson and Lipman, 1988). The statistical significance of sequence homologies was determined using IALIGN, an interactive version of the National Biomedical Research Foundation Align program (Dayhoff et al., 1983).

**Results**

**Colocalization of BIKI with Tubulin**

Subcellular immunolocalization of the BIKI gene product was determined using antibodies raised against a TrpE-BIKI fusion protein. The anti-BIKI antibodies react with polypeptides 57–60 kD in size, which are present in extracts of wild-type cells but absent in extracts of bikl-l::TRP1 strains containing a disruption of the BIKI gene. The BIKI polypeptides often resolve as multiple species on Western blots and are distinguishable from the α- and β-tubulin polypeptides, 50–53 kD in size, which are present in extracts of both BIKI and bikl-l::TRP1 strains (Fig. 1). Multiple species of BIKI found on Western blots may be the consequence of posttranslational modification or proteolysis.

In an unsynchronized culture of exponentially growing cells anti-BIKI antibodies stain structures that resemble intranuclear microtubules in 10–20% of the cells at 30°C and 30% of the cells at 14°C (results not shown). Enhanced immunolocalization of BIKI at 14°C could be the consequence of the greater stability of BIKI at low temperature or the detection of transient stages of growth in which BIKI antigen is accessible to antibody binding. Although staining of microtubule structures with anti-BIKI antibodies is detectable in cells containing a single copy of the BIKI gene, staining is more evident in a strain containing BIKI on the multicopy plasmid, pVB20. The steady state levels of the BIKI polypeptide are two- to threefold higher in a strain containing pVB20 than a strain containing a single chromosomal copy of BIKI (results not shown).

The failure to detect staining of microtubule structures in a majority of cells in an unsynchronized culture could be explained by the association of the BIKI protein with tubulin only during specific stages of the cell cycle. Thus the cell cycle dependence of BIKI staining was examined by synchronizing cells with α-factor to obtain a homogeneous population of cells arrested at G1 and subsequently releasing them from arrest by transfer to fresh medium without α-factor. After release from α-factor arrest, cells were grown at 14°C. The slow doubling time at 14°C (6 h) facilitated the examination of cells at discrete stages of the cell cycle. Samples from the culture at various times after α-factor release were stained with both the antitubulin and anti-BIKI antibody.

Immediately after release from α-factor arrest (results not shown) and at 1 h after release (Fig. 2), the anti-BIKI antibodies stained the cytoplasm of unbudded cells, whereas the 

![Figure 1. BIKI and tubulin polypeptides in cell extracts of homozygous BIKI/BIKI and bikl-l::TRP1,bikl-l::TRP1 diplodips. Western blots of total cellular proteins isolated from homzygous diploids (BIKI/BIKI) (+) (L4320) and bikl-l::TRP1,bikl-l::TRP1 (-) (L4324) were probed with anti-BIKI (BIKI Ab) and 206.1 and 345 antitubulin (TUB Ab) antibodies. BIKI resolved as two species; often three species are apparent. The mobility of size markers is indicated on the left.](image-url)
Figure 2. Fluorescence staining of synchronized wild-type cells with anti-BIK1 and antitubulin antibodies. Fluorescence images are of cells of strain S/B 551 double labeled with the anti-BIK1 (BIK1 Ab) (left) and the YOL1/34 antitubulin (TUB Ab) (right) antibodies, followed by staining with FITC- and Texas Red-conjugated secondary antibodies. Cells were grown for 1, 3, 4, 5, or 6 h after release from α-factor arrest at 14°C. Bar, 6 μm.
Genetic Interaction between BIK1 and Genes Required for Microtubule Function in Yeast

The immunolocalization studies suggested a possible physical association between BIK1 and tubulin. The phenotype of double mutants between biki-518 and various mutations in the tubulin genes provided genetic evidence for this physical association. Double mutant meiotic segregants were obtained by crossing a biki-518 strain with strains containing mutations in TUB1, TUB2, or TUB3. The double mutants were tested for their growth at permissive and restrictive temperatures and sensitivity to the drug benomyl, a member of the benzimidazole class of compounds, which cause microtubule depolymerization in vivo (Jacobs et al., 1988) and in vitro (Kilmartin, 1981) and a failure of microtubule-mediated events in vivo (Quinlan et al., 1980; Delgado and Conde, 1984; Jacobs et al., 1988).

The growth of the double mutants was assessed in two ways: (a) by scoring directly the growth of the double-mutant ascospores and (b) by determining survival of vegetative double-mutant cells upon loss of a plasmid containing either a functional BIK1 or TUB gene. The ascospore germination test was complicated by growth-enhancing suppressors that arose during germination or growth of the spore clones. These occurred frequently and made the primary phenotypes of the double mutants difficult to determine. The plasmid segregation test was more easily interpreted because it was possible to produce a homogeneous population of vegetative cells of the same genotype whose phenotype, upon loss of the plasmid, could be assessed. In the plasmid segregation test, we found that the majority of the double mutants did not grow at 30°C or had a slow growth phenotype (Table III). Epistasis relationships were examined in the viable double mutant strains. The biki-518 tub2-104 and biki-518 tub2-402 mutants exhibited benomyl sensitivity equivalent to the biki-518 strain, indicating that biki-518 is epistatic to tub2-104 and tub2-402, which as single mutants confer resistance to high levels of benomyl (Thomas, 1985; Hufnaker et al., 1988). A possible explanation for the epistasis relationships is that the biki-518 mutation alters the structural properties of tubulin such that tubulin binds benomyl in the double mutants, but not in the tub2-104 and tub2-402 single mutants.

A biki-518 strain was also crossed to strains carrying cinl, cin2, or cin4 mutations, which cause chromosome instability (Hoyt et al., 1989) and, like biki-518, cause cold sensitivity, hypersensitivity to benomyl, and lethality in combination with the tub1-1 allele (Stearns et al., 1990; Hoyt et al., 1990). The double mutants exhibited a slow growth phenotype at 30°C (Table III), a permissive temperature for growth of the single mutants, and also grew more slowly than the single mutants at 14°C. Both biki-518 cin1::HIS3 and biki-518 cin2::LEU2 strains exhibited variable levels of sensitivity to benomyl, suggesting that suppressors arise frequently in the double mutants. The biki-518 cin4::URA3 double mutant exhibited benomyl sensitivity equivalent to that of the cin4::URA3 parent, indicating cin4::URA3 is epistatic to biki-518.

BIKI Is Required for Normal Microtubule Structures

If BIK1 is a structural component of microtubules, as the immunolocalization and genetic studies suggest, absence of BIK1 should cause altered microtubule assembly. To investigate this possibility, microtubule structures in BIK1 and biki-1::TRPI cells were compared. Cells were synchronized with α-factor and then grown at 14°C, at which temperature the doubling time of biki-1::TRPI cells is approximately twice that of wild type cells (results not shown). In un budded BIK1 cells, cytoplasmic microtubules emanate from a single spindle pole body (Fig. 3 A). In BIK1 cells with nascent buds, a subset of microtubules appears to point toward the site of

**Table III. Growth of Double Mutants**

| Genotype                              | Ascospore | Vegetative cell |
|---------------------------------------|-----------|-----------------|
| Wild type                             | + + +     | + + +          |
| Single mutant                         | + + +     | + + +          |
| biki-518 tab1-1 (LA392)               | +         | -              |
| biki-518 tab2-105 (LA393)             | -         | -              |
| biki-518 tab2-401 (LA394)             | -         | +              |
| biki-518 tab2-403 (LA395)             | +         | +              |
| biki-518 tab2-404 (LA396)             | +         | +              |
| biki-518 tab2-405 (LA397)             | +         | +              |
| biki-518 tab2-104 (LA398)             | +         | +              |
| biki-518 tab2-402 (LA399)             | + + +     | +              |
| biki-518 tab3 (LA400)                 | + + +     | +              |
| biki-518 cin1::HIS3 (LA401)           | +         | +              |
| biki-518 cin2::LEU2 (LA402)           | +         | +              |
| biki-518 cin4::URA3 (LA403)           | +         | ND             |

* Ascospore segregants were obtained by sporulating the diploid strains L4392-L4403, indicated in parentheses, and were grown at 30°C. Minus indicates no growth. The plusses indicate a range of growth from very slow growth (+) to moderately slow growth (++) compared with wild-type and single mutants.

1 Vegetative cells containing plasmids were obtained by sporulating diploid strains transformed with various plasmids before sporulation: L4392-L4399, L4402, and L4403 were transformed with pRB327; L4400 was transformed with pRB327; L4401 was transformed with pRB1171. Minuses indicate inability to lose pRB20, pRB327, or pRB1171 spontaneously or on medium containing 5-fluoro-uracylic acid (Boeke et al., 1984) which selects for loss of the URA3-containing plasmids pVB20 and pRB1171. The plusses indicate a range of growth from very slow growth (+) to moderately slow growth (++) compared with growth of the wild type and single mutants after plasmid loss.

Refer to biki-1::TRPI, tab1-1, tab2 (any of the alleles listed), cin1::HIS3, cin2::LEU2, or cin4::URA3 none of which has a discernable growth defect at 30°C.
bud emergence (Fig. 3B). Cytoplasmic microtubules in bikl-l::TRP1 cells at the same stages of division are absent or abnormally short (Fig. 3, A and B). During bud formation in a BIK1 cell, the nucleus moves to the neck; the spindle pole body duplicates and moves to opposite poles, forming a short spindle. In small-budded BIKI cells, the cytoplasmic microtubules extend from one or both spindle pole bodies towards the neck of the bud (Fig. 3C). Cells with both sets of microtubules pointing toward the bud are routinely observed in synchronized populations of cells grown at 14°C but rarely in unsynchronized cells, suggesting this may be a transient stage of cell division. As the bud grows, the spindle lengths near the neck of the bud and cytoplasmic microtubules extend from one spindle pole body into the mother cell and from the other spindle pole body through the neck into the daughter cell (Fig. 3D). In bikl-l::TRP1 cells with buds, the cytoplasmic microtubules are usually absent; when they are visible, they are abnormally short (Fig. 3, A and B). During anaphase the spindle in BIK1 cells elongates until it extends from one pole in the mother cell to the opposite pole in the daughter cell; the cytoplasmic microtubules splay out from the spindle pole bodies which appear to abut the cell perimeter. bikl-l::TRP1 cells are quite different: the anaphase spindle is always shorter than that in BIK1 cells. Moreover spindle elongation often occurs within the confines of one member of a budded pair (mother or daughter), a situation never observed in wild type cells. In those bikl-l::TRP1 cells the spindle traverses the neck, it rarely (as contrasted with wild-type cells) extends the full length of the mother and daughter cells. As in earlier stages of division, cytoplasmic microtubules in bikl-l::TRP1 cells are absent or abnormally short (Fig. 3E).

BIKI Is Required for Proper Nuclear Segregation during Mitosis

bikl-l::TRP1 cells show aberrant nuclear segregation, consistent with mislocalization of the spindle during mitosis. At 30°C, 5-10% of the cells are anucleate or multinucleate and at 14°C, the fraction of multinucleate and anucleate cells increases to 10-20% of the total, consistent with the slow growth of bikl-l::TRP1 strains at low temperature. In BIK1 cultures grown at 30 or 14°C, 1% of the cells are multinucleate or anucleate. The basis of the defect in nuclear segregation during mitosis in bikl-l::TRP1 cells was determined by monitoring nuclear position in diploid cells (BIKI/BIKI and bikl-l::TRP1/bikl-l::TRP1) synchronized with α-factor. Cells were monitored for two cell-division cycles after release from α-factor arrest. The rates of bud formation and release were equivalent during this time period for BIK1/BIKI and bikl-l::TRP1/bikl-l::TRP1 cultures grown at 30 or 14°C (results not shown). Prior to anaphase, nuclear DNA is located either in the mother cell or spanning the neck between mother and daughter cells in a majority of BIK1/BIKI and bikl-l::TRP1/bikl-l::TRP1 cells grown at 30 and 14°C. A larger percentage of BIK1/BIKI cells have nuclear DNA spanning the neck compared with bikl-l::TRP1/bikl-l::TRP1 cells grown at either 30 or 14°C (Table IV). During anaphase, nuclear DNA segregates properly to mother and daughter cells in virtually all of the BIK1/bikl-l::TRP1 cells grown at 30 or 14°C. In contrast, nuclear DNA segregation occurs within the confines of the mother cell in a significant fraction of bikl-l::TRP1/bikl-l::TRP1 cells de-...
Loss of chromosome V was measured in cells from five independent colonies of each strain. As Table VI indicates, the frequency of chromosome loss in the bikl-1::TRP1/bikl-1::TRP1 diploids was on average 10-fold greater than in wild-type diploids grown at 30 or 14°C. The elevation of chromosome loss in bikl-1::TRP1/bikl-1::TRP1 diploids could be due to nondisjunction or to defects in DNA metabolism. To distinguish between these two possibilities, we used the frequency of mitotic recombination as a measure of defects in DNA metabolism. We found that the frequency of mitotic recombination in the bikl-1::TRP1/bikl-1::TRP1 strain was equivalent to that in the BIK1/BIK1 strain, when grown either at 30 or 14°C, suggesting that the former does not have enhanced DNA damage.

**Overexpression of the BIK1 Product Causes Cell Division Cycle Arrest**

The BIK1 promoter was replaced with the inducible GAL1 promoter to assess the effect of overexpression of BIK1 on cell growth. When a plasmid containing the GAL1-BIK1 fusion was introduced into a bikl-518/bikl-518 strain, the strain failed to grow on medium containing galactose but was able to grow on medium containing glucose. The effect of BIK1 overexpression was also examined by adding galactose to the GAL1-BIK1 strain growing in liquid medium containing raffinose, which does not induce or repress expression from the GAL1 promoter. 9 h after addition of galactose to the medium, strains containing the GAL1-BIK1 fusion plasmid had achieved less than one cell doubling before stopping proliferation, whereas strains containing control plasmids doubled approximately four times and continued to increase in cell number (Fig. 4 A). Western analysis demonstrated that in VBY82, the strain containing the GAL1-BIK1 fusion plasmid, expression of BIK1 was indeed regulated by galactose (Fig. 4 B). The BIK1 polypeptide did not accumulate in VBY82 cells prior to the addition of galactose to the medium. Two hours after addition of galactose to the medium, VBY82 had fourfold higher levels of the BIK1 polypeptide and eightfold higher levels by 9 h, compared with strain VBY79, which contained a plasmid with the BIK1 gene expressed from its own promoter.

4 h after the addition of galactose to the medium, >70% of the bikl-518/bikl-518 cells with the GAL1-BIK1 fusion plasmid had a distinctive cell division cycle morphology: they arrested division as large-budded cells containing a single nucleus located in or adjacent to the neck separating the mother cell from the bud. Before addition of galactose to the medium, a majority of large budded cells stained with antitubulin antibody had microtubule structures characteristic of a bikl-518/bikl-518 strain, i.e., a short anaphase spindle devoid of detectable cytoplasmic microtubules emanating from the spindle pole bodies. 2 h after the addition of galactose to the medium, a significant fraction of large budded bikl-518/bikl-518 GAL-BIK1 cells had long cytoplasmic microtubules. We were unable to distinguish whether the cytoplasmic microtubules extended from a single spindle pole body or from duplicated, unseparated spindle pole bodies (Fig. 5). At later times after the addition of galactose to the

### Table VI. Chromosome Loss and Mitotic Recombination

| Diploid strain     | Chromosome loss frequency (× 10⁻⁵) | Mitotic recombination frequency (× 10⁻⁵) |
|--------------------|------------------------------------|----------------------------------------|
|                    | 14°C | 30°C | 14°C | 30°C |
| BIK1/BIK           | 5.9  | 4.4  | 18.4 | 16.5 |
| bikl-1::TRP1/bikl-1::TRP1 | 60.5 | 45.8 | 23.0 | 14.5 |

Numbers listed are averages of five independent assays. The strains used were S/BS03 (BIK1/BIK1) and S/BS06 (bikl-1::TRP1/bikl-1::TRP1).
medium, 40–50% of the large budded cells contained residual or no microtubule structures (Fig. 5). These data suggest that overexpression of the BIK1 gene initially blocks spindle formation while promoting the formation of cytoplasmic microtubules, and then subsequently causes the loss of microtubule structures.

BIK1 Is Required for Nuclear Fusion

Cytological observations show that >90% of bik1 × bik1 zygotes have unfused nuclei even though the frequency of zygote formation is comparable to that in wild-type crosses. Genetic evidence for the failure of nuclear fusion in bik1 × bik1 crosses is demonstrated by the high frequency of cytokinetic in such crosses. Cytoductants are haploid exconjugants resulting from cell fusion and cytoplasmic mixing without nuclear fusion. The results in Table VII indicate that the frequency of diploid formation is 10-fold lower and the ratio of cytoductants-to-diploids is 10,000-fold greater in matings between two bikl-518 strains compared to that in matings in which one or both of the parents is BIK1. The high ratio of cytoductants to diploids in bikl-518 × bikl-518 matings indicates that diploid formation is blocked after cell fusion and before nuclear fusion. Nuclear fusion is impaired to a similar degree by the karl-1 mutation, which unilaterally blocks nuclear fusion (Rose and Fink, 1987).

We examined the microtubules in zygotes by indirect immunofluorescence using an antibody against tubulin. Immediately after cell fusion and prior to nuclear fusion, the spindle pole bodies are juxtaposed in a majority of zygotes regardless of the presence or absence of BIK1 product. Subsequent fusion of the spindle pole bodies is observed in a majority of zygotes containing the BIK1 product, but is rarely seen in zygotes lacking it. bikI × bikI zygotes with unfused spindle pole bodies, the nuclei have separated (Fig. 6).

Cytoplasmic microtubules in bikl × bikl zygotes are absent or abnormally short. The relatively small size of the cytoplasmic microtubules in a wild-type cell makes the assessment of this phenotype difficult. Therefore, the effects of the bikl-518 mutation in karl-1 cells were examined. karl-1 cells produce abnormally long cytoplasmic microtubules (Rose and Fink, 1987), making the distinction between the presence and absence of microtubules much clearer. We observed in a cross of bikl-518 karl-1 × bikl-518 than neither nucleus has discernable cytoplasmic microtubules (Fig. 7). This result clearly implicates BIK1 in the formation or stability of cytoplasmic microtubules.

Table VII. Diploid Frequency and Cytoductant to Diploid Ratio in Crosses of bikl-518 and karl-1 Mutants

| Parent MATa [rho-] Cyh+ | Parent MATα [rho+] Cyh+ | Diploid/Total* | Cytoductant/Diploid† |
|-------------------------|-------------------------|----------------|---------------------|
| BIK1                    | BIK1                    | 0.5            | 0.0004              |
| BIK1                    | bik1-518                | 0.6            | 0.002               |
| bik1-518                | BIK1                    | 0.32           | 0.001               |
| bik1-518                | bik1-518                | 0.02           | 3.2                 |
| BIK1karl-1              | BIK1karl-1              | 0.05           | 3.7                 |

* Prototrophic colonies/total colonies
† Cyh' [p+] colonies/prototrophic colonies

Strains used were L2964 (MATαBIK1), L2736 (MATαBIK1), JY195 (MATαbik1-518), L2751 (MATαbik1-518), L2965 (MATαkarl-1).
Sequence Homologies within the Putative Structural Domains of BIK1

The amino acid sequence of BIK1 deduced from the nucleotide sequence was determined previously (Trueheart et al., 1987). The Chou-Fasman and Garnier algorithms (Chou and Fasman, 1978; Garnier et al., 1978), used to predict protein secondary structure, suggest that BIK1 has three structural domains (Fig. 8). The amino-terminal region of 193 residues contains alternating regions predicted to form α-helices, β-sheets, β-turns, and random coils, which is characteristic of globular proteins. From amino acids 194 to 398 is a region predicted to be α-helical. The carboxy-terminal region, from amino acids 394 to 440, contains a high proportion of amino acids predicted to form β-turns.

A sequence at the amino terminus of BIK1, extending from amino acids 2 to 26, shows similarity to the mammalian microtubule-associated protein tau (Fig. 8). Within the tau sequence that shows similarity to BIK1 is an 18-amino acid motif that is repeated three times in tau (Lee et al., 1988). The region in tau containing these repeats and a homologous region in MAP2 are rich in basic amino acids and bind microtubules (Lewis et al., 1988; Lee et al., 1989; Lewis et al., 1989). Synthetic peptides corresponding to the acidic carboxy-terminal domain of tubulin bind both tau and MAP2 (Maccioni et al., 1988), suggesting that the basic regions in tau and MAP2 may interact electrostatically with the acidic domain of tubulin. The region in BIK1 which shows similarity to tau is basic; the entire region from amino acid 1 to 126 has a calculated pI of 11.3. In contrast, the region in BIK1 from amino acid 127 to 440 is acidic with a calculated pI of 4.9.

The carboxy terminus of BIK1, from amino acids 416 to 429, has the metal binding motif, C-X2-C-X-H-X4-C (X is...
Figure 8. Sequence homologies within putative structural domains of BIKI. The Garnier secondary structure predictions (Garnier et al., 1978) are shown above a schematic diagram of the BIKI polypeptide, which is divided into three putative domains. The scale indicates amino-acid position in the BIK1 polypeptide. In the amino-terminal domain (solid black) is a sequence that shows 40% identity to a region in tau. The alignment score, based on 1,000 randomized runs, is 6 SD from the mean. The solid bars designate amino acid identities and the colons designate similar amino acids. The sequence repeated three times in tau is shaded. The central region of BIK1 (white) appears to be $\alpha$-helical and shows 26% identity with the rod domain of the myosin alpha heavy chain from rabbit cardiac muscle. The homology to myosin is dispersed throughout this region (results not shown). The alignment score based on 100 randomized runs is 5 SD from the mean. In the carboxy terminus of BIK1 (hatched region) is a metal binding motif (shaded residues) found in retroviral gag-encoded nucleocapsid proteins of which several are listed (taken from Berg, 1986).

The $\alpha$-helical region of BIK1 shows similarity to the rod domains of several myosin heavy chains. BIK1 shows the greatest homology to the myosin $\alpha$ heavy chain from rabbit cardiac muscle (see legend to Fig. 8). Within the putative $\alpha$-helical region of BIK1 are 27 heptad repeats characteristic of myosin and intermediate filament proteins. Hydrophobic residues are located preferentially at the first and fourth positions of the repeat while charged residues preferentially occupy the remaining positions of the repeat (McLachlan and Karn, 1983) (Fig. 9). Two regions (amino acids 292–301 and 358–363) contain helix disrupting proline residues and interrupt the heptad repeats. Proteins containing heptad repeats form coiled-coil structures: $\alpha$-helices that coil around one another in a regular helical structure stabilized by interactions between hydrophobic residues on intertwined helices.

**Discussion**

The BIK1 protein of yeast displays properties expected of a structural component of the microtubule cytoskeleton. First of all, the BIK1 protein colocalizes with tubulin. In addition mutations in BIK1 cause aberrant nuclear segregation during mitosis, chromosome instability, and blocked nuclear fusion. These defects in microtubule function correlate with altered microtubule morphologies. The most dramatic morphological defect in bik1 cells is in the cytoplasmic microtubules, which are either absent or abnormally short. The location of the cytoplasmic microtubules in wild type cells and absence in nocodazole-treated cells and tub2 mutants defective in nuclear migration (Jacobs et al., 1988; Hufnaker et al., 1988) suggest they may be required to orient the spindle properly or to guide the nucleus into the bud. If so, defective cytoplasmic microtubules could give rise to the anucleate and multinucleate cells present in bik1 cultures. Elevated levels of chromosome loss in the bik1/*::TRP1 mutant are indicative of defective spindle function. The mitotic defects of bik1 null mutants are not lethal. Proteins functionally redundant with BIK1 could account for the viability of bik1 null mutants.

Mutations in BIK1 also affect the cytoplasmic microtubules during zygote formation. The absence of cytoplasmic microtubules observed in bik1/* bik1/* crosses may account for the absence of nuclear fusion in such matings. The cytoplasmic microtubules, which connect the spindle pole bodies on the haploid nuclei, are thought to pull the nuclei together (Byers and Goetsch, 1974, 1975; Meluh and Rose, 1990). One explanation for the failure of fusion in bik1/* crosses might be that the nuclei are unable to migrate toward each other early in zygote formation. Were this correct, we would have expected the nuclei of mating bik1/* cells to be located randomly in the zygotes. However, the nuclei and spindle pole bodies are appropriately juxtaposed early in zygote formation. The subsequent retreat of unfused haploid nuclei to midcell positions suggests that BIK1 is critical to a late step in migration that may be closely linked to nuclear fusion.

Overexpression of BIK1 from the GAL1 promoter has a profound effect on the microtubule cytoskeleton. Initially, overexpression of BIK1 results in the production of large-budded cells which contain extra-long cytoplasmic microtubules, but lack spindle microtubules. This phenotype contrasts with the absence of the cytoplasmic microtubules in the bik1/* mutant. Cells overexpressing BIK1 accomplish a single doubling and then cease dividing. Arrest in cell divi-

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**Figure 9.** Heptad repeats in BIK1. The histograms show the percentage of hydrophobic and charged residues in the positions (abcdefg) of the 27 heptad repeats in the $\alpha$-helical region of BIK1 extending from amino acid 194 to 398.
Double labeling of synchronized cells by indirect immunofluorescence using anti-BIK1 and antitubulin antibodies indicates BIK1 colocalizes with tubulin. The pattern of staining with BIK1 antibodies changes as cells progress through the cell cycle. BIK1 antibodies stain the cytoplasm of unbudded cells. We have not ruled out the possibility that levels of the BIK1 antigen change during the cell cycle or that the cytoplasmic staining with the BIK1 antibodies obscures staining of nuclear structures. The cytoplasmic staining is most likely not a consequence of treatment with a-factor, because BIK1 antibodies stain the cytoplasm of unbudded cells which were not treated with a-factor (results not shown). Colocalization of BIK1 with tubulin was observed during the initial stages of bud formation. Anti-BIK1 antibodies stained the spindle pole body most prominently, whereas staining of the mitotic spindle was variable. Several factors may be responsible for the variability of staining of the mitotic spindle: (a) BIK1 may dissociate from the spindle; (b) the BIK1 antigen may be unstable in cells during spindle formation; (c) the anti-BIK1 epitope may not be as accessible to binding antibody at this stage of division. We were unable to detect staining of the cytoplasmic microtubules with BIK1 antibodies during any stage of division. Either BIK1 does not associate with the cytoplasmic microtubules or staining of the cytoplasmic microtubules with BIK1 antibodies is below our level of detection. Colocalization of BIK1 with tubulin was observed in cells containing the BIK1 gene on a multicopy plasmid. Although the cytoplasmic localization of BIK1 during G1 may be the consequence of the overexpression of BIK1, it is unlikely that overexpression of BIK1 affects colocalization of BIK1 with tubulin. Changes in the distribution of the BIK1 polypeptide in cells at different stages of division raise the possibility that the localization of BIK1 may be regulated during the cell cycle.

Genetic interactions between BIK1 and the tubulin genes are consistent with the colocalization of BIK1 and tubulin. The synthetic lethality of bik1Δ and certain bik1Δ mutant strains and the poor growth of others suggests that the defects in microtubule function caused by mutations in BIK1 and the tubulin genes are additive. Similar reasoning can explain the poor growth of the bik1Δ cin1Δ double mutants. The similar phenotypes caused by mutations in the BIK1, TUB1, and CIN genes also suggest an interaction between these products.

The BIK1 protein has structural features similar to those of the neuronal MAPs, tau and MAP2. Tau and MAP2 contain homologous regions rich in basic amino acids which bind to microtubules in vitro (Lewis et al., 1988; Lee et al., 1989) and in vivo (Lewis et al., 1989). At the amino terminus of BIK1 is a sequence that shows similarity to a segment of the microtubule binding domain of tau. This region of BIK1 as well as the entire amino-terminal region of 126 amino acids is basic, with a calculated pI of 11.3. In contrast the carboxy-terminal half of BIK1 is acidic, with a calculated pI of 4.9. Recent evidence suggests that microtubule binding may occur by electrostatic interactions between tau or MAP2 and the acidic carboxy-terminus of tubulin (Maccioni et al., 1988). These observations taken together implicate the amino terminus of BIK1 in microtubule binding.

The central and carboxy-terminal domains of BIK1 may be involved in the assembly of BIK1 into a higher order structure and its association with other cellular components, respectively. Within the central α-helical domain of BIK1 is a heptad repeat characteristic of proteins which form extended coiled-coil structures (McLachlan and Karn, 1983). The carboxy terminus of BIK1 contains a cysteine-histidine region reminiscent of "zinc fingers" (Berg, 1986). This sequence, conserved in all retroviral nucleocapsid proteins (Berg, 1986; Katz and Jentoft, 1989), is implicated in retroviral RNA packaging (Meric and Goff, 1989) and has been shown to have nucleic acid annealing activity (Prats et al., 1988; Jentoft et al., 1988). Whether this sequence in BIK1 is involved in nucleic acid binding remains to be determined.

Association of BIK1 with tubulin may promote the nucleation or assembly of microtubules, coordinating spindle formation with the cell division cycle. The cytoplasmic microtubules, absent in the bik1Δ mutant, may be critical for positioning the spindle so that it elongates through the neck between mother and bud. Further studies of BIK1 may provide insight into how the formation of the mitotic spindle and cytoplasmic microtubules are temporally and spatially regulated.

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