Yeast Mutants That Produce a Novel Type of Ascus Containing Asci Instead of Spores

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ABSTRACT

Tetraploid yeast cells lacking *BFR1* or overexpressing an essential gene *BBP1* produce a novel type of ascus that contains asci instead of spores. We show here that the asci within an ascus likely arise because **a**/α spores undergo a second round of meiosis. Cells depleted of Bbp1p or lacking Bfr1p are defective in a number of processes such as nuclear segregation, bud formation, cytokinesis and nuclear spindle formation. Furthermore, deletion of *BFR1* or overexpression of *BBP1* leads to an increase in cell ploidy, indicating that Bfr1p and Bbp1p play roles in both the mitotic cell cycle and meiosis. Bfr1p and Bbp1p interact with each other in a two hybrid assay, further suggesting that they might form a complex important for cell cycle coordination.

All eukaryotic cell cycles are defined by two events: replication of chromosomes during S phase and their subsequent segregation during mitosis (Cross *et al.* 1989; Forsburg and Nurse 1991). A normal cell cycle consists of a number of steps mediated by macromolecular structures such as DNA polymerase, the spindle pole body (SPB), intranuclear and cytoplasmic microtubules and microtubule organization centers. Individual steps are closely regulated to ensure that cell growth, DNA replication, chromosome segregation and cell division are coupled (Hartwell and Weinert 1989; Roberts 1993; O'Connell and Nurse, 1994). In the yeast, *Saccharomyces cerevisiae,* the Cdc28 kinase, in association with various cyclins, serves as a master regulator that controls the progression of the cell cycle (Nasmyth, 1993). In addition, regulatory pathways called checkpoints monitor the cell cycle, and delay its progression in cells that are defective in a prior step in the cycle (Hartwell and Weinert 1989; Murray 1992). The effects of the checkpoint control are believed to be mediated through regulation of the Cdc28 kinase activity.

Because of the nature of the cell cycle, defects in either the individual steps in the cycle, or the regulatory pathways controlling the progression of the cycle, could lead to an uncoupling of the cell cycle events and the production of progeny that contain an abnormal complement of chromosomes. So far, mutations that lead to an increase in cell ploidy have been identified in genes required for the proper function of the mitotic spindle apparatus, *e.g., CDC31, KAR1, NDC1, SPA1, ESP1, CUT1, NUM1* (Schild *et al.* 1981; Thomas and Botstein 1986; Rose and Fink 1987; Baum *et al.* 1988; Snyder and Davis 1988; Uzawa *et al.* 1990; Kormanec *et al.,* 1991); for bud formation, *e.g., BEM2* (Chan and Botstein, 1993); or for cell cycle regulation, *e.g., rum1*$^+$, *p65cdc18, CDC16* and *CDC27* (Moreno and Nurse 1994; Nishitani and Nurse 1995; Heichman and Roberts 1996).

Meiosis is a specialized cell cycle in which two rounds of chromosome segregation follow a round of DNA replication (Honigberg *et al.* 1993). Many of the supramolecular structures and proteins required for the mitotic cell cycle are also important for meiosis, although their mitotic and meiotic functions are not necessarily in the same events (Weber and Byers 1992). In yeast, meiosis is triggered in response to nutritional signals and cell type, producing four spores enclosed in an ascus sac (Klapholz and Esposito 1981). DNA replication and other cellular events are repressed in spores until a nutritional signal triggers germination (Honigberg *et al.* 1993; Mitchell 1994). At present, little is known about the mechanism of postmeiotic repression. However, the phenotypes of the only mutants known to affect this process, the Drosophila *plutonium, pan gu* and *gnu,* suggest that some proteins required for mitotic cell cycle regulation may also play a role in postmeiotic repression (Freeman and Glover 1987; Shamanski and Orr-Weaver 1991; Axton *et al.* 1994).

We report in this paper that tetraploid yeast cells deleted of *BFR1* or overexpressing *BBP1* produce a novel ascus structure that contains asci instead of spores. The novel structure likely arises because **a**/α spores undergo a second round of meiosis. Deletion of *BFR1* or overexpression of *BBP1* also leads to an increase in cell ploidy. To our knowledge, these are the first two yeast genes known to produce such a novel ascus upon sporulation.

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TABLE 1

***S. cerevisiae* strains**

Strain	Genotype	Source
W303-1A	*Mat***a**, *ade2-1, can1-100, his3-11,15, leu2-3,112, trp1-1, ura3-1.*	R. Rothstein
W303	Isogenic **a**/α diploid strain.	R. Rothstein
W303T	Isogenic **a**/**a**/α/α tetraploid strain.	This study
W1089-1A	Isogenic to W303-1A, except *LEU2.*	R. Rothstein
R567	*Mat***a**/**a**/α/α, *his3-11,15, leu2-3,112.*	This study
ZXY21	Isogenic to W303, except *bfr1Δ::HIS3/BFR1.*	This study
ZXY21-1A	Isogenic to W303-1A, except *bfr1Δ::HIS3.*	This study
ZXY21-1C	Isogenic to W303.	This study
ZXY22	Isogenic to W303, except *bfr1Δ::HIS3/bfr1Δ::HIS3.*	This study
ZXY44	Isogenic to W303T, except *bfr1Δ::HIS3/bfr1Δ::HIS3/bfr1ΔHIS3/bfr1Δ::HIS3.*	This study
ZXY144L	Isogenic to ZXY44, except *bfr1Δ::LEU2* instead of *bfr1Δ::HIS3.*	This study
ZXY244	Isogenic to R567, except *bfr1Δ::HIS3/bfr1Δ::HIS3/bfr1Δ::HIS3/bfr1Δ::HIS3.*	This study
ZXY344L	Isogenic to ZXY244, except *bfr1Δ::LEU2* instead of *bfr1Δ::HIS3.*	This study
ZXY44-1A	Same as ZXY22.	This study
ZXY44-1B	*Mat***a**/**a**, otherwise the same as ZXY44-1A.	This study
ZXY42M	Isogenic to ZXY21, except *leu2-3, 112/LEU2.*	This study
ZXY43M	Triploid strain isogenic to W303, except *Mat***a**/α/α, *bfr1Δ::HIS3/bfr1Δ::HIS3/BFR1.*	This study
ZXY621R	Isogenic to W303, except *bbp1::TRP1/BBP1.*	This study
ZXY621R-1A	Isogenic to W303-1A, except *bbp1::TRP1.*	This study

MATERIALS AND METHODS

Strains, media and cell culture: *Escherichia coli* strain XL1 Blue was used for all DNA manipulation. Yeast strains are listed in Table 1. Tetraploid strain W303T was created by transforming W303 (the same colony used to create the heterozygous *bfr1/BFR1* strain ZXY21) with pGAL-HO, selecting for **a**/**a** and α/α diploids, and mating them (Herskowitz and Jensen 1991). Tetraploid strain R567 was created by mating R283 and R284, two diploid strains of opposite mating type. The ploidy of the strains was determined by fluorescence-activated cell sorting (FACS). Yeast culture and transformation were carried out according to standard procedures (Ito *et al.* 1983; Sherman *et al.* 1986).

All the strains were grown in glucose media prior to sporulation. Cells were washed twice with 2% KOAc, resuspended in 2% KOAc supplemented with nutritional requirements for auxotrophic strains (20% of the level for complete synthetic media, see Sherman 1991).

Plasmids: pBFR-BS: a 4-kb *Bam*HI-*Kpn*I DNA fragment containing *BFR1* is inserted into pBluescript KS(−) between the *Bam*HI and the *Kpn*I sites. pBFR-HIS: a fragment in pBFR-BS, corresponding to amino acids 151 to 294 of the Bfr1p, was replaced by a DNA fragment containing the yeast *HIS3* gene. pBFR-LEU: a 462-bp DNA fragment containing the −427 to +35 region of *BFR1,* and a second 400-bp fragment containing the 3′ region of *BFR1* starting from +1392 of *BFR1,* was generated by PCR and cloned into pBluescript KS(−) between the *Xba*I and the *Bam*HI, and the *Kpn*I and the *Sal*I sites. A *Hpa*I-*Sal*I DNA fragment containing the yeast *LEU2* gene was then cloned between the *Sma*I and the *Sal*I sites of the resulting plasmid. pBFR: a 3.5-kb *Kpn*I-*Bam*HI fragment including the *BFR1* ORF with the stop codon changed to GGA was fused in frame with a fragment encoding an epitope from the influenza hemagglutinin (HA, see Field *et al.* 1988) and cloned into pRS316. The resulting plasmid expresses HA-tagged *BFR1* from its own promoter. pBFR-GAL: the same HA-tagged *BFR1* open reading frame (ORF) was placed under the control of the inducible yeast GAL1 promoter in YCp50. pBFR-GB: containing a fusion between the DNA-binding domain of yeast GAL4 and the *BFR1* ORF, under the control of the ADH promoter. pBBP-BS: a 4-kb *Hin*dIII fragment containing *BBP1* was cloned into pBluescript KS(−) at the *Hin*dIII site. pBBP-BAM: a *Bam*HI site is created by PCR at the second and third codons of *BBP1* ORF, and a 1.3-kb *Bam*HI-*Hpa*I fragment containing the *BBP1* ORF minus the START codon was cloned into pRS316 between the *Bam*HI and the *Sma*I sites. pBBP-TRP: a 1.5-kb fragment containing the yeast *TRP1* gene was cloned into a *Hpa*I site in pBBP-BAM, at a position corresponding to amino acid 100 of Bbp1p. pBBP-C: a 3-kb *Xho*I-*Pst*I fragment containing the entire *BBP1* gene, including the promoter region, was cloned into pRS316 between the *Xho*I and the *Pst*I sites. pBBP-ADH-C: a 1.5-kb *Bam*HI fragment containing the yeast ADH promoter followed by a START codon and the HA epitope was excised from pAD5 (Field *et al.* 1988), and cloned into pBBP-BAM at the *Bam*HI site. This fused the HA epitope in frame with the *BBP1* ORF and placed the fusion gene under the control of the yeast ADH promoter. The same construct as in pBBP-ADH-C was cloned into pRS426 (Sikorski and Hieter 1989), and named pBBP-ADH. The HA-tagged *BBP1* ORF was also placed under the control of the yeast GAL1 promoter in pRS316, and named pBBP-GAL.

Isolation of *BFR1* and *BBP1*: *BFR1* was identified by screening a λ-Zap yeast genomic expression library with polyclonal antisera against potential nuclear localization sequence (NLS)-binding proteins, as described before (Shan *et al.,* 1994). Positive clones were used to generate probes to isolate the entire *BFR1* gene from a YCp50 yeast genomic library (a gift from Mark Rose, Princeton University, Princeton, NJ), using standard procedures (Sambrook *et al.* 1989).

BBP1 was identified by screening a yeast genomic two-hybrid library (a gift from Stanley Fields Department of Microbiology, SUNY, Stony Brook, NY) using pBFR-GB. The entire *BBP1* gene was isolated from the same YCp50 library as mentioned above. Dideoxy sequencing was done with Sequenase according to the manufacturer's procedure.

Disruption of *BFR1* and *BBP1*, and direct mating of spores with cells: Linear DNA fragments containing *bfr1Δ::HIS3*, *bfr1Δ::LEU2* or *bbp1::TRP1* were excised from pBFR-HIS, pBFR-LEU or pBBP-TRP (see Plasmids) and used to transform a diploid strain W303 (Rothstein 1983; Thomas and

Rothstein 1989). Transformants were analyzed by Southern blot for the presence of the deletion alleles. Heterozygous diploids were sporulated, and tetrads were dissected according to standard procedures. Spores were placed on top of several haploid cells of the desired mating type. Hybrids formed from mating were selected by restreaking the colonies on appropriate selective plates.

Fluorescence-activated cell sorting: FACS was done according to a published procedure (Hutter and Eipel 1979). Cells were fixed in 70% ethanol for 2 hr at room temperature. Fixed cells were treated with 1% RNase for 6 hr at 37°, followed by staining with 50 μg/ml propidium iodide overnight at 4°. Before analysis, cell suspensions were diluted 1:10 with 50 mM sodium phosphate, pH 7.2, 150 mM sodium chloride, and sonicated in a water bath for 30 min.

Staining with 4,6-diamidino-2-phenylindole (DAPI) and calcofluor: Yeast cells or asci were fixed in 70% ethanol for 1 hr at room temperature. Fixed cells or asci were washed once with water and resuspended in either 0.4 μg/ml of DAPI or 100 μg/ml of calcofluor. After 10 min at room temperature, cells or asci were pelleted, washed three to five times with water and viewed under a microscope. Alternatively, live cells or asci were directly pelleted and stained as above.

Indirect immunofluorescence and immunoblot analysis: These were carried out as described in earlier publications from this laboratory (see Shan *et al.* 1994). Whole yeast cell extracts were prepared by growing yeast to an OD_{600} of 0.8 and dissolving pelleted yeast cells directly in SDS gel loading buffer. Monoclonal anti-HA antibody 12CA5 was from Boehringer Mannheim. Monoclonal anti-tubulin antibody YOL1/34 was from Serotec, Ltd. FITC-conjugated goat anti-mouse IgG was from Jackson Laboratory. Alkaline phosphatase conjugated goat anti-mouse and goat anti-rabbit IgG were from BioRad.

RESULTS

Isolation of *BFR1*: In a search for potential receptors that bind nuclear localization sequences (NLS, see Robbins *et al.* 1991; Lee *et al.* 1991; Shan *et al.* 1994), we isolated a gene, *BFR1*, previously identified by Jackson and Képès as a multicopy suppressor of Brefeldin A-induced lethality. *BFR1* was implicated in secretion, nuclear segregation and cytokinesis and was not essential for cell viability (Jackson and Képès 1994). The affinity of Bfr1p toward the NLS is lower than that of the other NLS-binding proteins identified in the same screen, and it appeared nonspecific (data not shown). Thus, this affinity might not be biologically significant.

***BFR1* is required for the maintenance of normal ploidy in yeast cells:** We created *bfr1* strains by replacing a chromosomal copy of *BFR1* in a diploid strain W303 with a deletion allele *bfr1Δ::HIS3* (see materials and methods), followed by sporulation and tetrad dissection (Rothstein 1983). Microscopic examination revealed that *bfr1* cells were large in size, as compared with isogenic *BFR1* cells (see also Jackson and Képès 1994). A possible cause of an increase in cell size is an increase in ploidy. Thus, we analyzed the DNA content of *bfr1* cells by FACS. As shown in Figure 1, a heterozygous *bfr1*/*BFR1* diploid strain ZXY21 had a FACS profile of a diploid strain (compare with the wild-type diploid W303). However, the FACS profile of ZXY21-1A (*Mata* *bfr1*, derived from a *bfr1* spore of ZXY21) contained 1N, 2N and 4N peaks, suggesting that the culture contained both haploid and diploid cells (Figure 1, ZXY21-1A). Similarly, the FACS profile of ZXY22 (*Mata*/α, *bfr1*/*bfr1*) was indicative of the presence of both diploid and tetraploid cells (Figure 1, ZXY22). In contrast, a *Mata* *BFR1* culture from a *BFR1* spore of ZXY21 had a FACS profile of haploid cells (ZXY21-1C, compare with W303-1A). Finally, when *bfr1* cells of opposite mating type, derived from spores of ZXY21, were mated, tetraploid cells were obtained (see below). These results strongly suggest that *bfr1* cells increase their ploidy during growth.

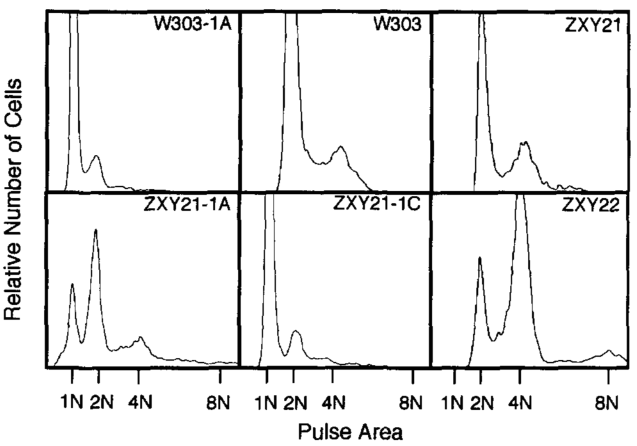

Figure 1.—FACS analysis of the DNA content of *bfr1* and *BFR1* yeast cells. Samples were prepared for FACS analysis as described in materials and methods. DNA content corresponding to those of haploid, diploid and tetraploid cells were labeled 1N, 2N and 4N, respectively: W303-1A, wild-type haploid strain; W303, wild-type diploid strain; ZXY21, a *bfr1*/*BFR1* heterozygous diploid strain isogenic to W303; ZXY21-1A, derived from a *bfr1* spore of ZXY21; ZXY21-1C, derived from a *BFR1* spore of ZXY21; ZXY22, a *bfr1* strain generated by mating two haploid *bfr1* cells of opposite mating type.

To demonstrate that the observed increase in ploidy was due to the lack of Bfr1p, we constructed pBFR-GAL, containing a *BFR1* ORF tagged with an epitope from the influenza hemagglutinin (HA) and controlled by the yeast GAL1 promoter. As shown in Figure 2, haploid *bfr1* cells carrying pBFR-GAL remained haploid when grown in galactose media (0 hr). After switching to glucose media, which represses the expression of *BFR1* from the plasmid, a gradual increase in the 2N peak, as well as the presence of a 4N peak, were observed (compare 0, 4, 10 and 20 hr). Immunoblot analysis showed that the HA-tagged Bfr1p was produced when cells carrying pBFR-GAL were grown in galactose media (Figure 2A, lane G), and was depleted within 4 hr of switching to glucose media (Figure 2A, lanes 1 to 4, and D). Thus, Bfr1p is required for the maintenance of cell ploidy in yeast, and in the absence of Bfr1p, a small percentage of cells in the culture increase their ploidy in each generation. Mutant *bfr1* cells arrested by α-factor or nocodazole showed no increase in their

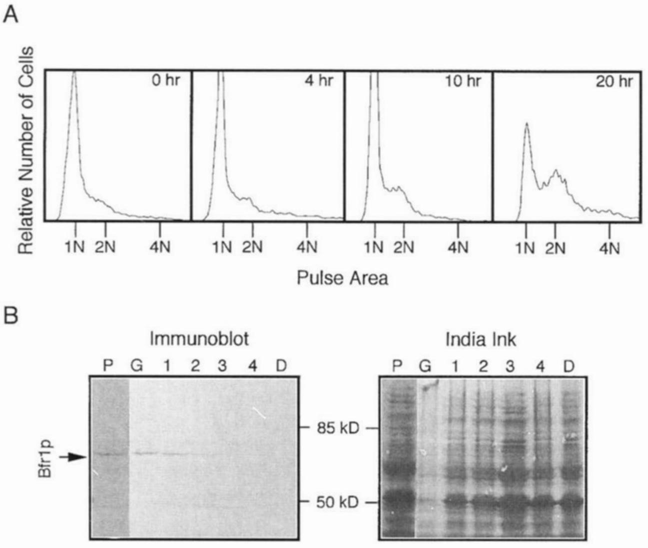

FIGURE 2.—Depletion of Bfr1p leads to an increase in cell ploidy. (A) FACS analysis of a haploid *bfr1* strain (ZXY21-1A) carrying pBFR-GAL, which expresses *HA-BFR1* from the GAL1 promoter. Cells were grown in galactose media to an OD_{600} of 0.2, and switched to glucose media. Samples were taken for FACS analysis after 0, 4, 10 and 20 hours in glucose media. (B) Immunoblot analysis of whole cell extracts made from ZXY21-1A carrying either pBFR (containing *HA-BFR1* controlled by the BFR1 promoter), or pBFR-GAL. Whole cell extracts were made as described in MATERIALS AND METHODS. HA-Bfr1p was detected with the anti-HA antibody 12CA5 (Immunoblot), and total protein was stained with India ink (India ink). Lane P, cells carrying pBFR. Lane G, cells carrying pBFR-GAL grown in media containing 2% galactose. Lanes 1–4, cells carrying pBFR-GAL switched to glucose media for 1–4 h. Lane D, cells carrying pBFR-GAL grown in glucose media.

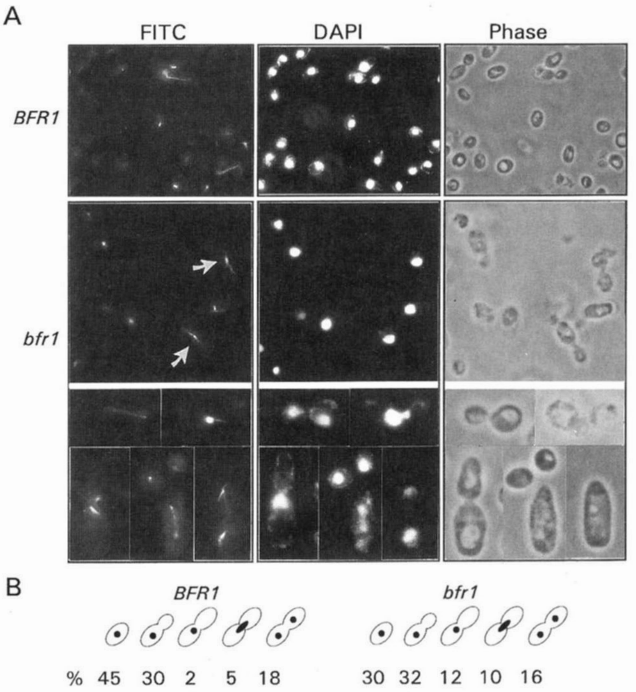

FIGURE 3.—Microtubule morphology and cell type distribution in cultures of *bfr1* and *BFR1* cells. (A) Indirect immunofluorescence of *BFR1* (ZXY21-1C) and *bfr1* (ZXY21-1A) cells, using anti-tubulin antibody. Arrows point to *bfr1* cells that have a large bud and one nucleus. FITC, FITC staining using the monoclonal anti-tubulin antibody YOL1/34 and FITC-conjugated donkey anti-rat IgG. DAPI, DAPI staining of DNA. Phase, phase contrast pictures of cells. (B) Distribution of cell type in cultures of *BFR1* and *bfr1* strains. The percentage of different types of cells were calculated by counting a total of 500 cells for each strain.

DNA content (data not shown), suggesting that *bfr1* cells do not replicate their DNA in the absence of mitosis or START.

A subpopulation of cells in a *bfr1* culture shows a defect in anaphase: In a *bfr1* culture, a small number of cells are anucleate or binucleate, suggesting that there might be a defect in the spindle apparatus (JACKSON and KÉPÈS 1994). We examined the microtubule morphology of *bfr1* cells by indirect immunofluorescence, using the monoclonal anti-tubulin antibody YOL1/34 (Figure 3A). Our results show that many large-budded *bfr1* cells have a single nucleus with a short spindle, either inside the mother cell or at the bud neck (Figure 3A, see arrows). Analysis of cell-type distribution showed that a *bfr1* culture contained significantly higher numbers of large-budded cells with a single nucleus than an isogenic *BFR1* culture (Figure 3B). In addition, some unbudded *bfr1* cells were observed to undergo nuclear division (see Figure 3A, *bfr1*).

A possible explanation for the observed difference in cell-type distribution is that anaphase is delayed in *bfr1* cells. To test this, we examined the microtubule morphology of *bfr1* cells synchronized with α-factor at different times during the cell cycle. The result showed that the majority of cells in a *bfr1* culture have microtubule morphology similar to that of the *BFR1* cells (data not shown). However, at a time when most of the *BFR1* cells have completed anaphase with two separated DNA masses that were connected by a long spindle, a small number of *bfr1* cells had only one DNA mass with a short spindle. These data suggest that a delay in anaphase occurs in a subpopulation of cells in a *bfr1* culture.

Isolation of *BBP1*: To better understand the cellular function of Bfr1p, we searched for proteins that interact with Bfr1p using the yeast two-hybrid system (FIELDS and SONG 1989). A positive clone was isolated that contained a previously unidentified gene encoding a protein of 384 amino acids, which we named *BBP1* (Bfr1 Binding Protein 1). The sequence information was deposited in GenBank under accession no. X92658. Bbp1p has no significant similarity to any proteins in the available databases. Its C-terminal portion has a region of 70 amino acids that contains a leucine every seven residues. This region is encoded by the fragment isolated from the two-hybrid library, thus likely binds Bfr1p.

A disrupted allele of *BBP1*, *bbp1*Δ :: *TRP1*, was created and used to disrupt one chromosomal copy of *BBP1* in the diploid strain W303 (Rothstein 1983). Three *bbp1/BBP1* heterozygous diploids were sporulated, and a total of 40 tetrads were dissected. No tetrad produced more than two colonies, and all the colonies were Trp$^-$, suggesting that *BBP1* is essential for cell growth. Microscopic examination revealed that the *bbp1* spores had formed microcolonies of eight to 16 cells before growth ceased. Tetrads of a heterozygous *bbp1/BBP1* strain ZXY621R carrying a *CEN* plasmid containing *BBP1* (pBBP-C) yielded a population of *TRP*$^+$, *bbp1*$^-$ colonies that all contained pBBP-C. Thus, *BBP1* on a plasmid can rescue the lethal phenotype of a *bbp1* strain.

Cells depleted of Bbp1p arrest with a G2 DNA content and a nonuniform morphology: A plasmid pBBP-GAL containing a HA-tagged *BBP1* ORF under the control of the yeast GAL1 promoter (see MATERIALS AND METHODS) was introduced into the heterozygous *bbp1/BBP1* strain ZXY621R. After sporulation and tetrad dissection, no Trp$^+$, Ura$^+$ colonies (*bbp1* carrying pBBP-GAL) were recovered on plates containing 2% galactose. This suggested that overexpression of *BBP1* is toxic. We found that media containing 1.5% galactose and 0.5% glucose (D/G media), which partially repressed expression of *BBP1* from the GAL1 promoter, allowed cells carrying pBBP-GAL to grow at a rate near that of wild-type cells.

When haploid *bbp1* cells carrying pBBP-GAL were transferred from D/G media to media containing 2% glucose to deplete Bbp1p, they continued to grow for about 16 to 18 hr before dying. Microscopic examination revealed that, after 16 hr in glucose media, only about 5% of *bbp1* cells carrying pBBP-GAL have a small bud, compared with 30% in a W303-1A (wild-type) culture (Figure 4A). Many cells are connected to form three or four cell units. When these cells are stained with calcofluor, the whole cell surface becomes brightly stained; bud scars and the chitin ring at the bud neck are difficult to detect (Figure 4A, calcofluor). DAPI staining shows that a large percentage of both unbudded and large-budded cells contain a single nucleus, although a small number of unbudded binucleate cells are observed (Figure 4A, DAPI). Rhodamine-phalloidin staining reveals that actin cables are no longer visible in these cells, and cortical actin patches are evenly distributed. A number of cells show faint and even staining by phalloidin (data not shown). Similar patterns of calcofluor and phalloidin staining have been reported for mutants defective in bud formation or cytokinesis (Flescher *et al.* 1993; Kim *et al.* 1994).

By FACS analysis, after 10 hr in glucose medium (about 4.5 generations), a much higher percentage of *bbp1* cells carrying pBBP-GAL have a 2N DNA content (Figure 4B, compare 0 and 10 hr panels). After 16 hr (six generations), most cells have a 2N DNA content and some have a 4N DNA content (Figure 4B, 16 hr). Thus, both large-budded cells and unbudded cells in the culture appear to have completed DNA replication.

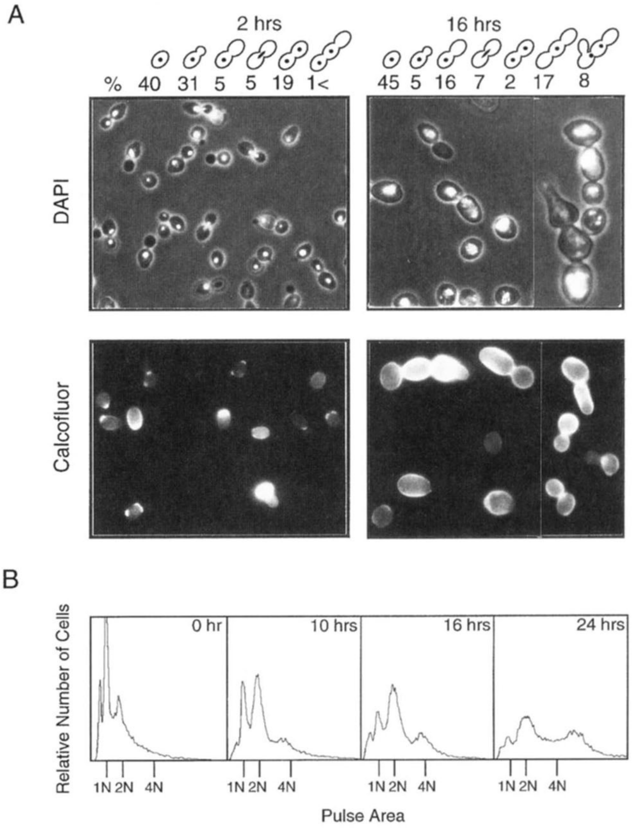

Figure 4.—Morphology and DNA content of cells depleted of Bbp1p. (A) Photographs of haploid *bbp1* cells carrying pBBP-GAL switched to glucose media for the indicated times. Cells were stained with DAPI or calcofluor, as described in MATERIALS AND METHODS. The percentage of different types of cells are indicated above the picture. The pictures were taken at the same magnification. (B) FACS profiles of the same strain switched to glucose media for 0, 10, 16 and 24 hours.

Cells depleted of Bbp1p are defective in the formation of the nuclear spindle: By indirect immunofluorescence using an anti-tubulin antibody, many large-budded cells lacking Bbp1p contained two SPBs, one in the mother and one in the daughter cell (Figure 5). However, the SPB in the daughter cell was not associated with DNA, but did associate with cytoplasmic microtubules (see arrow). About 30% of unbudded cells also had two spots that were stained by FITC even though DAPI staining revealed a single nucleus. No nuclear spindle was observed connecting the two SPBs in either the large-budded cells or the unbudded cells. A similar phenotype was observed in *ndc1* and *mps2* mutants (Winey *et al.* 1991; 1993), although we did not determine whether one of the SPBs was defective, as was the case for *ndc1* and *mps2* mutants. Taken together, our results suggest that depletion of Bbp1p leads to defects in bud formation and nuclear spindle formation, but not in DNA replication.

Overexpression of *BBP1* leads to slow cell growth and an increase in ploidy: To further test the toxic effect of *BBP1* overexpression, we constructed pBBP-

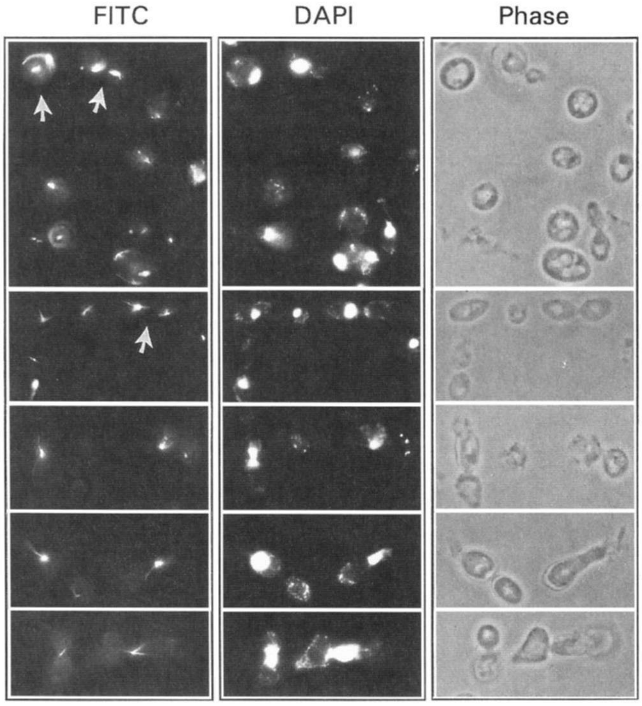

FIGURE 5.—Indirect immunofluorescence analysis of microtubules in cells depleted of Bbp1p. FITC, staining of microtubules using the monoclonal anti-tubulin antibody YOL1/34 and FITC-conjugated donkey anti-rat IgG. DAPI, staining of DNA. Phase, phase contrast picture of cells. Arrows point to cells that have two separated SPBs, but no spindle and only one spot stained by DAPI.

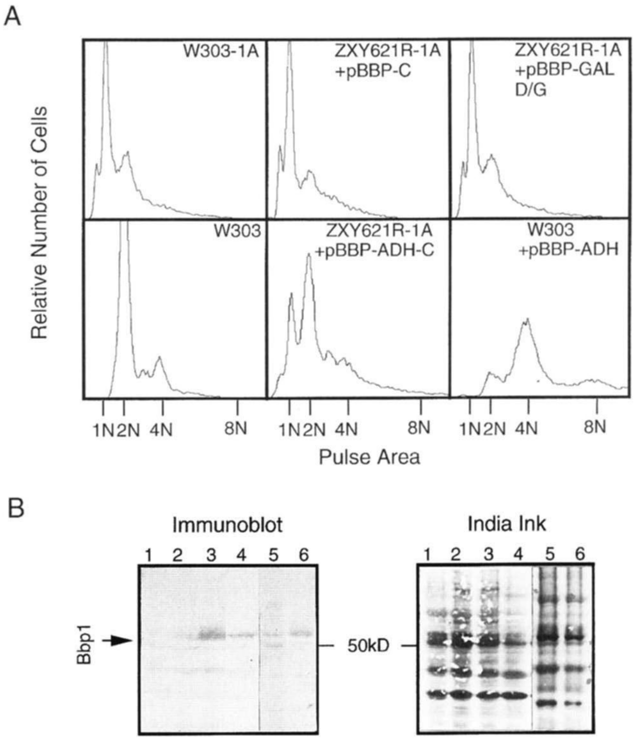

FIGURE 6.—Comparison of the DNA contents and the level of Bbp1p in yeast cells expressing *BBP1* under different conditions. (A) FACS profiles of cells expressing *BBP1* from different promoters. D/G indicates that cells were grown in media containing 0.5% glucose and 1.5% galactose. Otherwise, cells were grown in media containing 2% glucose. ZXY621R-1A: a haploid *bbp1* strain isogenic to W303-1A. pBBP-C: a *CEN* plasmid containing the entire *BBP1* gene. pBBP-GAL: a *CEN* plasmid containing *HA-BBP1* controlled by the GAL1 promoter. pBBP-ADH-C: a *CEN* plasmid containing *HA-BBP1* controlled by the ADH promoter. pBBP-ADH: the same as pBBP-ADH-C except a 2μ plasmid. (B) the level of Bbp1p in cells carrying the above plasmids. Immunoblot, the levels of Bbp1p detected by the anti-HA antibody 12CA5. India ink, staining of total proteins by india ink. Cells were grown in media containing 2% glucose, unless indicated otherwise. Lane 1, W303; lane 2, ZXY621R-1A carrying pBBP-GAL grown in D/G media; lane 3, ZXY621R-1A carrying pBBP-GAL grown in media containing 2% galactose for 3 hours; lane 4, ZXY621R-1A carrying pBBP-ADH-C; lane 5, W303 carrying pBBP-ADH-C; lane 6, ZXY621R-1A carrying pBBP-ADH.

ADH, a yeast 2μ plasmid, and pBBP-ADH-C, a *CEN* plasmid. Both contained *HA-BBP1* controlled by the yeast ADH promoter. Wild-type and *bbp1* cells carrying either pBBP-ADH or pBBP-ADH-C grow at a slower rate than wild-type cells carrying the vector plasmid without *BBP1,* confirming that overexpression of *BBP1* is harmful to yeast. Microscopic examination revealed that wild-type cells carrying pBBP-ADH or pBBP-ADH-C are larger in size. Some of them are binucleate and others are abnormally elongated in shape and unable to undergo cytokinesis, which is similar to the phenotypes observed in *bfr1* cells (data not shown).

The effect of *BBP1* overexpression on cell ploidy was analyzed by FACS. Overexpression of *BBP1* from the ADH promoter leads to an increase in cell ploidy (Figure 6A, ZXY621R-1A + pBBP-ADH-C and W303 + pBBP-ADH), whereas expression of *BBP1* from its own promoter (ZXY621R-1A + pBBP-C), or from the GAL1 promoter in D/G media (ZXY621R-1A + pBBP-GAL in D/G), has no effect on cell ploidy. Similar to *bfr1* cells, cells overexpressing *BBP1,* when arrested by α-factor or nocodazole, do not continue to increase their DNA content (data not shown).

To confirm that the observed phenotypes are due to the presence of excess Bbp1p, we analyzed the level of Bbp1p in cells carrying various *BBP1*-containing plasmids by immunoblotting, using the anti-HA antibody (Figure 6B). Cells carrying pBBP-GAL, when grown in D/G media, contained a low level of Bbp1p (lane 2) that increases to a high level after 3 hr in 2% galactose media (lane 3). Cells carrying pBBP-ADH-C or pBBP-ADH also contained higher levels of Bbp1p than cells carrying pBBP-GAL grown in D/G media (compare lanes 4–6 with lanes 2 and 3). Cells expressing HA-BBP1 from its own promoter produced no detectable signal on immunoblots (data not shown).

A novel type of ascus is observed when tetraploid *bfr1* cells are sporulated: To analyze the role of Bfr1p in meiosis, we sporulated a homozygous *bfr1* strain derived from mating of *bfr1* cells of opposite mating type (ZXY44). Asci began to form in the culture after 30 hr,

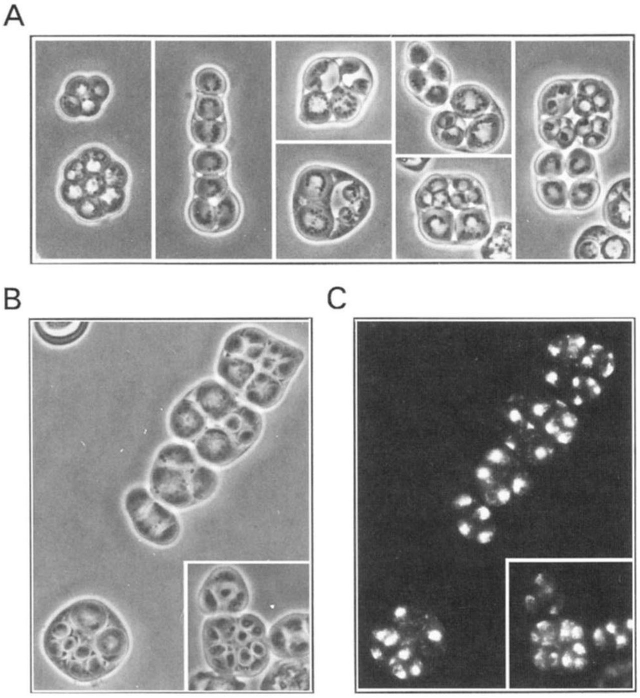

FIGURE 7.—A novel type of ascus is produced by a *bfr1* strain ZXY44. (A) Different types of asci in a sporulating culture of ZXY44 cells, including five that contained internal asci at different stages of development. (B) Five fixed asci containing between zero to four completely formed internal asci. Asci were fixed and stained as described in MATERIALS AND METHODS. (C), DAPI staining of the same five asci as in B.

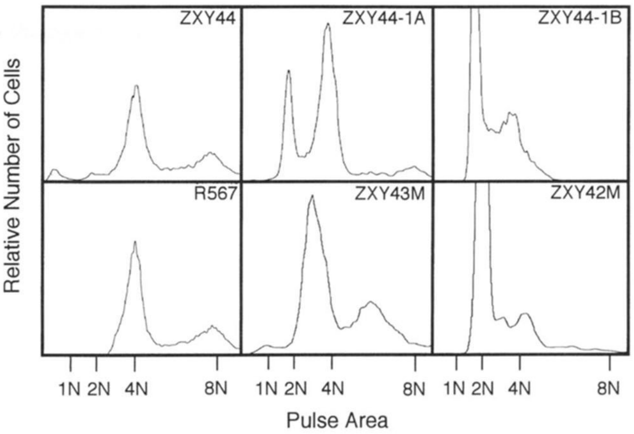

FIGURE 8.—DNA content of the *bfr1* strain ZXY44 and its derivatives. ZXY44 was generated from mating of two *bfr1* cells of opposite mating type; ZXY44-1A is from a ZXY44 spore that is *MAT***a**/α; ZXY44-1B is from a ZXY44 spore that is *MAT***a**/**a**; R567 is a *BFR1* tetraploid strain; ZXY42M is generated by direct mating between a spore in an internal ascus produced by ZXY44 and a W1089-1A cell; ZXY43M is generated by direct mating between a spore inside a normal ascus produced by ZXY44 and a W1089-1A cell.

and up to 80% of cells formed normal asci in 3 days. However, after 5 days we observed that many spores appeared to undergo further sporulation, resulting in the production of a novel type of ascus that contained between one to four complete asci. Figure 7A shows different types of asci observed in a sporulating culture of ZXY44: five of them contained internal asci at different stages of development. Each spore within the internal asci has a nucleus, as analyzed by DAPI staining (Figure 7, B and C). Up to 20% of the asci in a ZXY44 culture contained internal asci. To our knowledge, this type of ascus has not been previously reported, and is different from an ascus that contains eight or more spores which is observed when bi or multinucleate cells undergo sporulation (see Figure 7A for an example of a normal ascus containing more than four spores).

Because *bfr1* cells increase their ploidy, we analyzed the ploidy of ZXY44 (homozygous *bfr1*) by FACS and found that ZXY44 was tetraploid (Figure 8, ZXY44). To confirm this, 50 normal ZXY44 asci were dissected after 3 days, and the mating type of spores determined. The viability of spores was high (92%). Of the 40 asci that yielded four live spores, 23 produced only nonmating spores that formed colonies capable of sporulating; 15 produced two nonmating spores plus one **a** type and one α type spore; two tetrads produced two **a** and two α spores. FACS analysis suggested that these spores formed colonies containing diploid and tetraploid cells (Figure 8, ZXY44-1A and ZXY44-1B). When the spores were mated directly with wild-type haploid **a** cells (W1089-1A), the resulting hybrids were all triploid (Figure 8, ZXY43M). These results confirmed that ZXY44 was tetraploid.

The novel ascus likely arises because *bfr1* a/α spores undergo a second round of meiosis: Since ZXY44 is tetraploid, an explanation for the origin of the novel asci is that diploid *bfr1* spores can undergo a second round of meiosis to form the internal asci (Figure 9). If this is true, the spores inside the internal asci should be haploid. To test this, we dissected five novel asci in a ZXY44 culture, and mated the spores inside all of the internal asci directly with W1089-1A cells. Half of the spores in each internal ascus were able to mate, suggesting that both **a** and α spores were present in equal numbers. FACS analysis indicated that the resulting hybrids were all diploids (Figure 8, ZXY42M). This was further confirmed by sporulating four individual hybrids and analyzing the mating type of the spores. In all four cases, every tetrad produced two **a** and two α spores (spore viability >90%), in agreement with that expected from a diploid strain. Thus the original spores within the internal asci are indeed haploid and the internal asci within an ascus appear to be the products of two rounds of meiosis.

The above data also suggested that only **a**/α spores were able to undergo a second round of meiosis. We have shown earlier that after three days, before the formation of the novel asci, the spores produced by ZXY44 cells were all diploids. Thus the second round of DNA replication likely occurred after the formation of spores from the first round of meiosis. This notion is further

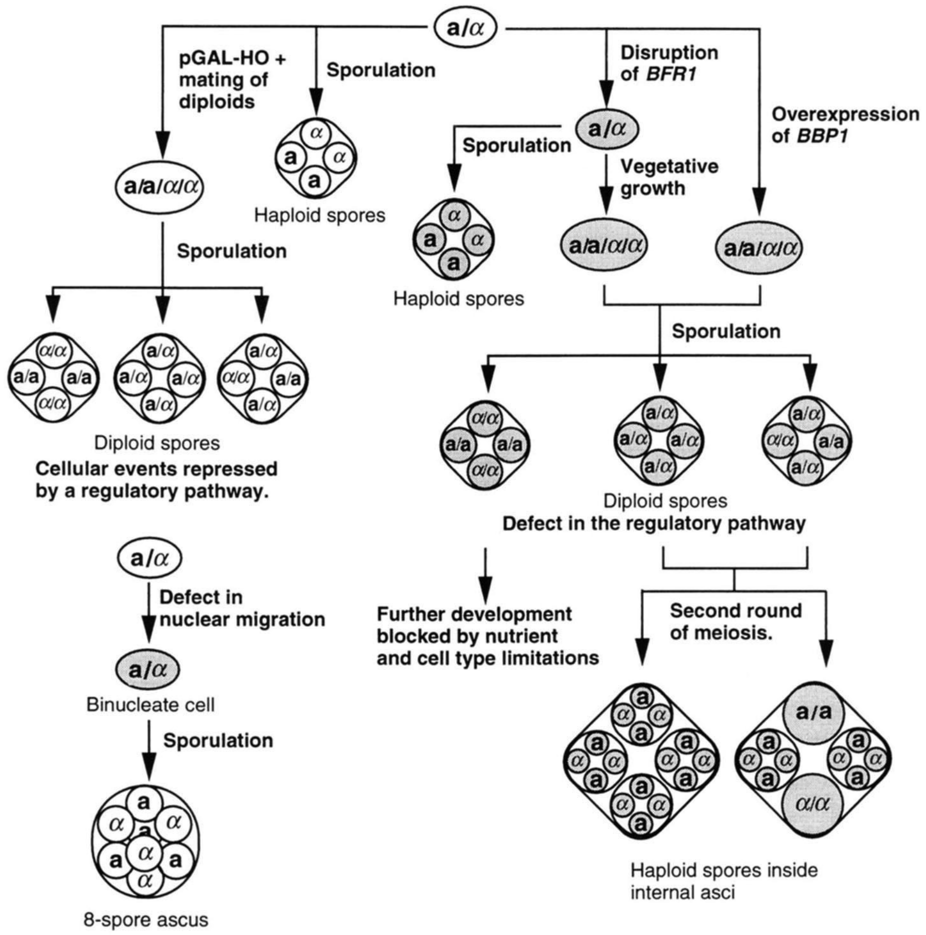

FIGURE 9.—A schematic diagram showing a possible mechanism that accounts for the production of the observed novel ascus structure. Events leading to the production of asci containing eight spores are also shown. Yeast cells are represented by ovals, spores by circles, and tetrads by diamonds. Wild-type strains are white and mutant strains shaded. Tetraploid wild-type strains were constructed by transformation of diploid strains with pGAL-HO, as described in MATERIALS AND METHODS.

supported by the fact that some asci contain both "normal" spores and internal asci, indicating that the initiation of the second round of meiosis happened after the completion of the first round (Figure 9). However, the possibility that the novel asci arise due to some *bfr1* cells entering meiosis directly from G2 so that they would undergo two complete sets of meiotic divisions but only one round of DNA synthesis cannot be ruled out.

We also sporulated a culture of ZXY44-1A, which contained both diploid and tetraploid cells. After 5 days, the spores inside the normal asci in the culture were mated with W1089-1A cells, and six resulting hybrids were analyzed by FACS. Five were shown to be diploid and one triploid, indicating that both haploid and diploid spores were produced by ZXY44-1A cells. This suggests that diploid *bfr1* cells undergo only one round of meiosis to produce haploid spores.

To confirm that the observed phenotype was due to the disruption of *BFR1*, we replaced most of *BFR1* ORF, corresponding to residue 11 to 462 of Bfr1p, with the yeast *LEU2* gene (*bfr1Δ::LEU2*). Homozygous tetraploid *bfr1* strains in two different strain backgrounds, W303 and LL20, were created with both *bfr1Δ::HIS3* and *bfr1Δ::LEU2* (ZXY144L, ZXY244 and ZXY344L, see Table 1). All homozygous *bfr1* tetraploid strains produced novel asci upon sporulation. We also created two homozygous *BFR1* tetraploid strains W303T and R567 that are isogenic to the *bfr1* tetraploids (see MATERIALS AND METHODS). Under identical conditions, these tetraploid strains produced only normal tetrads.

Overexpression of *BBP1* leads to the production of the novel asci upon sporulation: Because overexpression of *BBP1* and disruption of *BFR1* produce similar phenotypes in vegetatively growing yeast, we sporulated diploid W303 cells carrying pBBP-ADH to observe whether *BBP1* overexpression also leads to the production of novel asci. By FACS analysis the culture used contained more than 85% tetraploid cells. As shown in Figure 10, after 5 days approximately 5% of the asci in the culture contain internal asci. Thus, overexpression of *BBP1* from the ADH-promoter leads to a meiotic phenotype similar to that observed in cells lacking *BFR1*. Figure 9 summarizes the observed meiotic phenotype in cells lacking *BFR1* or overexpressing *BBP1* and

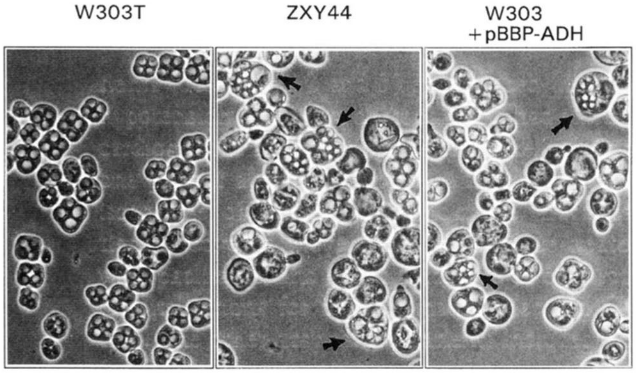

FIGURE 10.—Phase contrast photographs of sporulation cultures of W303T and ZXY44 yeast cells and W303 cells carrying pBBP-ADH. Arrows point to novel asci containing asci inside.

presents a possible mechanism to explain the production of novel asci (see DISCUSSION for more details).

DISCUSSION

We have shown that yeast *BFR1* and a novel essential gene, *BBP1,* play important roles in both the mitotic cell cycle and meiosis. Cells lacking Bfr1p or overproducing Bbp1p are defective in coordinating DNA replication with cell division, resulting in an increase in cell ploidy during growth. The most striking phenotype of these mutants is the production, upon sporulation of tetraploid mutant cells, of a novel type of ascus containing complete asci instead of individual spores. Our data suggest that mutant **a**/α spores were able to undergo a second round of meiosis, leading to the production of the internal asci observed.

As shown in Figure 9, in wild-type cells, spores are stable for a long period of time in the absence of a nutritional signal that triggers germination, and **a**/α spores produced by wild-type tetraploid cells do not undergo further meiosis. This argues that a repression pathway exists to prevent the initiation of further DNA replication and other cell cycle events in spores. Our results indicate that Bfr1p and Bbp1p play an important role in preventing cell cycle events in spores. Thus Bfr1p and Bbp1p are potential candidates as components of the repression pathway. In this view, the observed phenotype arises because the absence of Bfr1p or the presence of excess Bbp1p leads to a defect in the repression pathway. Since nutritional conditions in sporulation media favor the initiation of meiosis rather than the mitotic cell cycle, mutant **a**/α spores will undergo a second round of meiosis, whereas **a** and α type spores will not initiate meiosis due to cell type limitation.

An alternative possibility is that Bfr1p and Bbp1p play a role in regulating entry into meiosis. In this view, the absence of Bfr1p or the presence of excess Bbp1p results in yeast cells entering meiosis from G2 without a round of meiotic DNA synthesis, but still undergoing meiotic divisions to form diploid spores. The spores would then initiate a normal round of meiosis (including DNA synthesis) to form haploid spores within internal asci. This model (G2 entry) still requires that there are two rounds of meiosis. The difference between this model and that of postmeiotic repression, is that the two rounds of meiosis in the G2 entry model are asymmetric, the first round without DNA synthesis, and the second round with DNA synthesis.

So far, the only known mutants that affect postmeiotic repression are the Drosophila mutants, *plutonium, pan gu* and *gnu.* They produce eggs that undergo multiple DNA replications after the completion of meiosis (FREEMAN and GLOVER 1987; SHAMANSKI and ORR-WEAVER 1991). Interestingly, the fertilized mutant eggs also undergo multiple rounds of DNA replication without mitosis early in development. Thus, the Drosophila mutants share a common feature with yeast mutants described in this paper: both the mitotic and the meiotic phenotype involve extra rounds of DNA replication. *plutonium* encodes a small ankyrin repeat protein that has no sequence similarity to either Bfr1p or Bbp1p (AXTON *et al.* 1994).

A difference between the *S. cerevisiae* and the Drosophila mutants is that the mutant eggs replicate their DNA without division, whereas the mutant spores undergo divisions to produce viable spores. A simple explanation is that multiple factors are involved in postmeiotic repression and mutations in different factors lead to different phenotypes. Alternatively, this may indicate that replication and division are repressed by different pathways in Drosophila, but by a single pathway in yeast (see HONIGBERG *et al.* 1993). This difference may reflect the fact that spores only need to respond to a nutritional signal to begin the mitotic cell cycle, whereas fertilization is needed for Drosophila eggs to begin normal development.

Deletion of *BFR1* or overexpression of *BBP1* leads to a gradual increase in ploidy during the mitotic cell cycle, but in contrast to some mutants that show an increase in ploidy when arrested in G1 (MORENO and NURSE 1994; HEICHMAN and ROBERTS 1996), these cells do not continue to replicate their DNA in the absence of mitosis or START. We favor the idea that Bfr1p and Bbp1p are involved in coordinating cell cycle events in addition to their roles in meiosis. In each cell cycle a small number of mutant cells may start replication without bud formation and/or proper assembly of the spindle apparatus, which could then lead to an increase in ploidy. In agreement with this, we observed a marked increase in the number of large-budded cells with a single nucleus as well as unbudded binucleate cells in a *bfr1* culture. Further evidence supporting the idea that these two proteins are involved in cell cycle coordination comes from the fact that cells depleted of Bbp1p are defective in bud formation, cytokinesis and spindle assembly, but DNA replication is normal, indicating an uncoupling of cell cycle events.

Alternatively, Bfr1p and Bbp1p might perform independent functions in more than one cellular process, and the increase in ploidy might reflect a defect in a specific function rather than a loss of cell cycle coordination. For example, a mutation in *BEM2,* a gene required for bud formation (KIM *et al.* 1994), results in an increase in ploidy, which led to a suggestion by Chan and Botstein (1993) that chromosome gain or increase-in-ploidy may be a common phenotype for mutants defective in bud formation or growth. Another potential cause of an increase in ploidy is a defect in the mitotic spindle apparatus (SCHILD *et al.* 1981; THOMAS and BOTSTEIN 1986; ROSE and FINK 1987; BAUM *et al.* 1988; SNYDER and DAVIS 1988; UZAWA *et al.* 1990; KORMANEC *et al.* 1991; SCHROER 1994). Our data do suggest that cells lacking *BFR1* or overexpressing *BBP1* are defective in bud formation, cytokinesis and nuclear spindle formation.

Deletion of *BFR1* has previously been shown to accentuate the late-Golgi transport defect of a mutant in the secretion pathway, *sec21.* However, Bfr1p does not appear to have a direct role in the secretion process because disruption of *BFR1* does not lead to a detectable secretion defect (JACKSON and KÉPÈS 1994). Interestingly, depletion of Bbp1p leads to a delocalized distribution of chitin, suggesting that although secretion is not blocked secretory vesicles are not directed to the bud site. One possibility is that *bfr1* and *bbp1* mutants are defective in cell cycle dependent organization of the actin cytoskeleton (WELCH *et al.* 1994), which leads to a defect in polarized secretion.

Our data from the two-hybrid assay suggest that Bfr1p and Bbp1p interact physically with each other, perhaps forming a complex either by themselves or with other proteins. The similarities between the observed phenotypes of cells lacking *BFR1* or cells overexpressing *BBP1* support this idea. Furthermore, the level of Bbp1p appears to be critical. Perhaps in the absence of Bfr1p or in the presence of an excessive amount of Bbp1p, the complex becomes nonfunctional or toxic to the cell.

The dual phenotypes in both meiotic and mitotic cell cycles, observed in cells lacking *BFR1* or overexpressing *BBP1,* raise the intriguing question of whether other mutants showing an increase-in-ploidy phenotype might also show a similar defect in meiosis. Detailed analysis of the cellular functions of these two proteins, as well as the identification of other proteins that interact with them should provide new insights into the regulation of meiosis and the coordination of cell cycle events in yeast.

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LITERATURE CITED

AXTON, J. M., F. L. SHAMANSKI, L. M. YOUNG, D. S. HENDERSON, J. B. BOYD *et al.,* 1994 The inhibitor of DNA replication encoded by the Drosophila gene *plutonium* is a small, ankyrin repeat protein. EMBO J. **13:** 462–470.

BAUM, P., C. YIP, L. GOETSCH and B. BYERS, 1988 A yeast gene essential for regulation of spindle pole duplication. Mol. Cell. Biol. **8:** 5386–5397.

CHAN, C. S. M., and D. BOTSTEIN, 1993 Isolation and characterization of chromosome-gain and increase-in-ploidy mutants in yeast. Genetics **135:** 677–691.

CROSS, F., J. ROBERTS and H. WEINTRAUB, 1989 Simple and complex cell cycles. Annu. Rev. Cell Biol. **5:** 341–395.

FIELD, J., J.-I. NIKAWA, D. BROEK, B. MACDONALD, L. RODGERS *et al.,* 1988 Purification of a RAS-responsive adenylyl cyclase complex from *Saccharomyces cerevisiae* by use of an epitope addition method. Mol. Cell. Biol. **8:** 2159–2165.

FIELDS, S., and O.-K. SONG, 1989 A novel genetic system to detect protein-protein interactions. Nature **340:** 245–246.

FLESCHER, E. G., K. MADDEN and M. SNYDER, 1993 Components required for cytokinesis are important for bud site selection in yeast. J. Cell Biol. **122:** 373–386.

FORSBURG, S. L., and P. NURSE, 1991 Cell cycle regulation in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe.* Annu. Rev. Cell Biol. **7:** 227–256.

FREEMAN, M., and D. M. GLOVER, 1987 The *gnu* mutation of Drosophila causes inappropriate DNA synthesis in unfertilized and fertilized eggs. Genes Dev. **1:** 924–930.

HARTWELL, L. H., and T. WEINERT, 1989 Checkpoints: controls that ensure the order of cell cycle events. Science **246:** 629–634.

HEICHMAN, K. A., and J. M. ROBERTS, 1996 The yeast *CDC16* and *CDC27* genes restrict DNA replication to once per cell cycle. Cell **85:** 39–48.

HERSKOWITZ, I., and R. E. JENSEN, 1991 Putting the HO gene to work: practical uses for mating type switching. Methods Enzymol. **194:** 132–146.

HONIGBERG, S. M., R. M. MCCARROLL and R. E. ESPOSITO, 1993 Regulatory mechanisms in meiosis. Curr. Opin. Cell Biol. **5:** 219–225.

HUTTER, K. J., and H. E. EIPEL, 1979 Microbial determinations by flow cytometry. J. Gen. Microbiol. **113:** 369–375.

ITO, H., Y. JUKUDA, K. MURATA and A. KIMURA, 1983 Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153:** 163–168.

JACKSON, C. L., and F. KÉPÈS, 1994 *BFR1,* a multicopy suppressor of Brefeldin A-induced lethality, is implicated in secretion and nuclear segregation in *Saccharomyces cerevisiae.* Genetics **137:** 423–437.

KIM, Y.-J., L. FRANCISO, G.-C. CHEN, E. MARCOTTE and C. S. M. CHAN, 1994 Control of cellular morphogenesis by the Ipl2/Bem2 GTPase-activating protein: possible role of protein phosphorylation. J. Cell Biol. **127:** 1381–1394.

KLAPHOLZ, S., and R. E. ESPOSITO, 1981 Meiosis and ascospore development, pp. 211–287 in *The Molecular Biology of The Yeast Saccharomyces: Life Cycle and Inheritance,* edited by J. M. STRATHERN, E. W. JONES and J. R. BROACH. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

KORMANEC, J., I. SCHAAFF-GERSTENSCHLAGER, F. K. ZIMMERMANN, D. PERECKO and H. KUNTZEL, 1991 Nuclear migration in *Saccharomyces cerevisiae* is controlled by the highly repetitive 313kDa NUM1 protein. Mol. Gen. Genet. **230:** 277–287.

LEE, W-C., Z. XUE and T. MELESE, 1991 The *NSR1* gene encodes a protein that specifically binds nuclear localization sequences and has two RNA recognition motifs. J. Cell Biol. **113:** 1–12.

LEW, D. J., and S. I. REED, 1995 A cell cycle checkpoint monitors cell morphogenesis in budding yeast. J. Cell Biol. **129:** 739–749.

MITCHELL, A. P., 1994 Control of meiotic gene expression in *Saccharomyces cerevisiae.* Microbiol. Rev. **58:** 56–70.

MORENO, S., and P. NURSE, 1994 Regulation of progression through the G1 phase of the cell cycle by the *rum1*$^+$ gene. Nature **367:** 236–242.

MURRAY, A., 1992 Creative blocks: cell-cycle checkpoints and feedback controls. Nature **359:** 599–604.

NASMYTH, K., 1993 Control of the yeast cell cycle by the Cdc28 protein kinase. Curr. Opin. Cell Biol. **5:** 166–179.

NISHITANI, H., and P. NURSE, 1995 p65*cdc18* plays a major role controlling the initiation of DNA replication in fission yeast. Cell **83:** 397–405.

O'CONNELL, M. J., and P. NURSE, 1994 How cells know they are in G1 or G2. Curr. Opin. Cell Biol. **6:** 867–871.

ROBBINS, J., S. M. DILWORTH, R. A. LASKEY and C. DINGWALL, 1991 Two interdependent basic domains in nucleoplasmin nuclear targeting sequence: identification of a class of bipartite nuclear targeting sequence. Cell **64:** 615–623.

ROBERTS, J., 1993 Turning DNA replication on and off. Curr. Opin. Cell Biol. **5:** 201–206.

ROSE, M. D., and J. R. FINK, 1987 *KAR1,* a gene required for function of both intranuclear and extranuclear microtubules in yeast. Cell **48:** 1047–1060.

ROTHSTEIN, R. J., 1983 One-step gene disruption in yeast. Methods Enzymol. **101:** 203–211.

SAMBROOK, J., E. F. FRITSCH, and T. MANIATIS, 1989 *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

SCHILD, D., H. N. ANANTHASWAMY and R. K. MORTIMER, 1981 An endomitotic effect of a cell cycle mutation of *Saccharomyces cerevisiae.* Genetics **97:** 551–562.

SCHROER, T. A., 1994 New insights into the interaction of cytoplasmic dynein with the actin related protein, *Arp*1. J. Cell Biol. **127:** 1–4.

SETHI, N., M. C. MONTEAGUDO, D. KOSHLAND, E. HOGAN and D. J. BURKE, 1991 The *CDC20* gene product of *Saccharomyces cerevisiae,* a β-transducin homolog, is required for a subset of microtubule-dependent cellular processes. Mol. Cell. Biol. **11:** 5592–5602.

SHAMANSKI, F. L., and T. L. ORR-WEAVER, 1991 The Drosophila *plutonium* and *pan gu* genes regulate entry into S phase at fertilization. Cell **66:** 1289–1300.

SHAN, X., Z. XUE, and T. MELESE, 1994 Yeast *NP146* encodes a novel prolyl *cis-trans* isomerase that is located in the nucleolus. J. Cell Biol. **126:** 853–862.

SHERMAN, F., 1991 Getting started with yeast. Methods Enzymol. **194:** 3–20.

SHERMAN, F., J. B. HICKS and G. R. FINK, 1986 *Methods of Yeast Genetics.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

SIKORSKI, R., and P. HIETER, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae.* Genetics **122:** 19–27.

SIKORSKI, R. S., M. S. BOGUSKI, M. GOEBL and P. HIETER, 1990 A repeating amino acid motif in *CDC23* defines a family of proteins and a new relationship among genes required for mitosis and RNA synthesis. Cell **60:** 307–317.

SNYDER, M., and R. W. DAVIS, 1988 *SPA1:* a gene important for chromosome segregation and other mitotic functions in *S. cerevisiae.* Cell **54:** 743–754.

THOMAS, B. J., and R. J. ROTHSTEIN, 1989 Elevated recombination rates in transcriptionally active DNA. Cell **56:** 619–630.

THOMAS, J. H., and D. BOTSTEIN, 1986 A gene required for the separation of chromosomes on the spindle apparatus in yeast. Cell **44:** 65–76.

UZAWA, S., I. SAMEJIMA, T. HIRANO, K. TANAKA and M. YANAGIDA, 1990 The fission yeast *cut1*$^+$ gene regulates spindle pole body duplication and has homology to the budding yeast *ESP1* gene. Cell **62:** 913–925.

WEBER, L., and B. BYERS, 1992 A *RAD9*-dependent checkpoint blocks meiosis of *cdc13* yeast cells. Genetics **131:** 55–62.

WELCH, M. D., D. A. HOLTZMAN and D. G. DRUBIN, 1994 The yeast actin cytoskeleton. Curr. Opin. Cell Biol. **6:** 110–119.

WINEY, M., L. GOETSCH, P. BAUM and B. BYERS, 1991 *MPS1* and *MPS2*: novel yeast genes defining distinct steps of spindle pole body duplication. J. Cell Biol. **114:** 745–754.

WINEY, M., M. A. HOYT, C. CHAN, L. GOETSCH, D. BOTSTEIN *et al.,* 1993 *NDC1:* a nuclear periphery component required for yeast spindle pole body duplication. J. Cell Biol. **122:** 743–751.