Mutation in the *bimD* Gene of *Aspergillus nidulans* Confers a Conditional Mitotic Block and Sensitivity to DNA Damaging Agents

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ABSTRACT

Mutation in the *bimD* gene of *Aspergillus nidulans* results in a mitotic block in anaphase characterized by a defective mitosis. Mutation in *bimD* also confers, at temperatures permissive for the mitotic arrest phenotype, an increased sensitivity to DNA damaging agents, including methyl methanesulfonate and ultraviolet light. In order to better understand the relationship between DNA damage and mitotic progression, we cloned the *bimD* gene from Aspergillus. A cosmid containing the *bimD* gene was identified among pools of cosmids by cotransformation with the nutritional selective *pyrG* gene of a strain carrying the recessive, temperature-sensitive lethal *bimD6* mutation. The *bimD* gene encodes a predicted polypeptide of 166,000 daltons in mass and contains amino acid sequence motifs similar to those found in some DNA-binding transcription factors. These sequences include a basic domain followed by a leucine zipper, which together are called a bZIP motif, and a carboxyl-terminal domain enriched in acidic amino acids. Overexpression of the wild-type *bimD* protein resulted in an arrest of the nuclear division cycle that was reversible and determined to be in either the G_1 or S phase of the cell cycle. Our data suggest that *bimD* may play an essential regulatory role relating to DNA metabolism which is required for a successful mitosis.

REGULATION of entry into mitosis has been shown to occur by phosphorylation of various cellular substrates by protein kinases including the $p34^{cdc2}$ protein kinase of *Schizoaccharomyces pombe* and its homologs in many organisms and the NIMA protein kinase of *Aspergillus nidulans* (MACNEILL, CREANER and NURSE 1991; OSMANI, MAY and MORRIS 1987). For example, nuclear envelope breakdown during mitosis occurs as a result of phosphorylation of nuclear lamins by $p34^{cdc2}$ kinase. Nuclear lamins have been shown to be phosphorylated by $p34^{cdc2}$ both *in vitro* and when lamins are introduced into fission yeast (ENOCH *et al.* 1991; PETER *et al.* 1990, 1991). Other cellular substrates phosphorylated by $p34^{cdc2}$ kinase at mitosis include caldesmin and chromatin high mobility group protein I in mammalian cells (YAMAKITA, YAMASHIRO and MATSUMURA 1992; NISSEN, LANGAN and REEVES 1991). Nucleolin in mammalian cells and histone H1 are also substrates for $p34^{cdc2}$ kinase (BELENGUER *et al.* 1990; ARION *et al.* 1988). As a regulator of entry into mitosis, the $p34^{cdc2}$ protein kinase potentially responds to a variety of signals to ensure that entry into mitosis does not occur at an inappropriate time. These signals include incomplete DNA synthesis, the presence of damaged DNA and an incomplete mitotic spindle. The failure to halt progression into mitosis in response to any of these signals may result in a defective or catastrophic mitosis which ultimately would lead to cell death. The $p34^{cdc2}$ kinase is regulated by both phosphorylation and dephosphorylation events in a seemingly redundant system of control (LUNDGREN *et al.* 1991; RUSSEL and NURSE 1986, 1987). This complex regulatory system seems to exist, however, because of the many signals to which this kinase must respond to control entry into mitosis. For example, in *S. pombe*, the pathways by which *cdc2* is inhibited in response to DNA damage induced by γ-radiation appears to be different from the pathway by which incompletely replicated DNA inhibits *cdc2* (ROWLEY, HUDSON and YOUNG 1992). Mitotic delay caused by γ-radiation-induced damage requires the *wee1* protein kinase, which is a negative regulator of *cdc2* function (RUSSEL and NURSE 1987). Mitotic delay in response to incomplete DNA synthesis, however, does not require *wee1* (ENOCH and NURSE 1990; ENOCH, CARR and NURSE 1992). In *Saccharomyces cerevisiae*, delay of progression through mitosis in response to the absence of a mitotic spindle appears to be due to inhibition of inactivation of $p34^{CDC28}$, the homologous kinase to *S. pombe* $p34^{cdc2}$ (HOYT, TOTIS and ROBERTS 1991; LI and MURRAY 1991). Genes which affect mitotic progression in response to DNA damage are known such as the *RAD9* gene of *S. cerevisiae* (WEINERT and HARTWELL 1988; SCHIESTL *et al.* 1989) and the mammalian p53 (KASTAN *et al.* 1991), yet it is unknown if these genes act through the $p34^{cdc2}$ kinase to affect progression into mitosis. However, p53 and $p34^{cdc2}$ may cooperate to inhibit

cell growth, based on effects of expression of p53 and the human CDC2Hs in *S. cerevisieae* (NIGRO *et al.* 1992). Interestingly, p53 is a sequence-specific DNA-binding protein which can act as a transcriptional activator (FARMER *et al.* 1992; BARGONETTI *et al.* 1991; KERN *et al.* 1991; RAYCROFT, WU and LOZANO 1990; FIELDS and JANG 1990), and *RAD9* has homology to *jun*, a DNA-binding transcription factor (SCHIESTL *et al.* 1989). This may suggest similar mechanisms by which these two proteins affect mitotic progression in response to DNA damage.

Genetic analysis of mutations which result in either a failure to enter or exit mitosis or result in a defective mitosis can potentially identify genes involved in controlling mitotic progression, genes whose products are components of the mitotic apparatus, and genes which control production of these components. In *A. nidulans* this approach has identified genes involved in control of entry into and exit from mitosis (MORRIS 1976; OSMANI, MAY and MORRIS 1987; OSMANI, PU and MORRIS 1988; OSMANI *et al.* 1988; ENGLE *et al.* 1990; DOONAN and MORRIS 1989). It has also identified genes whose products are apparently novel structural components important for mitosis (MAY *et al.* 1992; ENOS and MORRIS 1990; O'DONNELL *et al.* 1991). Of particular interest would be mutations which affect two processes whose interrelationships are not fully understood, such as DNA metabolism and mitosis. Such mutations could identify genes which act to ensure that chromosomes are properly segregated at mitosis.

Mutations in the *RAD9* checkpoint gene of *S. cerevisiae* allow cells to enter mitosis in the presence of damaged DNA, yet the only mitotic defect resulting from a complete absence of the *RAD9* gene product is an increase in the rate of chromosome loss (HARTWELL and WEINERT 1989). Further, it is not known how *RAD9* acts to delay mitosis in response to damaged DNA (WEINERT and HARTWELL 1988). Mutations in the *bimE* gene of Aspergillus and in the hamster RCC1 gene result in premature chromosome condensation and entry into mitosis (NISHIMOTO, EILEN and BASILICO 1978; OSMANI *et al.* 1988). However, whereas mutations in both the RCC1 gene and in *pim1*, the *S. pombe* RCC1 homolog, seem to cause premature activation of H1 kinase, this does not seem to be the case for *PRP20*, the *S. cerevisiae* RCC1 homolog (MATSUMOTO and BEACH 1991; CLARK *et al.* 1991). The mechanism, therefore, by which cells delay chromosome condensation prior to entry into mitosis is not completely understood. Therefore, it is of interest to study genes which are involved in both mitosis and aspects of DNA metabolism, as this will aid in determining how cells ultimately segregate genetic information into two identical nuclei at mitosis.

In this study we describe the *bimD* gene of *A. nidulans*, mutations in which result in a conditional mitotic arrest and an increased sensitivity to DNA-damaging agents which is seen under conditions permissive for the mitotic defect. Additionally, the mitotic block is characterized by an abnormal, catastrophic mitosis and a decrease in the number of microtubules in the mitotic spindle (OAKLEY 1981). We describe the cloning and characterization of the *bimD* gene. We present evidence to suggest that this gene performs an essential function which is required for a successful mitosis. This function may be in DNA metabolism, possibly as a DNA-binding protein.

MATERIALS AND METHODS

Aspergillus strains, culture conditions, and transformation: *A. nidulans* strains used in this study were D6.2: *bimD6; pyrG89; wA3; sC12*, used for complementation of the *bimD6* mutation, the 2n strain, GM24/GM26: *pyrG89 riboA1 yA2; nicB8; fwA1/pyrG89 pabaA1; AcrA1; benA22 riboB2*, used for disruption of the *bimD* gene. The strains used for tests for methyl methanesulfonate (MMS) and UV sensitivity were M4053: *riboA1; sC12; bimD6 pyroA4; nicA2; chaA1*, M4055: *riboA1; sC12; pyroA4; nicA2; chA1* and M3930: *pabaA1; uvsB110; nicA2; chaA1*. The strain SO30: *nimT23 cnxE16 wA2; nicA2; choA1; pabaA1*, was used for anti-tubulin immunofluorescence. *pyrG89* strains were grown on media containing 2% malt extract, 0.2% peptone, 1% dextrose, trace elements (COVE 1977), 5 mM uridine, 10 mM uracil, 1 μg/ml pyrodoxine, 8.8 μg/ml riboflavin. Transformation of *A. nidulans* was performed as described previously (MAY 1989). For growth in liquid media and microscopic studies strains were grown in 0.5% yeast extract, 20 mM dextrose, trace elements, 5 mM uridine, and 10 mM uracil. Genetic methods and other media employed were those described previously (KÄFER 1977; PONTECORVO 1953).

Plasmid construction: The plasmid pbimD12 used for the *bimD* gene disruption was made by subcloning a 2.0-kb *EcoRI-BglII* internal *bimD* genomic fragment (see Figure 5) into the vector pRG4 which contains the *pyr-4* gene of *Neurospora crassa* which complements the *pyrG89* mutation of *A. nidulans* (WARING, MAY and MORRIS 1989). The plasmid pAL3bimD was constructed by placing a 4.8-kb, full-length *bimD* cDNA insert under the control of the inducible *alcA* promoter in the vector pAL3 which contains the *pyr-4* gene of *N. crassa* as a nutritional marker (WARING, MAY and MORRIS 1989).

Cloning of the *bimD* gene, isolation of cDNAs and sequence determination: To clone the *bimD* gene pools of 40 linkage group IV-specific *A. nidulans* cosmid clones were transformed along with the plasmid ppyrG into the strain D6.2. The cosmid library was constructed in the vector pWE15 (BRODY *et al.* 1991). Once we identified a pool containing complementing activity we transformed each cosmid in that pool individually, using DNA prepared from a DNA minipreparation protocol (VOLLMER and YANOFSKY 1986). Three cDNA libraries were screened with restriction fragments of *bimD* genomic clones to identify overlapping *bimD* cDNA clones, a λZAP and UniZAP library (Stratagene, La Jolla, California; MAY *et al.* 1992) and a λgt10 library (OSMANI, PU and MORRIS 1988). A near full-length cDNA, which was overlapping with the other, shorter clones, was isolated from the λgt10 library. Sequencing of *bimD* cDNAs was performed using the T7 DNA polymerase system (Sequenase, U.S. Biochemical Corp., Cleveland)

using single stranded templates in M13mp18 and M13mp19 (EHINGER, DENISON and MAY 1990). Sequences were assembled with the aid of the MBCR computer facility at Baylor College of Medicine. DNA gel electrophoresis and genomic Southern analysis were performed as described previously (MAY *et al.* 1987). The sequences presented in this manuscript have been deposited in GenBank and assigned accession no. L03200.

Anti-tubuin immunofluorescense and DAPI staining of *bimD6* and *nimT23* strains: The strain SO30 was grown for 12 hr at the permissive temperature of 25° then transferred to the restrictive temperature of 42° for 3 hr to block cells in G_2 (O'CONNELL *et al.* 1992). The cells were then returned to the permissive temperature for 15 min after which time they were prepared for staining with anti-tubulin antibody and 4,6-diamidino-2-phenylindole (DAPI). The strain D6.2 was grown at the permissive temperature of 25° for for 12 hr and then transferred to the restrictive temperature for 2 hr after which time the cells were prepared for anti-tubulin antibody and DAPI staining. The cells were fixed for 45 min in 1 × MTSB, 10% dimethylsulfoxide (DMSO), 8% formaldehyde (MTSB = 100 mM Pipes, pH 6.7, 25 mM EGTA, 5 mM $MgSO_4$). The cells were then digested in 0.5 × MTSB, 5% Driselase, 2% Novozyme, 50% egg white for 70 min. Extraction was then carried out in 1 × MTSB with 0.1% Nonidet P-40, 10% DMSO for 60 sec. Anti-tubulin antibody DM1A was used at a dilution of 1:100 and for 45 min. Fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse antibody was then reacted against the cells at a dilution of 1:200 for 45 min.

Tests for mutagen sensitivity: For MMS treatment, conidia were treated in NaK-phosphate buffer (0.05 M, pH 7.0) for 2 hr at 32° and then plated onto rich medium to determine survival (KÄFER and MAYOR 1986). For UV treatment, conidia were plated on complete medium (CM) and irradiated on the plates after preincubation for 6 hr at 28° (KÄFER and MAYOR 1986). UV dose rate was 1.6 $J/m^2/sec$.

Other materials: Restriction endonucleases and DNA modifying enzymes were from New England Biolabs, Promega Biotechnology or Boehringer Mannheim and used according to the manufacturer's instructions. Other reagents were from Sigma Chemical Co. (St. Louis, Misssouri) or Fisher Scientific Co. (Houston, Texas).

RESULTS

Genetic mapping of the *bimD5* mutation: Our strategy to clone the *bimD* gene was potentially dependent upon a precise linkage group assignment of the mutation and possibly upon mapping to its chromosomal location. Our linkage group assignment by mitotic mapping experiments confirmed the original assignment of linkage group IV. Attempts to map the *bimD5* mutation meiotically within linkage group IV failed to detect linkage to any of the markers in commonly used meiotic mapping strains. The left arm of linkage group IV contains a gap between the genes *uvsH* and *methG*. Using suitable markers and mitotic crossing over we were able to place the *bimD5* mutation on the left arm of linkage group IV. Subsequent crosses to *uvsH* mutant strains confirmed this location of *bimD* to the left arm of linkage group IV and placed *bimD* and *uvsH* 2.5% recombination from one another. The small distance between *bimD* and *uvsH* suggested that the two could be the same gene, particularly since mutations in both genes confer mutagen sensitivity (Käfer and Mayor 1986). We know, however, that this is not the case, as a *bimD* genomic clone does not complement a *uvsH77* mutant strain.

***bimD6* mutant strains have increased sensitivity, relative to wild type, to UV light and MMS:** During the course of our genetic mapping it was determined that *bimD6* mutant strains had increased sensitivity to MMS, an agent used to test for *uvs* mutations. This sensitivity to MMS of *bimD6* mutants was independent of the temperature of growth, as it was observed at the permissive temperature for growth. Both the *bimD5* and *bimD6* mutant alleles exhibited this phenotype. The sensitivity of a strain carrying the *bimD6* mutation is greater than a wild type strain and is similar to a strain carrying the *uvsB110* mutation (Figure 1, upper panel). The sensitivity of *uvsB* strains has been characterized previously (KÄFER and MAYOR 1986). We next wanted to determine if this sensitivity of *bimD6* strains was unique to some property of MMS or if it was related to this agent's DNA damaging ability. We therefore examined the sensitivity of a *bimD6* mutant strain to UV compared to a wild-type strain. The *bimD6* mutation also conferred a sensitivity to UV light which was greater than wild-type sensitivity and less than that of a *uvsB110* strain (Figure 1, lower panel).

Mitotic defect of *bimD6* mutant strains: The *bimD5* and *bimD6* mutations were originally isolated as conditionally lethal mitotic mutants (MORRIS 1976). We have characterized the nature of the mitotic defect of the *bimD6* mutation in greater detail. Following a shift to restrictive temperature the chromosomal mitotic index (CMI, percentage of cells in mitosis) of a *bimD6* strain increases to approximately 40% at 2 hr, after which an increase in the number of cells with abnormal nuclear morphology is seen. In contrast, the CMI of a wild-type strain remains at approximately 4% in the same experiment (data not shown).

Examination of nuclei with the DNA-specific dye DAPI and of mitotic spindles by anti-tubulin immunofluorescence showed that the chromatin in relation to the spindle is not wild-type in appearance in a *bimD6* mutant strain at restrictive temperature (Figure 2). Normally in mitosis, chromatin is highly condensed and forms two well defined masses in late anaphase and telophase (Figure 2, b and d) which resides at opposite ends of the mitotic spindle (Figure 2, a and c; the cell shown in a and b contains two spindles). In contrast, in the defective mitosis of a *bimD6* mutant strain, the condensed chromatin lies randomly along the spindle and does not form two discrete masses at either end of the spindle. We often observe in the *bimD6* mutant cells three or four discrete chromatin

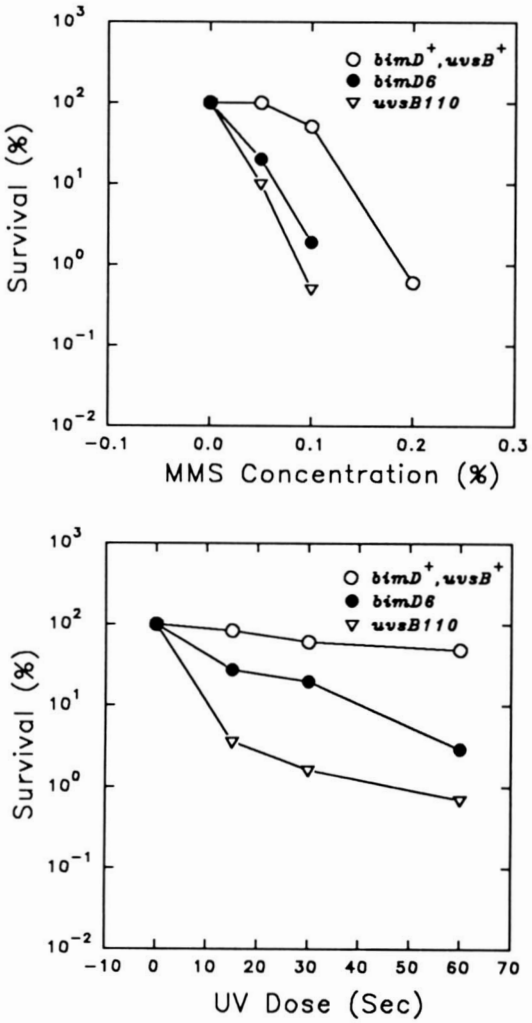

FIGURE 1.—Comparison of sensitivity to MMS and UV of *bimD6*, *uvsB110*, and wild-type strains. (Upper panel) Survival when conidia are treated with MMS in liquid buffer and then spread onto plates; (lower panel) survival when conidia are pregerminated on CM plates prior to UV irradiation of the growing cells on the plates. See MATERIALS AND METHODS for the details of the treatments and of the strains used.

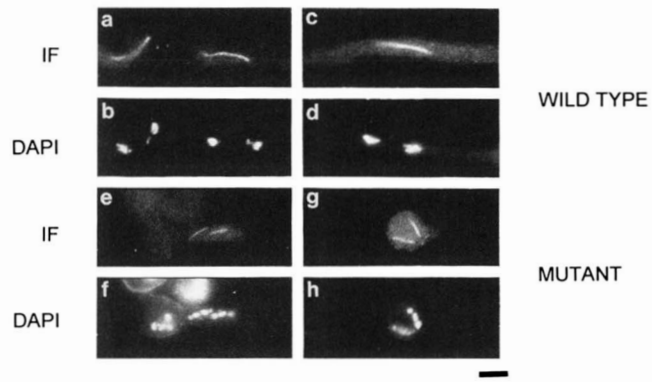

FIGURE 2.—Microtubule and chromosomal appearance of a wild-type mitosis and of a *bimD6* mutant strain defective mitosis. Conidia of strain SO30, a *nimT23* strain (panels a–d) were germinated at permissive temperature for 12 hr then shifted to restrictive temperature for 3 hr at which time the cells are blocked in G_2. The cells were then shifted to the permissive temperature at which time the cells progress through mitosis. The *nimT23* strain was used to obtain a population of cells undergoing a synchronous, successful mitosis to compare with the abnormal mitosis of a *bimD6* strain (O'CONNELL *et al.* 1992). Conidia of D6.2, a *bimD6* strain (panels e–h) were germinated at permissive temperature for 12 hr then shifted to restrictive temperature for 2 hr at which time samples were prepared for immunofluorescense and DAPI staining as described in MATERIALS AND METHODS. Panels a, c, e and g are cells stained with anti-tubulin antibody to visualize the mitotic spindles (see MATERIALS AND METHODS). Panels b, d, f and h are cells stained with DAPI to visualize the nuclei. Scale bar = 5 μm.

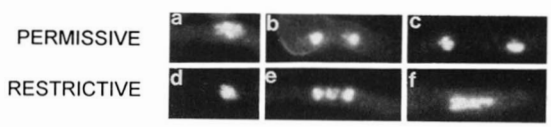

FIGURE 3.—Mitotic appearance of a *bimD6* strain at permissive and restrictive temperature. This figure shows representative cells from the classes of mitotic figures quantitated in Table 1. Panels a, b and c show metaphase, anaphase and telophase mitotic figures, respectively, of D6.2 cells grown at permissive temperature. Panel d shows an metaphase mitotic configuration of a D6.2 cell at restrictive temperature. Panels e and f show anaphase cells at restrictive temperature. Scale bar = 5 μm.

masses (Figure 2, f and h) along the length of the spindles (Figure 2, e and g; both cells shown contain two spindles). Spindle elongation never occurs in the *bimD6* mutant strain to the degree seen in the control strain in which telophase spindles appear highly elongated. The *bimD6* mutant mitosis appears therefore to proceed into anaphase and never progresses beyond that point. The catastrophic mitosis seen at 42° is prevented by addition of benomyl to the media at restrictive temperature before shifting cells from permissive temperature (data not shown). Benomyl arrests cells in mitosis with condensed chromatin but does not allow progression through mitosis, as no mitotic spindle is present due to microtubule depolymerization. This is additional evidence that the nuclear defects seen in the mutant are the result of a defective anaphase separation.

To quantitate the effects of the *bimD6* mutation on mitosis, a *bimD6* mutant strain was grown at permissive temperature and some of the cells transferred to restrictive temperature for 2 hr. After 2 hr, cells from each culture were analyzed to determine which of three stages in mitosis (metaphase, anaphase or telophase) the cells were in. Metaphase cells were identified as having a single, highly condensed chromatin mass and a short mitotic spindle. Anaphase cells show some separation between chromatin masses and a spindle which is elongated relative to the metaphase spindle. Telophase cells exhibit two chromatin masses more widely separated than anaphase cells and a highly elongated spindle. *bimD6* cells at permissive temperature showed all three stages of mitosis, metaphase (Figure 3a), anaphase (Figure 3b) and telophase (Figure 3c). Of the cells at permissive temperature examined, 55% were metaphase, 3% were anaphase

TABLE 1

Quantitation of mitotic stages of a *bimD6* mutant strain at permissive and restrictive temperatures

Growth temperature	Stage (%)		
	Metaphase	Anaphase	Telophase
Permissive	55	3	42
Restrictive	6	94	0

Cells of a *bimD6* mutant strain were germinated at 25°. Some of the cells were left at 25° (Permissive) and some of the cells were shifted to 42° (Restrictive). One hundred mitotic cells at each temperature were examined to determine which of three stages of mitosis, metaphase, anaphase, or telophase, the cells were in. Examples of the stages present at both temperatures are shown in Figure 3.

and 42% were telophase (Table 1). Metaphase cells were also present in the *bimD6* culture at restrictive temperature (Figure 3d). However, these cells only represented 6% of the cells seen (Table 1). In contrast, none of the cells at restrictive temperature were telophase (Table 1). The predominant class of cells at restrictive temperature were anaphase, with 94% of the cells in this stage (Table 1). However, the anaphase configurations were abnormal, in that a large, single chromatin mass (Figure 3f) or many discrete masses (Figure 3e) were seen in the mutant as opposed to the two seen in a wild-type anaphase (Figure 3b).

The relationship of the chromatin to the mitotic spindle in the *bimD6* mutant suggests a defect of attachment of the chromosomes to the spindle and is consistent with previously published information demonstrating a lack of spindle pole body to kinetochore microtubules in the mutant at restrictive temperature (OAKLEY 1981).

Cloning of the *bimD* gene: To clone the *bimD* gene, we cotransformed pools of linkage group IV specific cosmids with the *pyrG* gene as a selective nutritional marker into a *bimD6, pyrG89* double mutant strain of *A. nidulans*. Using pools of 40 cosmid clones in cotransformation experiments we were able to identify a pool that had complementing activity for the *bimD6* mutation. The individual cosmid clones for this pool were cotransformed to identify a single cosmid that contained complementing activity. From the identified cosmid, a 12.7-kb *Kpn*I band was found to contain the complementing activity (Figure 4).

We analyzed restriction fragments of this 12.7-kb *Kpn*I fragment for their ability to complement the *bimD6* mutation and for the number and size of messenger RNA species they hybridized with on Northern transfers of polyadenylated RNA (Figure 4). Using this approach we identified a transcriptional unit of approximately 5 kb that was most likely responsible for the complementing activity. That the *bimD* gene, and not a suppressor gene, was contained on the 12.7-kb fragment was determined by two-step gene replacement (MILLER, MILLER and TIMBERLAKE 1985). A

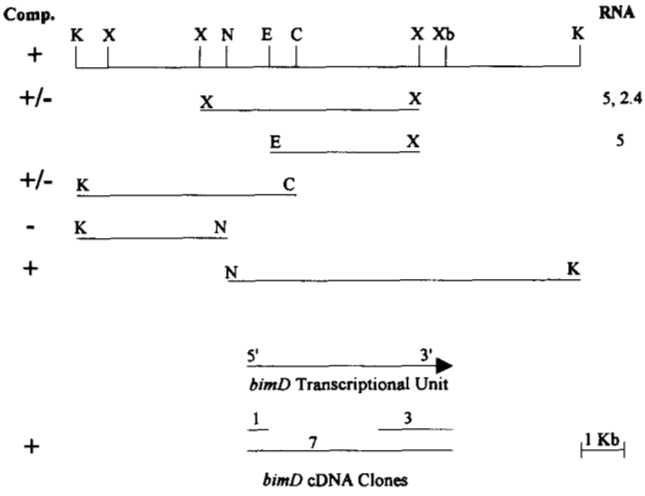

FIGURE 4.—*bimD* genomic clone analysis and isolation of *bimDc* DNAs. The ability of various subclones to complement the *bimD6* mutation is indicated at the left side and the size of RNA species, in thousands of nucleotides, detected on Northern blots is indicated at the right side. As the larger *Nco*I/*Kpn*I fragment but not the smaller complements the *bimD6* mutation, an unidentified transcriptional unit of 2,400 nucleotides is present upstream of the *bimD* gene. Complementation activity of the various fragments was scored as no (−), a few (+/−), or many (+) ts$^+$ transformants. The location and direction of the *bimD* transcriptional unit is indicated below the map along with the location of the overlapping cDNA clones used to determine the sequence of *bimD*. The restriction sites indicated are K, *Kpn*I; X, *Xho*I; N, *Nco*I; E, *Eco*RI; C, *Cla*I; Xb, *Xba*I.

plasmid containing the 12.7-kb *Kpn*I fragment and the nutritional selective marker the *pyr-4* gene of *N. crassa* was transformed into a *bimD6, pyrG89* mutant strain. A transformant was identified by Southern analysis which had intergrated a single copy of the plasmid at *bimD*, producing a tandem repeat of this 12.7-kb fragment. This strain was then cured of the transforming plasmid using 5-fluoro-orotic acid eviction at permissive temperature (MAY *et al.* 1992). Recombination between the tandem repeat created by the transforming circular plasmid and the target sequences on the chromosome can result in the loss of the plasmid sequences from the chromosome, leaving either the original mutant sequences or the wild-type sequences. Following plasmid eviction, strains that were wild-type for growth at restrictive temperature and strains that were temperature sensitive were obtained. Two apparent replacement strains were then out crossed to another wild-type strain to show that the heat sensitive *bimD6* mutation in the original transformant strain was not uncovered, thus indicating that a suppressor of the *bimD6* mutation was not inadvertantly cloned and was responsible for complementation of the heat sensitivity. A final and more definitive proof that the approximately 5-kb transcriptional unit contained within the 12.7-kb *Kpn*I fragment was the *bimD* gene was that a full-length cDNA clone was able to rescue the heat sensitivity of the *bimD6* mutation when cotransformed with the *pyrG*

gene. In this experiment rescue of the heat sensitivity was presumably by repair of the mutant allele with the wild-type information from the plasmid containing the cDNA, which lacks promoter activity.

Sequence of the *bimD* cDNA and derived amino acid sequence suggests a DNA binding function for the *bimD* protein: Three overlapping cDNA clones were isolated for the nearly 5-kb *bimD* transcriptional unit (Figure 4). The nucleotide sequence and derived amino acid sequence of these three overlapping cDNA clones was determined (Figure 5). The longest cDNA clone, #7 in Figure 4, was 4885 bp in length. It was this cDNA clone that was used to rescue a *bimD6* mutant strain in the cotransformation experiments described in the preceding section. The predicted open reading frame was 1506 amino acids in length. Near the beginning of this open reading frame was a methionine that could initiate translation of a protein with a predicted molecular mass of 166,295 daltons. This has been confirmed by reacting a *bimD* polyclonal antisera to a Western blot of protein made from a strain of *A. nidulans* which was made to overexpress *bimD*. An immunoreactive band of the predicted molecular weight is recognized by the polyclonal antisera (data not shown). Complementary DNA clone #7 is likely to be nearly full-length because genomic sequences from the *Nco*I to the *Xba*I sites (Figure 4) are sufficient to complement the *bimD6* mutation when integrated at the *trpC* gene (data not shown). Thus these sequences must contain the entire *bimD* trascriptional unit. In addition a termination codon at base 49 of the cDNA sequence (Figure 5) is upstream of and in frame with the presumptive initiation codon.

Analysis of the amino acid sequence predicted by the open reading frame for features found in other proteins yielded the following results. Three putative nuclear localization signals were found in the predicted polypeptide (Figure 5, single underline). The last 140 amino acid residues of the predicted polypeptide were enriched in acidic amino acids, 28% *vs.* 14% over the entire polypeptide. The overall distribution of basic amino acid residues in this same region was similar, 14%, to that found for the entire polypeptide, 15%. It was also noted that there was a leucine zipper motif that was preceded by a basic domain which was similar to that found in the bZIP class of transcription factors (LANDSHULZ, JOHNSON and MCKNIGHT 1988) (Figure 5, double underline). This region of the *bimD* protein, BIMD, is aligned in Figure 6 with known bZIP proteins. Highlighted in Figure 6 are amino acids which BIMD shares with one or more of the bZIP proteins. The region of BIMD containing the heptad leucine repeats is separated from the basic region by an additional two amino acids as compared to the other leucine zipper proteins. The bZIP proteins shown illustrate both the similarities and the differences between members of this class of proteins. For example in the leucine zipper region, some of the bZIP proteins have in place of the leucine residues, conservative replacements such as isoleucine, methionine and tryptophan. BIMD has two leucine residues in the first and second positions and isoleucines in the third and fourth positions. The *bimD* basic region is most similar to GCN4 and *cpc-1*, both fungal genes. As no additional sequence similarity was found between *bimD* and other genes, we conclude that *bimD* is a novel gene whose product is involved in mitosis. A *bimD* genomic fragment was used to probe a Southern blot under conditions of low stringency showed that *bimD* is present as a single copy gene in the *A. nidulans* genome (data not shown).

***bimD* is an essential gene:** A diploid strain of *A. nidulans* was transformed with the circular disrupting plasmid pbimD12 and a strain heterozygous for the *bimD* gene disruption based on Southern analysis was obtained. This strain was haploidized on medium that would support the growth of all possible haploid genotypes and the resulting segregants were analyzed genotypically by Southern transfer. A total of 50 presumptive haploids were analyzed for their genotypes of which 9 were diploid as determined by retention of their ability to grow on minimal medium, and 34 did not carry the integrated plasmid, and therefore were wild type for *bimD*, as they required uridine for growth. The remaining 7 had the transforming plasmid for the gene disruption, as they were able to grow on media minus uridine, but were presumably haploid as they were not able to grow on minimal medium. Southern analysis of genomic DNA from this last class of transformants showed that they were still heterozygous for the gene disruption. This result suggested that those strains still having the integrated plasmid were disomic for linkage group IV. In addition these strains conidiated poorly and had abnormal colony morphology on medium selective for the transforming plasmid, characteristics of disomic strains. We can exclude the possibility that the truncated protein produced from the 3′ deleted gene was a poison peptide because the diploid heterozygous for the gene disruption was normal, even in the absence of selection for the nutrional markers used to construct it. Similarly, we would expect that the disomic strains produced during haploidization would not be viable had the truncated polypeptide been deleterious to the growth of the strain. Furthermore, we would not have expected to recover these disomic strains for the same reasons. These data strongly suggest that *bimD* is an essential gene.

Overexpression of *bimD* leads to a specific and reversible block in the nuclear division cycle: The similarity of *bimD* to certain transcription factors suggested to us that *bimD* could perform a regulatory

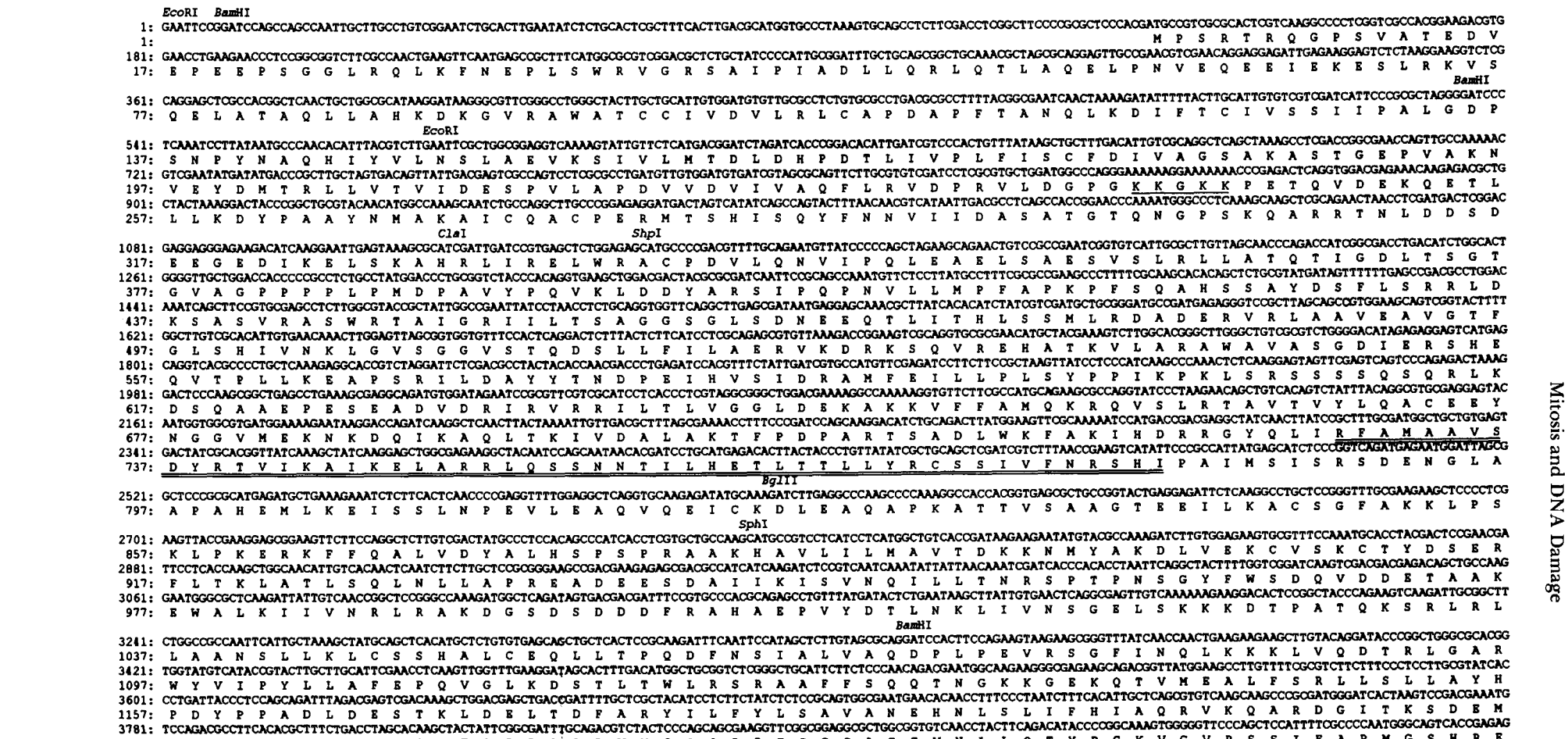

FIGURE 5.—*bimD* nucleotide sequence and derived amino acid sequence. Nucleotide sequence was obtained from overlapping cDNA clones. The entire sequence was determined on both strands. The leucine zipper and basic region are indicated by the double underline. This region is shown in greater detail in Figure 6. Consensus nuclear localization signals are indicated by the single underline. The numbers of the first nucleotide and amino acids of each line are indicated on the right. The positions of some restriction sites are indicated above the nucleotide sequence.

```
                    BASIC DOMAIN                    LEUCINE ZIPPER
                                                  L     L     L     L     L
BIMD      RRGYQLIRFAMAAVSDYRTVIKAIKELARRLQSSNNTILHETLTTLLYRCSSIVFNRSHIPAIMSIS
GCN4            SSDPAALKRARNTEAARRSRARKLQRMKQ--LEDKVEELLSKNYHLENEVARLKKLVGER
CPC1      YPYHVGAPSDVVAMKRARNTLAARKSRERKAQRLEE--LEAKIEELIAERDRWKNLALAHGASTE
C\EBP     KAKSVDKNSNEYRVRRERNNIAVRKSRDKAKQRNVE--TQQKVLELTSDNDRLRKRVEQLSRELDTL
CREB      PTQPAEEAARKREVRLMKNREAARECRRKKKEYVKC--LENRVAVLENQNKTLIEELKALKDLYCHK
HBP1            ERELKKQKRLSNRESARRSRLRKQAECEE--LGQRAEALKSENSSLRIELDRIKKEYEEL
Cys-3           RLAAEEDKRKRNTAASARFRIKKKQREQA--LEKSAKEMSEKVTQLEGRIQALETENKYL
CRE-BP1         PDEKRRKFLERNRAAASRCRQKRKVWVQS--LEKKAEDLSSLNGQLQSEVTLLRNEVAQL
ATF1      QTTKTDDPQLKREIRLMKNREA-RECRRKKKEYVKC--LENRVAVLENQNKTLIEELKTLKDLYSNK
ATF2      RRAANEDPDEKRRKVLERNRAAASRCRQKRKVWVQS--LEKKAEDLSSLNGQLQSEVTLLRNEVAKL
BZLF         LEECDSELEIKRYKNRVASRKCRAKFKQLLQH--YREVAAAKSSENQRLLLLKQMSPSLDVDS
DBP       VQVPEEQKDEKYWSRRYKNNEAAKRSRDARRLKENQ--ISVRAAFLEKENALLRQEVVAVQELSHYR
E4BP4     EFIPDEKKDAMYWEKRRKNNEAAKRSREKRRLNDLV--LENKLIALGEENATLKAELLSLKLKFGLI
MET4      GFEKKQLIKKELGDDDEDLLIQSKKSHQKKKLKEKE--LESSIHELTEIAASLQKRIHTLETENKLL
JUN             SQERIKAERKRMRNRIAASKCRKRKLERIAR--LEEKVDTLKAQNSELASTANMLTEQVAQL
FOS             EERRRIRRIRRERNKMAAAKCRNRRRELTDT--LQAETDQLEDKKSALQTEIANLLKEKEKL
YAP1            DLDPETKQKRTAQNRAAQRAFRERKERKMKE--LEKKVQSLESIQQQNEVEATFLRDQLITL
TGA1            SKPVEKVLRRLAQNREAARKSRLRKKAYVQQ--LENSKLKLIQLEQELERARKQGMCVGGGV
Opaque2         MPTEERVRKRKESNRESARRSRYRKAAHLKE--LEDQVAQLKAENSCLLRRIAALNQKYNKA
```

FIGURE 6.—Comparison of *bimDb* ZIP domain to known bZIP proteins. The bZIP region of *bimD* is alligned here with members of the bZIP family of proteins. Leucine residues are indicated above the heptad leucine repeats. The proteins shown are C\EBP (LANDSHULTZ, JOHNSON and MCKNIGHT 1988), GCN4, JUN, FOS (LANDSHULTZ *et al.* 1988), CREB (GONZALEZ *et al.* 1989), HBP1 (GUILTINAN, MARCOTTE and QUATRANO 1990), Cys-3 (FU and MARZLUF 1990), CRE-BP1 (MAEKAWA *et al.* 1989), CPC1 (PALUH *et al.* 1988), ATF1, ATF2 (HAI *et al.* 1989), BZLF (FARRELL *et al.* 1989), DBP (MUELLER, MAIRE and SCHIBLER 1990), E4BP4 (COWELL, SKINNER and HURST 1992), MET4 (THOMAS, JACQUEMIN and SURDIN-KERJAN 1992), YAP1 (MOYE-ROWLEY, HARSHMAN and PARKER 1989), Opaque2 (TROPSHA *et al.* 1991).

function in the cell. The mitotic defect at restrictive temperature suggested that BIMD might positively regulate the expression of components required for mitosis. In the absence of functional BIMD at restrictive temperature, these components might not be made, resulting in a defective mitosis. If this were the case, then overexpression of the BIMD might induce mitotic events in the cell. To test this hypothesis, the full-length *bimD* cDNA was placed under the control of the regulatable *alcA* promoter in the vector pAL3 (see MATERIALS AND METHODS for details of construction) (WARING, MAY and MORRIS 1989). This *alcA* driven *bimD* cDNA was transformed into a wild- type strain of *A. nidulans* and the effects of overexpression were determined by testing primary transformants on replicate plates containing either ethanol as an inducer of *alcA*, or glucose as a repressor for *alcA*. All transformants tested were inhibited for growth on ethanol relative to their growth on glucose. Thus it appeared that overexpression of BIMD might have an effect on cell growth or viability. Southern analysis of these transformants showed that inhibition of growth correlated with copy number of the integrated plasmid. A strain with a single copy of the plasmid was less inhibited for growth on ethanol than a strain with five copies. Microsopic analysis of the strain which had integrated five copies of the plasmid showed that germination in ethanol led to cells with a single nucleus (Figure 7, white bar, 14-hr growth) while a control strain had undergone one nuclear division in the same time (black bar, 14-hr growth). However, the nuclei in these arrested cells did not appear mitotic, but rather appeared interphase (not shown). Thus, overexpression of BIMD did not induce mitotic events, but rather produced a block in interphase.

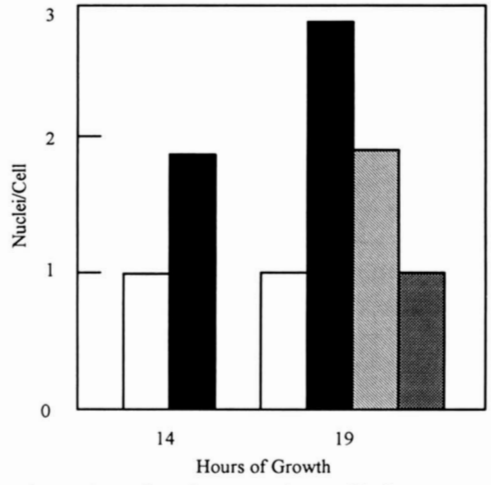

FIGURE 7.—The cell cycle arrest due to *bimD* overexpression is reversible and occurs in G_1 or S. A BIMD overexpressing strain, GR5::pAL3bimD.10 (white bar, hatched bars) and a control transformant, GR5::ppyrG.1 (black bar) were germinated for 14 hr in ethanol (inducing) medium and samples taken for microscopic determination of nuclear number. At this time cells were maintained in ethanol for an additional 5 hr (white bar and black bar, 19 hr) or transferred to glucose (repressing) medium (bar hatched with single line) or glucose medium and 90 mM hydroxyurea (bar hatched with double lines) for an additional 5 hr of growth (19 hr total) samples were taken for analysis. Values shown are an average of 100 cells counted for each condition.

As the cell cycle arrest by overexpression of BIMD could have resulted from a toxicity of the protein at high levels and not represent a specific function of BIMD, we next examined whether cells that had been arrested by BIMD overexpression could recover from the block. If the cells recovered from the block, we also wanted to determine at what stage in the cell cycle the arrest occurred. Cells blocked by overexpression were washed into repressing medium in the

presence and absence of hydroxyurea, an inhibitor of DNA synthesis that arrests cells in S phase (BERGEN, UPSHALL and MORRIS 1984). If the cells arrested by overexpression of BIMD were in G_1 or S then those cells washed into repressing medium with hydroxyurea would remain uninucleate. In contrast, if these cells had arrested in G_2, they would undergo a single nuclear division and arrest as binucleate cells. Cells that had been washed into medium lacking hydroxyurea act as controls to demonstrate that the block caused by overexpression of BIMD was reversible and therefore less likely to be a nonspecific effect. We observed that cells arrested for the nuclear division cycle by BIMD overexpression were viable when washed into repressing medium and able to undergo nuclear division in the absence of hydroxyurea (bar hatched with single lines, 19-hr growth). In contrast, cells washed into repressing medium with hydroxyurea did not undergo nuclear division, suggesting that they had been blocked in either G_1 or S (bar hatched with double lines, 19-hr growth). Control cells maintained in ethanol had an average of three nuclei per cell at this time (black bar, 19-hr growth) and *bimD* overexpressing cells maintained in ethanol still had not divided at this time (white bar, 19-hr growth). Thus, the cell cycle arrest caused by BIMD overexpression is less likely to be a nonspecific, toxic effect on the cell, as it is fully reversible. Furthermore, the growth arrest of the cell is at a specific point in the cell cycle, as cells are blocked uniformly in either G_1 or S and not randomly throughout the cell cycle as indicated by the release into hydroxyurea experiments.

The plasmid, pAL3bimD, used in our BIMD overexpression experiments carried the *pyr-4* gene of *N. crassa* as a selectable, nutritional marker, whereas our control in this experiment was transformed with the *pyrG* gene of *A. nidulans*. We examined the possibility that the nuclear division arrest of the pAL3bimD transformant when grown in inducing medium (minimal medium with ethanol) was due to the reduced efficiency of the *pyr-4* gene in complementing the *pyrG89* mutation relative to the *pyrG* gene. We tested this by germination of the pAL3bimD and *pyrG* transformants in repressing medium (minimal medium plus acetate). In this control experiment, the pAL3bimD transformant and the *pyrG* transformant underwent nuclear division at the same time and grew equally well (data not shown). Thus, the nuclear division arrest seen when the pAL3bimD transformant is germinated in ethanol medium is specific to the induction of BIMD overexpression.

Germination of the pAL3bimD transformant in repressing medium followed by transfer to inducing medium does not result in a cell cycle arrest although the strain is slowed in growth relative to a strain which is not transferred from inducing medium. The lack of a cell cycle arrest in this experiment may be due to the fact that a large amount of the BIMD protein has to accumulate in the G_1 arrested conidia in order to achieve the cell cycle block.

DISCUSSION

Our early models for BIMD function predicted that BIMD was a structural protein, possibly a kinetochore protein or a component of the mitotic spindle required to stabilize kinetochore microtubules. As determined by electron microscopy, the apparent absense of kinetochore microtubules from the mitotic spindle in the mutant supported this early conclusion (OAKLEY 1981). Anti-tubulin immunofluorescence and nuclear staining with DAPI could support this conclusion also, as in the mutant the chromatin is distributed randomly along the spindle rather than being separated into two discrete masses as in a wild-type mitosis (Figures 2 and 3). The *bimD6* mitotic block occurs during anaphase and is characterized by incomplete chromatin separation and a mitotic spindle which does not elongate to the degree seen in a normal telophase. However, some spindle elongation does occur, as shorter, metaphase spindles are also seen at restrictive temperature. This suggests that kinetochore microtubule depolymerization during anaphase A is not required for anaphase B spindle elongation, as the kinetochore microtubules are absent from the spindle in the mutant. Rather, a checkpoint may exist in the cell which prevents further spindle elongation, anaphase B, in the absence of complete anaphase A separation of chromatin, which normally occurs by kinetochore microtubule shortening.

The presence of a putative DNA-binding motif could also indicate a role for BIMD as a kinetochore protein. This would explain the defect in chromatin separation of *bimD6* mutants. The centromere binding protein CBF1 contains a helix-loop-helix motif (CAI and DAVIS 1990). Other centromere binding proteins may contain other DNA-binding motifs. The presense in BIMD, however, of an acidic region found in some transcriptional activators in addition to a bZIP motif suggests a regulatory role in the cell (WHITE *et al.* 1992). The results from our BIMD overexpression experiments would seem to support this conclusion. Expression of a gene out of context has been used in a number of systems to examine the consequences to cellular proliferation and development. For example, overexpression of the *nimA* gene of *A. nidulans* causes premature entry into mitosis (OSMANI, PU and MORRIS 1988) and in *S. pombe* overexpression of p53 blocks growth (BISCHOFF, CASSO and BEACH 1992). We reasoned that if BIMD acts as a regulator of gene expression, the overexpression of the protein in a wild type cell may produce a specific effect on mitosis or cell

cycle progression. If BIMD acts to positively regulate the cell cycle by regulating expression of genes required for mitosis, then overexpression may induce mitotic like events. The fact, however, that overexpression results in a cell cycle arrest in G_1 or S suggests that BIMD may act as a negative regulator of cell cycle progression. BIMD may act to negatively regulate components required for mitosis, and overexpression may prevent the production of these components and therefore prevents mitosis from occurring. Alternatively, *bimD* may be a bifunctional protein, carrying out one function in S phase, regulatory for example and another, perhaps structural role in mitosis. For example, the CBF1 protein of *S. cerevisiae* acts as a centromere binding protein and may act as a transcriptional regulator required for methionine prototrophy (CAI and DAVIS 1990).

Although we cannot rule out the possibility that the arrest due to BIMD overexpression is an effect unrelated to BIMD function, the reversibility and specificity of the block argues against this possibility. We also cannot be certain from the sequence similarities to bZIP proteins that BIMD is truly a member of this class of proteins. Additional experiments will be necessary to determine whether BIMD is a bZIP protein. However, we mention these elements because they may be important and because the presence of these sequence elements has guided us in our models for BIMD function and permitted us to develop testable hypotheses.

The sensitivity of *bimD6* mutants to DNA damaging agents suggests that BIMD could regulate the transcription of genes required for DNA repair. One such gene has been identified in *S. cerevisiae*, encoding the photolyase regulatory protein (PRP), which mediates the transcriptional induction of the DNA repair gene *PHR1* in response to DNA damage (SEBASTIAN and SANCAR 1991). Alternatively, BIMD may itself play a more direct role in the repair of DNA damage. The increased sensitivity of *bimD6* strains to DNA-damaging agents is not seen in another of the *bim* mutants tested, *bimB3*, and is therefore not likely to be a general phenotype of *bim* mutants. Conversely, no mutagen sensitive *uvs* or *mus* alleles exhibit a conditional mitotic phenotype (KÄFER and MAYOR 1986; ZHAO and KÄFER 1992). It is likely that the sensitivity of *bimD6* mutant strains to DNA-damaging agents at temperatures permissive for the mitotic defect is the result of partial loss of *bimD* function of the mutant gene product at permissive temperature and the mitotic defect seen at restrictive temperature the result of a complete loss of *bimD* function. Evidence for this is that MMS treatment of *bimD6* cells results in a catastrophic mitosis similar to that seen at restrictive temperature (data not shown). *bimD* function may therefore be required at S phase to carry out a role related to DNA metabolism, but the absense of its function may not be seen until M phase, when an abnormal mitosis occurs. The absence of kinetochore microtubules in this case could be due to a failure to assemble a kinetochore structure if DNA damage persists. The *bimD* gene product does not appear to have a checkpoint function, responding to DNA damage like the RAD9 gene product of *S. cerevisiae*, as *bimD6* mutant cells, like wild-type Aspergillus cells, show a cell cycle delay after DNA damage (data not shown).

The *bimD* gene product is a novel component of the cell involved in mitotic progression. In addition, its role seems to involve mitotic progression and repair of DNA damage. The relationships between these two events are not completely understood. Further understanding the role of *bimD* in the cell should enhance our understanding of these processes.

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LITERATURE CITED

ARION, D., L. MEIJER, L. BRIZUELA and D. BEACH, 1988 cdc2 is a component of the M phase-specific histone H1 kinase: evidence for identity with MPF. Cell **55:** 371–378.

BARGONETTI, J., P. N. FRIEDMAN, S. E. KERN, B. VOGELSTEIN and C. PRIVES, 1991 Wild-type but not mutant p53 immunopurified proteins bind to sequences adjacent to the SV40 origin of replication. Cell **65:** 1083–1091.

BELENGUER, P., F. M. CAIZERGUES, J. C. LABBE, M. DOREE and F. AMALRIC, 1990 Mitosis-specific phosphorylation of nucleolin by p34cdc2 protein kinase. Mol. Cell. Biol. **10:** 3607–3618.

BERGEN, L. G., A. UPSHALL and N. R. MORRIS, 1984 S-phase, G2, and nuclear division mutants of Aspergillus nidulans. J. Bacteriol. **159:** 114–119.

BISCHOFF, J. R., D. CASSO and D. BEACH, 1992 Human p53 inhibits growth in *Schizosaccharomyces pombe*. Mol. Cell. Biol. **12:** 1405–1411.

BRODY, H., J. GRIFFITH, A. J. CUTICCHIA, J. ARNOLD and W. E. TIMBERLAKE, 1991 Chromosome-specific recombinant DNA libraries from the fungus *Aspergillus nidulans*. Nucleic Acids Res. **19:** 3105–3109..

CAI, M., and R. W. DAVIS, 1990 Yeast centromere binding protein CBF1, of the Helix-Loop-Helix protein family, is required for chromosome stability and methionine prototrophy. Cell **61:** 437–446.

CLARK, K., M. OHTSUBO, T. NISHIMOTO, M. GOEBL and G. SPRAGUE, 1991 The yeast SRM1 protein and human RCC1 protein share analogous functions. Cell Regul. **2:** 781–792.

COVE, D. J., 1977 The genetics of *Aspergillus nidulans*, pp. 81–95 in *The Genetics and Physiology of Aspergillus nidulans*, edited by J. E. SMITH and J. A. PATEMAN. Academic Press, London.

COWELL, I. G., A. SKINNER and H. C. HURST, 1992 E4B4: transcriptional repression by a novel member of the bZIP family of transcription factors. Mol. Cell. Biol. **12:** 3070–3077.

DOONAN, J. H., and N. R. MORRIS, 1989 The *bimG* gene of *Aspergillus nidulans*, required for completion of anaphase, encodes a homolog of mammalian phosphoprotein phosphatase 1. Cell **57:** 987–996.

EHINGER, A., S. H. DENISON and G. S. MAY, 1990 Sequence, organization and expression of the core histone genes of *Aspergillus nidulans*. Mol. Gen. Genet. **222:** 416–424.

ENGLE, D. B., S. A. OSMANI, A. H. OSMANI, S. ROSBOROUGH, X. XIANG and N. R. MORRIS, 1990 A negative regulator of mitosis in Aspergillus is putative membrane spanning protein. J. Biol. Chem. **265:** 16132–16137.

ENOCH, T., A. M. CARR and P. NURSE, 1992 Fission yeast genes involved in coupling mitosis to DNA completion of DNA replication. Genes Dev. **6:** 2035–2046.

ENOCH, T., and P. NURSE, 1990 Mutation of fission yeast cell cycle control genes abolishes dependence of mitosis on DNA replication. Cell **60:** 665–673.

ENOCH, T., P. MATTHIAS, P. NURSE and E. A. HIGG, 1991 $p34^{cdc2}$ acts as a lamin kinase is fission yeast. J. Cell Biol. **112:** 797–807.

ENOS, A. P., and N. R. MORRIS, 1990 Mutation of a gene that encodes a kinesin-like protein blocks nuclear division in *A. nidulans*. Cell **60:** 1019–1027.

FARMER, G., J. BARGONETTI, H. ZHU, P. FRIEDMAN, R. PRYMER and C. PRIVES, 1992 Wild-type p53 activates transcription *in vitro*. Nature **358:** 83–86.

FARRELL, P. J., D. T. ROWE, C. M. ROONEY and T. KOUZARIDES, 1989 Epstein-Barr virus BZLF1 trans-activator specifically binds to a consensus AP-1 site and is related to *c-fos*. EMBO J. **8:** 127–132.

FIELDS, S., and S. K. JANG, 1990 Presence of a potent transcription activating sequence in the p53 protein. Science **249:** 1046–1049.

FU, Y.-H., and G. A. MARZLUF, 1990 *cys-3* the positive-acting sulfur regulatory gene of *Neurospora crassa*, encodes a sequence-specific DNA-binding protein. J. Biol. Chem. **265:** 11942–11947.

GONZALEZ, G. A., K. K. YAMAMOTO, W. H. FISCHER, D. KARR, P. MANZEL, W. BIGGS, W. W. VALE and M. R. MONTIMINY, 1989 A cluster of phosphorylation sites on the c-AMP regulated nuclear factor CREB predicted from its sequence. Nature **337:** 749–752.

GUILTINAN, M. J., W. R. MARCOTTE and R. S. QUATRANO, 1990 A plant leucine zipper protein that recognizes an abscisic acid response element. Science **250:** 267–271.

HAI, T., F. LIU, W. J. COUKOS and M. R. GREEN, 1989 Transcription factor ATF1 clones: an extensive family of leucine zipper proteins able to selectively form DNA-binding heterodimers. Genes Dev. **3:** 2083–2090.

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

HOYT, M. A., L. TOTIS and B. T. ROBERTS, 1991 *S. cerevisiae* genes required for cell cycle arrest in response to loss of microtubule function. Cell **66:** 507–517.

KÄFER E., 1977 Meiotic and mitotic recombination in *Aspergillus* and its chromosomal aberrations. Adv. Genet. **19:** A1-A23.

KÄFER, E., and O. MAYOR, 1986 Genetic analysis of DNA repair in *Aspergillus*: evidence for different types of MMS-sensitive hyperrec mutants. Mutat. Res. **161:** 119–134.

KASTAN, M. B., O. ONYEKWERE, D. SIDRANSKY, B. VOGELSTEIN and R. W. CRAIG, 1991 Participation of p53 protein in the cellular response to DNA damage. Cancer Res. **51:** 6304–6311.

KERN, S. E., K. W. KINZLER, A. BRUSKIN, D. JAROSZ, P. FRIEDMAN, C. PRIVES and B. VOGELSTEIN, 1991 Identification of p53 as a sequence-specific DNA-binding protein. Science **252:** 1708–1711.

LANDSHULZ, W. H., P. F. JOHNSON and S. L. MCKNIGHT, 1988 The leucine zipper: a hypothetical structure common to a new class of DNA binding proteins. Science **240:** 1759–1764.

LANDSHULZ, W. H., P. F. JOHNSON, E. Y. ASASHI, B. J. GRAVES and S. L. MCKNIGHT, 1988 Isolation of a recombinant copy of the gene encoding C/EBP. Genes Dev. **2:** 786–800.

LI, R., and A. W. MURRAY, 1991 Feedback control of mitosis in budding yeast. Cell **66:** 519–531.

LUNDGREN, K., N. WALNORTH, R. BOOHER, M. DEMBSKI, M. KIRSCHNER and D. BEACH, 1991 *mik1* and *wee1* cooperate in the inhibitory tyrosine phosphorylation of cdc2. Cell **64:** 1111–1122.

MACNEILL, S. A., J. CREANER and P. NURSE, 1991 Isolation, characterization and molecular cloning of new mutant alleles of the fission yeast $p34^{cdc2+}$ protein kinase gene: identification of new temperature sensitive G2-arresting alleles. Mol. Gen. Genet. **229:** 109–118.

MAEKAWA, T., H. SAKURA, C. KANEI-ISHII, T. SUDO, T. YOSHIMURA, J. FUJISAWA, M. YOSHIDA and S. ISHII, 1989 Leucine zipper structure of the protein CRE-BP1 binding to the cyclic AMP response element in brain. EMBO J. **8:** 2023–2028.

MATSUMOTO, T., and D. BEACH, 1991 Premature initiation of mitosis in yeast lacking RCC1 or an interacting GTPase. Cell **66:** 347–360.

MAY, G. S., 1989 The highly divergent β-tubulins of *Aspergillus nidulans* are functionally interchangeable. J. Cell Biol. **109:** 2267–2274.

MAY, G. S., M.-L. S TSANG, H. SMITH, S. FIDEL and N. R. MORRIS, 1987 *Aspergillus nidulans* β-tubulins are unusually divergent. Gene **55:** 231–243.

MAY, G. S., C. A. MCGOLDRICK, C. L. HOLT and S. H. DENISON, 1992 The *bimB3* mutation of *Aspergillus nidulans* uncouples DNA replication from the completion of mitosis. J. Biol. Chem. **267:** 15737–15743.

MILLER, B. L., K. Y. MILLER and W. E. TIMBERLAKE, 1985 Direct and indirect gene replacements in *Aspergillus nidulans*. Mol. Cell. Biol. **5:** 1714–1721.

MORRIS, N. R., 1976 Mitotic mutants of *Aspergillus nidulans*. Genet. Res. **26:** 237–254.

MOYE-ROWLEY, W. S., K. P. HARSHMAN and C. S. PARKER, 1989 Yeast YAP1 encodes a novel form of the jun family of transcriptional activator proteins. Genes Dev. **3:** 283–292.

MUELLER, C. R., P. MAIRE and U. SCHIBLER, 1990 DBP, a liver-enriched transcriptional activator, is expressed late in ontogeny and its tissue specificity is determined posttranscriptionally. Cell **61:** 279–291.

NIGRO, J. M., R. SIKORSKI, S. I. REED and B. VOGELSTEIN, 1992 Human p53 and CDC2Hs genes combine to inhibit the proliferation of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **12:** 1357–1365.

NISHIMOTO, T., E. EILEN and C. BASILICO, 1978 Premature chromosome condensation in a ts DNA mutant of BHK cells. Cell **15:** 475–483.

NISSEN, M. S., T. A. LANGAN and R. REEVES, 1991 Phosphorylation by *cdc2* kinase modulates DNA binding activity of high mobility group 1 nonhiston chromatin protein. J. Biol. Chem. **266:** 19945–19952.

OAKLEY, B. R., 1981 Mitotic Mutants, pp. 181–196 in *Mitosis and Cytokinesis*, edited by A. M. ZIMMERMAN. Academic Press, New York.

O'CONNELL, M. J., A. H. OSMANI, N. R. MORRIS and S. A. OSMANI, 1992 An extra copy of $nimE^{cyclin\ B}$ elevates pre-MPF levels and partially suppresses mutation of $nimT^{cdc25}$ in *Aspergillus nidulans*. EMBO J. **11:** 2139–2149.

O'DONNELL, K. L., A. H. OSMANI, S. A. OSMANI and N. R. MORRIS, 1991 *bimA* encodes a member of the tetratricopeptide repeat family of proteins and is required for the completion of mitosis in *Aspergillus nidulans*. J. Cell Sci. **99:** 711–719.

OSMANI, S. A., G. S. MAY and N. R. MORRIS, 1987 Regulation of the mRNA levels of *nimA*, a gene required for the G2-M transition in Aspergillus nidulans. J. Cell Biol. **104:** 1495–1504.

OSMANI, S. A., R. T. PU and R. N. MORRIS, 1988 Mitotic induc-

tion and maintenance by overexpression of a G2-specific gene that encodes a potential protein kinase. Cell **53:** 237–244.

OSMANI, S. A., D. B. ENGLE, J. H. DOONAN and N. R. MORRIS, 1988 Spindle formation and chromatin condensation in cells blocked at interphase by mutation of a negative cell cycle control gene. Cell **52:** 241–251.

PALUH, J. L., M. J. ORBACH, T. L. LEGERTON and C. YANOFSKY, 1988 The cross pathway control gene of *Neurospora crassa*, *cpc-1* encodes a protein similar to GCN4 of yeast and the DNA-binding domain of the oncogene v-*jun*-encoded protein. Proc. Natl. Acad. Sci. USA **85:** 3728–3732.

PETER, M., J. NAKAGAWA, M. DOREE, J. C. LABBE and E. A. NIGG, 1990 *In vitro* disassembly of the nuclear lamina and M-phase-specific phosphorylation of lamins by *cdc2* kinase. Cell **61:** 591–602.

PETER, M., E. HEITLINGER, M. HÄNER, U. AEBI and E. A. NIGG, 1991 Disassembly of *in vitro* formed lamin head-to-tail polymers by CDC2 kinase. EMBO J. **10:** 1535–1544.

PONTECORVO, G., J. A. ROPER, C. M. HEMMONS, K. D. MACDONALD and A. W. J. BUFTON, 1953 The Genetics of *Aspergillus nidulans*. Adv. Genet. **5:** 141–238.

RAYCROFT, L., H. WU and G. LOZANO, 1990 Transcriptional activation by wild-type but not transforming mutants of the p53 anti-oncogene. Science **249:** 1049–1051.

ROWLEY, R., J. HUDSON and P. G. YOUNG, 1992 The *wee1* protein kinase is required for radiation-induced mitotic delay. Nature **356:** 353–355.

RUSSEL, P., and P. NURSE, 1986 cdc25$^+$ functions as an inducer of the mitotic control of fission yeast. Cell **45:** 145–153.

RUSSEL, P., and P. NURSE, 1987 Negative Regulation of of mitosis by *wee1*$^+$, a gene encoding a protein kinase homolog. Cell **49:** 559–567.

SEBASTIAN, J., and G. B. SANCAR, 1991 A damage-responsive DNA binding protein regulates transcription of the yeast DNA repair gene *PHR1*. Proc. Natl. Acad. Sci. USA **88:** 11251–11255.

SCHIESTL, R. H., P. REYNOLDS, S. PRAKASH and L. PRAKASH, 1989 Cloning and sequence analysis of the *S. cerivisiae RAD9* gene and further evidence that its product is required for cell cycle arrest induced by DNA damage. Mol. Cell. Biol. **9:** 1882–1896.

THOMAS, D., I. JACQUEMIN and Y. SURDIN-KERJAN, 1992 *MET4*: a leucine zipper protein and centromere-binding factor 1 are both required for transcriptional activation of sulfur metabolism in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **12:** 1719–1727.

TROPSHA, A., P. J. BOWEN, F. K. BROWN and J. S. KIZER, 1991 Do interhelical side chain-backbone hydrogen bonds participate in formation of leucine zipper coiled coils? Proc. Natl. Acad. Sci. USA **88:** 9488–9492.

VOLLMER, S. J., and C. YANOFSKY, 1986 Efficient cloning of genes of *Neurospora crassa*. Proc. Natl. Acad. Sci. USA **83:** 4869–4873.

WARING, R. B., G. S. MAY and N. R. MORRIS, 1989 Characterization of an inducible expression system in *Aspergillus nidulans* using *alcA* and tubulin-coding genes. Gene **79:** 119–130.

WEINERT, T. A., and L. H. HARTWELL, 1988 The *RAD9* gene controls the cell cycle response to DNA damage in *Saccharomyces cerevisiae*. Science **241:** 317–322.

WHITE, J., C. BROU, J. WU, Y. LUTZ, V. MONCOLLIN and P. CHAMBON, 1992 The acidic transcriptional activator GAL-VP16 acts on preformed template-committed complexes. EMBO J. **11:** 2229–2240.

YAMAKITA, Y., S. YAMASHIRO and F. MATSUMURA, 1992 Characterization of mitotically phophorylated caldesmon. J. Biol. Chem. **267:** 12022–12029.

ZHAO, P., and E. KÄFER, 1992 Effects of mutagen-sensitive *mus* mutations on spontaneous mitotic recombination in *Aspergillus*. Genetics **130:** 717–728.

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