MOLECULAR AND CELLULAR BIOLOGY, July 1995, p. 3685–3696 Vol. 15, No. 7
0270-7306/95/$04.00+0

Bdf1, a Yeast Chromosomal Protein Required for Sporulation

PENELOPE CHUA AND G. SHIRLEEN ROEDER*

Department of Biology, Yale University, New Haven, Connecticut 06520-8103

Received 13 December 1994/Returned for modification 6 February 1995/Accepted 6 April 1995

The *BDF1* gene of *Saccharomyces cerevisiae* is required for sporulation. Under starvation conditions, most cells from the *bdf1* null mutant fail to undergo one or both meiotic divisions, and there is an absolute defect in spore formation. The Bdf1 protein localizes to the nucleus throughout all stages of the mitotic and meiotic cell cycles. Analysis of spread meiotic nuclei reveals that the Bdf1 protein is localized fairly uniformly along chromosomes, except that it is excluded specifically from the nucleolus. A *bdf1* null mutant displays a reduced rate of vegetative growth and sensitivity to a DNA-damaging agent. The *BDF1* gene encodes a 77-kDa protein that contains two bromodomains, sequence motifs of unknown function. Separation-of-function alleles suggest that only one of the two bromodomains is required for sporulation, whereas both are required for Bdf1 function in vegetative cells. We propose that the Bdf1 protein is a component of chromatin and that the mitotic and meiotic defects of the *bdf1* null mutant result from alterations in chromatin structure.

Diploid eukaryotic organisms can undergo two different types of cell division, mitosis and meiosis. During mitosis and the second division of meiosis, sister chromatids separate and segregate, while homologous chromosomes behave independently. In contrast, during the first division of meiosis, homologous chromosomes pair and recombine with each other and then segregate to opposite poles.

Although many of the proteins required for a successful meiotic cell cycle are synthesized specifically during meiosis, proteins present in both vegetative and meiotic cells play important roles as well (65). Some proteins serve identical functions during the two types of cell division, but others act differentially. For example, several *CDC* gene products of *Saccharomyces cerevisiae* are required for the G_1-to-S transition in the mitotic cell cycle, but some of these act after DNA replication during meiosis (64). Still other gene products serve functions of different importance in the two types of cell cycle. For instance, genes in the yeast *RAD50* series are required for recombinational repair of DNA damage but are otherwise dispensable for vegetative growth (17). However, these genes are essential for the generation of viable meiotic products, because recombination is required for reductional chromosome segregation (53).

Chromosomes undergo a dramatic reorganization during prophase I of meiosis I, as homologous chromosomes align with each other to form an elaborate proteinaceous structure called the synaptonemal complex (75). The complex consists of two lateral elements and an intervening central region; each lateral element represents the protein backbone of one pair of condensed sister chromatids. The DNA is organized as a series of chromatin loops, each attached at its base to a lateral element. Not surprisingly, many of the structural components of the synaptonemal complex are synthesized only in meiotic cells (9, 27, 31, 44, 69). However, the synaptonemal complex is built upon preexisting mitotic chromosomes; consequently, the chromosomes of vegetative and meiotic cells must share many structural components. For example, topoisomerase II is a component of the mitotic chromosome scaffold (12, 19) and of meiotic chromosome cores (38, 45).

In this report, we demonstrate that the Bdf1 protein of *S. cerevisiae* is a novel component of mitotic and meiotic chromosomes. A unique aspect of the Bdf1 localization pattern is its exclusion specifically from the nucleolus. The *BDF1* gene product is absolutely required for progression through the meiotic cell cycle, and it is important, but not essential, for vegetative growth. We argue that the effects of the *bdf1* null mutation on cell cycle progression are indirect consequences of changes in chromatin structure.

MATERIALS AND METHODS

Screen for meiotic recombination mutants. A screen for mutants defective in meiotic recombination was performed as described by Menees and Roeder (43), starting with strain CY32A2 (Table 1). A mutant isolate (PC8) that displays a decreased level of meiotic recombination and is also sensitive to the DNA-damaging agent methyl methanesulfonate (MMS) was chosen for study. Further analysis revealed that the MMS-sensitive phenotype is due to a mutation in a single gene (*BDF1*) and does not cosegregate with the mutation(s) conferring the recombination defect.

Plasmids. *Escherichia coli* XL-1Blue (Stratagene) was used for all plasmid constructions. Plasmids were constructed by using standard procedures (60).

pCB10 is the original *bdf1*-complementing plasmid carrying approximately 15 kb of yeast DNA from a genomic library. The library was provided by Forrest Spencer and Philip Hieter and consists of *Sau*3A partial digest fragments of yeast DNA inserted at the *Bam*HI site of a YCp50 (51) derivative in which the *URA3* gene has been replaced by *LEU2*. pCB31 contains *BDF1* as a 4.6-kb *Eco*RI fragment cloned into the *Eco*RI site in pHSS6 (30). pCB45 is a transposon insertion mutant, derived from pCB31, in which the transposon is located 159 bp upstream from the 3′ end of the *BDF1* coding region. pCB74 was derived from pCB45 by deleting *BDF1* coding sequences between the *Bam*HI site in the gene and the *Bam*HI site in the transposon. The resulting plasmid contains sequences upstream and downstream of *BDF1* interrupted by the *LEU2* gene from the transposon, with almost all of the *BDF1* coding region and approximately half of the transposon removed. pCB74 was cut with *Not*I prior to transformation into yeast cells and used to construct *bdf1*::*LEU2* strains by one-step gene disruption (58).

pCB140 contains the 2.5-kb *Hin*dIII fragment containing the *HO* gene from pJH84 (34) at the *Hin*dIII site of pBS M13$^+$ (Stratagene). pCB140 was used to construct pCB154 in two steps. First, the approximately 450-bp *Eco*RV-*Eco*RI fragment consisting of the polylinker (which contains a *Pst*I site) together with 398 bp of sequences downstream of the *HO* gene was removed. Second, the 0.5-kb *Pst*I-*Bam*HI fragment containing *HO* coding sequences near the 5′ end of the gene was replaced with the 2.1-kb *Bam*HI-*Nsi*I fragment containing the *URA3* gene from YCp50 (51). pCB154 was used to disrupt the *HO* gene in one step (58) by targeting with *Bgl*II (which cuts 391 bp downstream of the *Bam*HI site) and *Hin*dIII and selecting for Ura$^+$ transformants.

pCB156 contains the *THR1* gene (61) on a 3.1-kb *Bam*HI fragment cloned into the *Bam*HI site in pBS M13$^+$. To construct the *thr1-Nco* allele, pCB156 was cut with *Nco*I, and the sticky ends were filled in and religated. The 3.1-kb *Bam*HI

* Corresponding author. Mailing address: Department of Biology, Yale University, P. O. Box 208103, New Haven, CT 06520-8103. Phone: (203) 432-3501. Fax: (203) 432-3263. Electronic mail address: shirleen_roeder@quickmail.yale.edu.

TABLE 1. Yeast strains used in this study[a]

Strain	Genotype
CY32A2	*MAT***a** *CRY1 CEN3(TRP1) leu2-3 his4-280 J10A*::*URA3 spo13*::*ADE2 trp1 ura3-1 ade2-1*
	*MAT*α *cry1 CEN3 leu2-3,112 his4-260*
PC8	CY32A2 but *bdf1-1 recX*
BR2171-7B	*MAT***a** *leu2 HO trp1-1 arg4-8 thr1-4 ura3-1 ade2-1*
	*MAT*α *leu2 HO trp1-1 arg4-8 thr1-4 ura3-1 ade2-1*
PC336	BR2171-7B but *bdf1*::*lacZ*
	BDF1
PC465a	*MAT***a** *leu2 HO trp1-1 arg4-8 thr1-4 ura3-1 ade2-1 bdf1*::*LEU2 mei4*::*URA3*
	*MAT*α *leu2 HO trp1-1 arg4-8 thr1-4 ura3-1 ade2-1 bdf1*::*LEU2 mei4*::*URA3*
PC543	*MAT***a** *leu2 his4-260 ho*::*ura3 trp1-1 arg4-8 thr1-Nco ura3-1 ade2-1 bdf1*::*LEU2 red1*::*lacZ*
	*MAT*α *leu2 his4-Hpa ho*::*ura3 trp1-1 arg4-8 thr1-4 ura3-1 ade2-1 BDF1 RED1*
PC531	PC543 but *bdf1*::*LEU2*
	bdf1::*LEU2*
PC608	*MAT***a** *leu2 his4-Hpa ho*::*ura3-1 trp1-1 spo13*::*URA3 arg4-8 thr1-Nco ura3-1 ade2-1 bdf1*::*LEU2*
	*MAT*α *leu2 HIS4 ho*::*ura3-1 trp1-1 spo13*::*URA3 arg4-8 thr1-4 ura3-1 ade2-1 BDF1*
PC606	PC608 but *bdf1*::*LEU2*
	bdf1::*LEU2*
PC621	*MAT***a** *leu2 HIS4 ho*::*ura3-1 trp1-1 arg4-8 thr1-4 ura3-1 ade2-1 bdf1*::*LEU2*
	*MAT*α *leu2 his4-260 ho*::*ura3-1 trp1-1 arg4-8 thr1-4 ura3-1 ade2-1 bdf1*::*LEU2*
PC622	PC621 but *bdf1*::*LEU2*
	BDF1
PC440	*MAT*α *leu2 ho*::*ura3-1 trp1-1 arg4-8 thr1-4 ura3-1 ade2-1*
PC469	PC440 but *bdf1*::*LEU2*
PC623	PC440 but *rad9*::*LEU2*
PC624	PC440 but *rad52*::*LEU2*

[a] BR2171-7B, PC336, PC465a, PC543, PC531, PC608, PC606, PC621, and PC622 are isogenic. PC440 is a haploid meiotic segregant of a diploid isogenic with BR2171-7B; PC469, PC623, and PC624 were derived from PC440 by transformation. The *bdf1*::*lacZ* fusion gene in PC336 was generated by transposon mutagenesis; *lacZ* is fused to codon 418 in the *BDF1* coding region.

fragment containing *thr1-Nco* was then inserted into the *Bam*HI site of YIp5 to create pCB162. The *thr1-Nco* allele was introduced into yeast by two-step gene replacement (58), using pCB162 targeted with *Sac*I.

R37 and R1259 are YIp5 derivatives containing the *his4-260* (10) and *his4-Hpa* (74) mutations. They were used to introduce the mutant *HIS4* alleles into yeast strains by two-step gene replacement (58).

pB69 contains a transposon insertion in the *RED1* gene in which the *lacZ* gene is fused in frame to *RED1* (56). The wild-type *RED1* gene was replaced by the *red1*::*lacZ* gene by transforming with pB69 cut with *Not*I and selecting for the *LEU2* gene in the transposon.

pCB152 contains *BDF1* coding sequences fused in frame to the glutathione *S*-transferase (GST) gene in the expression vector pGEX-KG (24). This plasmid was made in three steps. First, an *Eco*RI linker was inserted at the *Sna*BI site 3 nucleotides upstream of the start codon in the *BDF1* gene in pCB31. Second, the 3.1-kb *Eco*RI fragment containing the entire *BDF1* gene from pCB31 was inserted into the *Eco*RI site in pGEX-KG. Finally, the vector was cut with *Stu*I, which removes approximately two-thirds of the *BDF1* coding sequences (including the bromodomains) and religated. This process creates an in-frame fusion of the *GST* gene and the 5′ and 3′ regions of the *BDF1* coding region in pGEX-KG.

The *rad9*::*LEU2* mutation was introduced by transformation with pTWO13 (76). The *rad52*::*LEU2* mutation was introduced by transformation with pSM20 obtained from David Schild.

Yeast strains and genetic manipulations. Yeast strains and genotypes are listed in Table 1. BR2171-7B was obtained from Beth Rockmill (56). Yeast transformations were carried out by the lithium acetate procedure (33). For all experiments, cells were grown in YEPAD medium, which is YPD (63) supplemented with adenine.

All yeast strains used to characterize the *bdf1*::*LEU2* mutant were derived from the homothallic strain, BR2171-7B. Stable haploid derivatives were obtained by disrupting one copy of the *HO* gene in the diploid parent with pCB154 and then sporulating a transformant and dissecting tetrads. The haploid segregants were made Ura$^-$ by selection on medium containing 5-fluoro-orotic acid (63), and the *his4-260* and *his4-Hpa* alleles were then introduced. A *thr1-4* haploid derivative was transformed to Thr$^+$ with pCB156, and the *thr1-Nco* allele was then introduced. The *bdf1*::*LEU2* null mutation was introduced into yeast cells by one-step gene disruption (58), using pCB74 digested with *Not*I. To alleviate problems caused by the mitotic growth defect in *bdf1* mutants, *bdf1*::*LEU2* haploids were obtained by first transforming wild-type diploids and then sporulating the transformants and dissecting tetrads to obtain Leu$^+$ segregants. To construct *bdf1*::*LEU2* strains carrying the *red1*::*lacZ* or *mei4*::*URA3* mutation, a *MAT*α *bdf1*::*LEU2* haploid was crossed to *MAT***a** *red1*::*lacZ* haploid or a *MAT***a** *mei4*::*URA3* haploid, and the resulting diploids were sporulated. Tetrads were then dissected to obtain haploid segregants carrying both disruptions. Since the haploid parents are isogenic, all segregants from these crosses are isogenic except at the *BDF1*, *RED1*, and *MEI4* loci.

Transposon mutagenesis. pCB31 was subjected to transposon mutagenesis (30), using derivatives of the bacterial mTn*3* transposon containing *lacZ* coding sequences and either *LEU2* or *URA3*. The transposon insertions were mapped by restriction digest analysis and introduced into BR2171-7B (Table 1) by one-step gene replacement after digestion with *Not*I. The effect of each insertion was analyzed by sporulating the transformants and dissecting tetrads; cells from viable Leu$^+$ or Ura$^+$ (depending on the transposon) spore colonies were tested for MMS sensitivity and sporulation proficiency. To obtain a diploid heterozygous for a double mutation in the *BDF1* gene, BR2171-7B was transformed sequentially with a *URA3*-marked and a *LEU2*-marked transposon. Diploid transformants were then dissected to identify those in which both transposons had inserted into the same copy of the *BDF1* gene.

DNA sequencing and sequence analysis. Sequencing was carried out by using a Sequenase version 2.0 kit (United States Biochemical Corp., Cleveland, Ohio), using T7 polymerase on double-stranded DNA templates as specified by the manufacturer. A collection of different transposon insertion mutants in pCB31 was used to obtain sequence information, using primers complementary to the ends of the transposon. Sequences on both strands were determined by using transposons inserted in both orientations and positioned at approximately 200-bp intervals.

The computer software used for sequence management and analysis was Geneworks version 2.2 (IntelliGenetics Inc., Mountain View, Calif.). The PAM-250 scoring matrix was used to assess protein similarity. The method used to build a phylogenetic tree of bromodomains was the unweighted pair group method with arithmetic mean. All sequences were scored against one another, and the two most closely related sequences were aligned first. This and subsequent subalignments were scored against the remaining sequences, and the next-best pair (which might be two sequences, a subalignment and a sequence, or two subalignments) was removed from the list and aligned. This process was repeated until a single alignment of all sequences remained. Default parameters were used; the cost to open a gap was 5, and the cost to lengthen a gap was 25.

Anti-Bdf1 antibodies. The protein encoded by pCB152 was produced in *E. coli* XL-1Blue and purified as described by Smith and Johnson (66) and Guan and Dixon (24). The purified protein is a GST-Bdf1 fusion protein that includes the first 33 N-terminal amino acids of Bdf1 fused to the last 236 C-terminal amino acids of Bdf1. The GST-Bdf1 chimeric protein was used as an antigen to raise antibodies in rabbits, and the resulting antibodies were affinity purified (67). A 1:50 to 1:100 dilution of the purified antibodies was used in immunolocalization experiments.

Measurements of MMS sensitivity, growth rate, and sporulation. To measure sensitivity to MMS, saturated overnight cultures of PC440, PC469, PC623, and

PC624 were diluted 10-fold into YEPAD medium containing 0.004% MMS. After incubation with shaking at 30°C for 6 h, cells were washed twice, and then appropriate dilutions were plated on YEPAD.

To measure growth rates, saturated overnight cultures of PC621 and PC622 were diluted 125-fold into YEPAD and incubated with shaking at 30°C. The optical density at 600 nm was determined at regular intervals. Doubling times were calculated after cells had reached logarithmic growth phase.

To assess sporulation, the frequency of asci relative to total (sporulated and unsporulated) cells was determined with a light microscope. At least 100 cells were scored from each strain.

Measurements of recombination. To measure recombination, strains PC531, PC543, PC606, and PC608 were grown and sporulated as follows. Saturated cultures grown overnight in YEPAD were diluted in 3 volumes of YEPAD and grown at 30°C for 12 h with vigorous shaking. The cultures were then diluted 1:10 into 2% potassium acetate and incubated with shaking at 30°C.

To measure gene conversion, cultures of PC531 and PC543 were plated on synthetic complete medium and on medium lacking threonine or histidine to select for prototrophic recombinants. Colonies were counted after 3 days. Premeiotic frequencies were calculated as the median frequencies obtained from three independent cultures of each strain. To calculate the rate of meiotic recombination, the mitotic frequency was subtracted from the corresponding meiotic value for each culture, and the corrected frequencies from three independent cultures were then averaged.

To measure crossing over, cultures of strains PC606 and PC608 were incubated in sporulation medium for 2 days and then plated on medium lacking threonine to enrich for cells that had undergone commitment to meiotic recombination. The strains used for this experiment carry the *spo13* mutation, and so even wild-type cells that sporulate remain diploid and are therefore comparable to the diploid products of the unsporulated *bdf1* mutant. Approximately 800 Thr^+ recombinants from each culture were scored for homozygosis at *HIS4* and *MAT*; single and double crossovers were scored as follows. The His^- nonmating colonies were classified as single recombinants, and the number of these was doubled to account for *HIS4*/*HIS4 MAT***a**/*MAT*α cells. The His^+ α-mating class was counted as single recombinants, except that the number was reduced by the number of His^- **a**-mating colonies (reductional segregants), since there should be an equal number of nonrecombinant segregants of genotype *HIS4*/*HIS4 MAT*α/*MAT*α. Similarly, the His^+ **a**-mating colonies were classified as single recombinants except that the number was reduced by the number of His^- α-mating double recombinants, since there should be an equal number of *HIS4*/*HIS4 MAT***a**/*MAT***a** double recombinants. To obtain the total number of nonparental ditypes due to four-strand double crossovers, the number of His^- α-mating colonies was doubled to account for the reciprocal *HIS4*/*HIS4 MAT***a**/*MAT***a** recombinants. Map distance was calculated by summing the single crossovers and six times the number of nonparental ditypes and then dividing by the total number of colonies examined.

Cytology. To obtain spread chromosome preparations, strains were grown and sporulated as described above for meiotic recombination assays. Except where noted, meiotic cultures were harvested, and chromosomes were spread by the method of Dresser and Giroux (11) as modified by Engebrecht and Roeder (14). For examination by fluorescence microscopy, chromosomes spread on polylysine-coated slides were incubated with antibodies as described by Sym et al. (69). Mouse antinucleolus antibodies (78) were provided by Michael Snyder, and rabbit anti-β-galactosidase antibodies were purchased from Cappel Laboratories. Secondary antibodies were conjugated with either Texas red or fluorescein isothiocyanate. Chromatin was stained with 4′6-diamidino-2-phenylindole (DAPI).

Vegetative cells from late log-phase cultures were fixed for 1 h in 3.7% formaldehyde and prepared for immunocytology as described by Pringle et al. (55). To monitor the nuclear divisions, cultures of PC543 and PC531 grown overnight in YEPAD were diluted in 10 volumes of YEPAD and grown at 30°C for 12 h with vigorous shaking. The cultures were then diluted 1:10 into 2% potassium acetate and incubated with shaking at 30°C. Cells were stained with DAPI and examined in a fluorescence microscope as described by Thompson and Roeder (73).

Nucleotide sequence accession number. The *BDF1* DNA sequence is available from GenBank under accession number U18116.

RESULTS

Isolation, cloning, and mapping of *BDF1*. The *BDF1* gene was identified by a mutation conferring sensitivity to MMS (see Materials and Methods). The wild-type *BDF1* gene was cloned by transforming the *bdf1-1* mutant with a yeast genomic library and screening for complementation of the MMS-sensitive phenotype. A 4.6-kb *Eco*RI fragment with complementing activity was subjected to transposon mutagenesis in bacteria, and the resulting insertion mutations were transformed into yeast cells (30). By scoring the MMS sensitivity of cells carrying different transposon insertions, the gene was localized to a region of approximately 2.3 kb (Fig. 1).

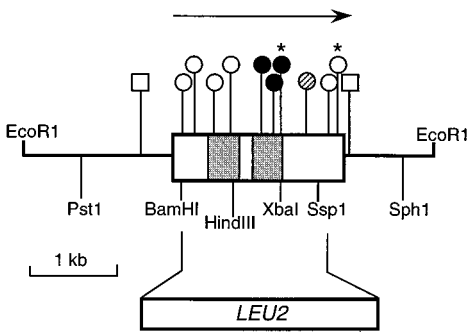


FIG. 1. The *BDF1* gene. The open reading frame is represented by the open box; the arrow above indicates the direction of transcription and translation. The shaded boxes indicate the two bromodomains. The region of the gene deleted and replaced with *LEU2* to make the *bdf1*::*LEU2* null mutation is indicated. The open circles indicate transposon insertions that confer an MMS-sensitive phenotype but do not impair sporulation (~85% in both wild-type and mutant strains). The squares indicate insertions that do not disrupt the *BDF1* gene. Insertion mutants in which sporulation is abolished (<1%) or reduced (~10%) are indicated by the black circles and hatched circles, respectively. Transposon insertions that create in-frame *lacZ* fusions are indicated by asterisks.

To establish the chromosomal position of the *BDF1* gene, radioactively labeled *BDF1* DNA was used to probe a blot of electrophoretically separated yeast chromosomes (8) as well as a blot of ordered phage clones of yeast genomic DNA (50). The gene is located on chromosome XII between *URA4* and *ILV5*. A partial sequence of the *BDF1* gene was obtained by Widner and Wickner (77) during sequencing of the *SKI2* gene, which maps 26 centimorgans centromere proximal of the *URA4* gene.

***BDF1* encodes a 77-kDa protein with two bromodomains.** The DNA sequence of the 2.3-kb region containing the *BDF1* gene is presented in Fig. 2. The sequence contains a single open reading frame of 2,058 bp that is predicted to encode a protein 685 amino acids in length with a molecular mass of 77 kDa (Fig. 2). There are no consensus splice sites within the gene, indicating that the mRNA is not spliced.

Analysis of the Bdf1 protein sequence revealed two internal regions, about 80 amino acids in length, that are 39% identical and 62% similar (Fig. 2). A search of protein databases identified numerous proteins containing regions of homology to the Bdf1 repeats. This protein sequence motif has been called a bromodomain (25), and its function remains unknown. The bromodomain exists in a single copy in some proteins but in two copies in several others. An evolutionary tree showing the sequence relationships between the Bdf1 bromodomains and those of other proteins is shown in Fig. 3.

In addition to the conserved bromodomain sequences, homology was also detected between the last 165 amino acids in Bdf1 and the carboxy termini of the *Drosophila* Fsh1 protein and the human OrfX and Ring3 proteins. The homology is strongest between Bdf1 and OrfX (Fig. 2). Whereas the *Drosophila* Fsh1 protein is homologous to the human OrfX and Ring3 proteins throughout their entire lengths, the homology between Bdf1 and Fsh1/OrfX/Ring3 is confined to the bromodomains and the last 165 amino acids.

The Bdf1 protein localizes to chromosomes. A GST-Bdf1 fusion protein was produced in and purified from bacteria (see Materials and Methods), and this hybrid protein was used as an antigen to raise antibodies in rabbits. The resulting antibodies were used to localize the Bdf1 protein in whole vegetative cells

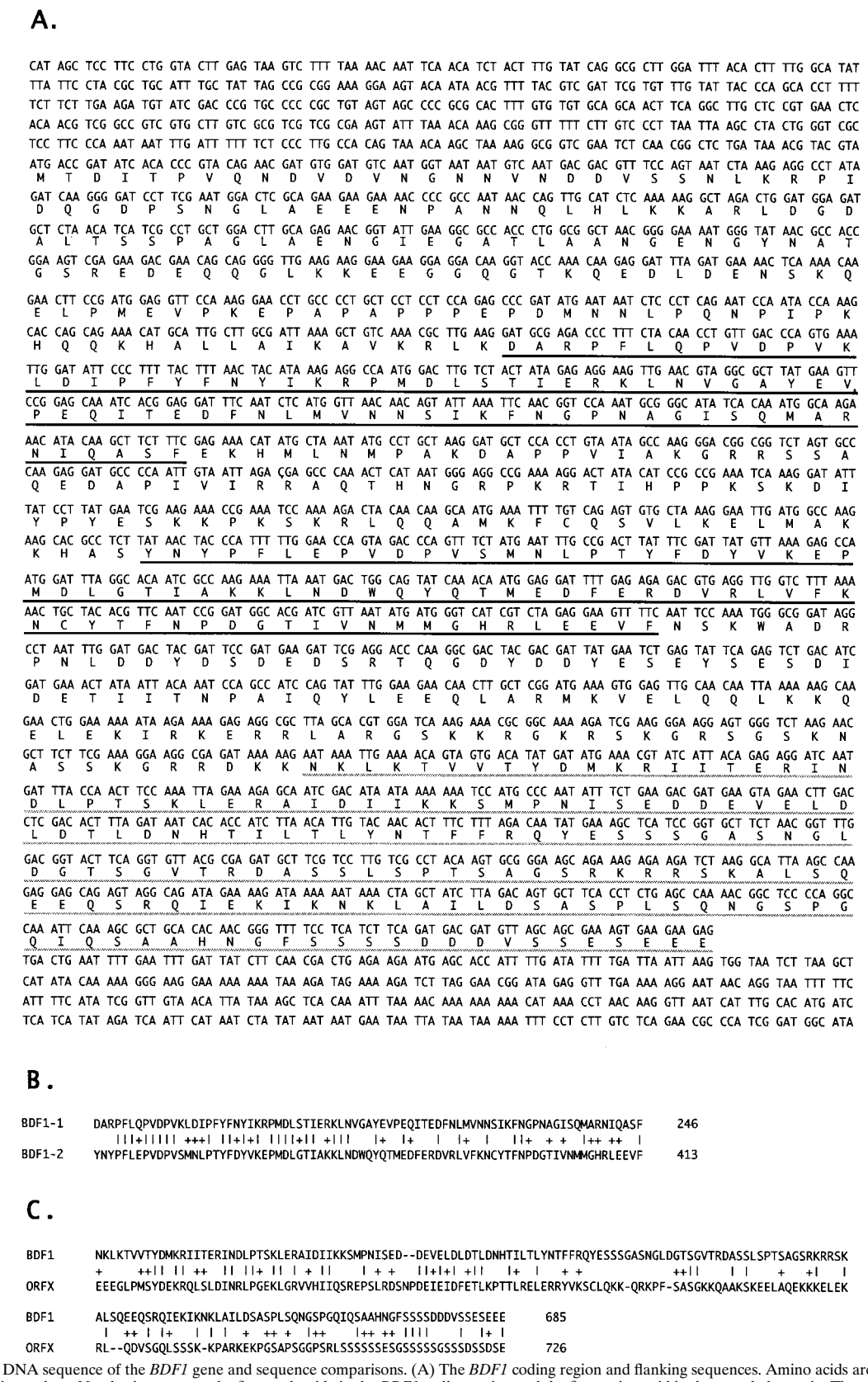


FIG. 2. DNA sequence of the *BDF1* gene and sequence comparisons. (A) The *BDF1* coding region and flanking sequences. Amino acids are indicated beneath the corresponding codons. Numbering starts at the first nucleotide in the *BDF1* coding region and the first amino acid in the encoded protein. The solid underlines indicate the two bromodomains; the additional region of homology to the OrfX, Fsh1, and Ring3 proteins is indicated by the shaded underline. (B) Comparison of two Bdf1 bromodomains. (C) Comparison of the region of homology between Bdf1 and OrfX outside the bromodomains. In panels B and C, identical amino acids are indicated by vertical lines and similar residues are indicated by plus signs; the numbers indicate the position within the protein of the last amino acid presented.

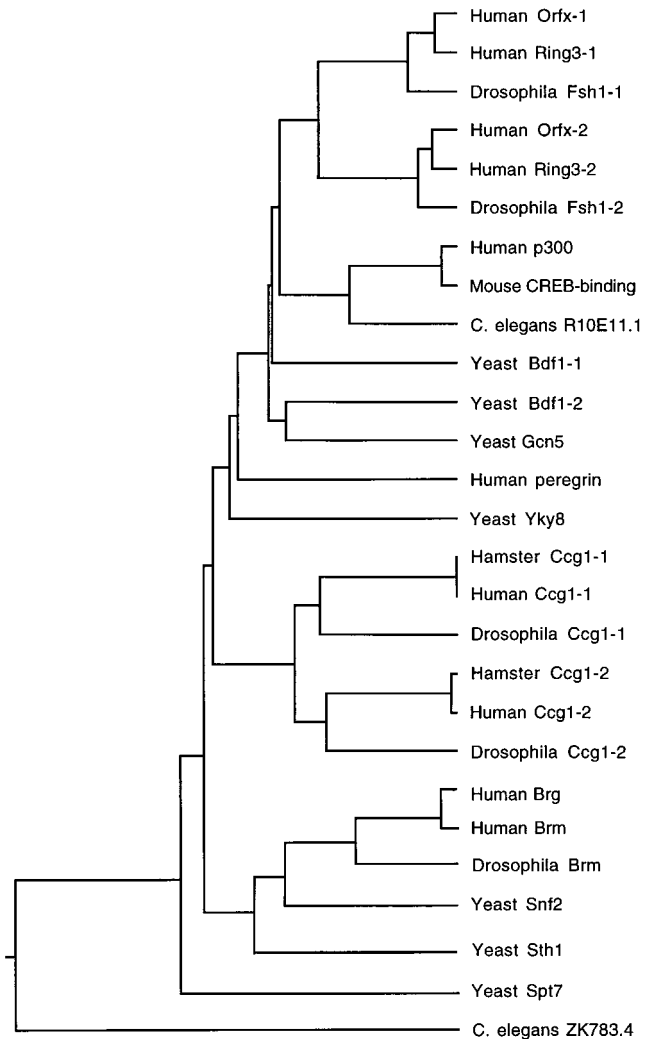


FIG. 3. Phylogenetic tree of bromodomains. All sequences were retrieved from the GenBank database. For proteins that contain two bromodomains, the first and second repeats are indicated by the numbers 1 and 2 following the hyphens. Bromodomain sequences analyzed were 79 amino acids in length with endpoints as defined by Haynes et al. (25). Different alignments are obtained when the bromodomain is assumed to be 87 amino acids in length (42).

(Fig. 4). Cells were stained simultaneously with DAPI, a DNA-binding dye, and with antitubulin antibodies. Anti-Bdf1 antibodies stain the nuclei of cells with short, intermediate, and long spindles, indicating that the Bdf1 protein localizes to the nucleus at all stages of the mitotic cell cycle. Staining of the nucleus with anti-Bdf1 antibodies is also observed in meiotic cells; nuclear staining is evident in mononucleate, binucleate, and tetranucleate cells (data not shown), indicating that Bdf1 is localized to the nucleus throughout the meiotic cell cycle. Anti-Bdf1 antibodies are specific for the Bdf1 protein, as demonstrated by their failure to stain cells from a *bdf1* null mutant (Fig. 4).

To determine whether the Bdf1 protein is associated specifically with chromosomes, spreads of meiotic chromosomes were prepared and incubated with anti-Bdf1 antibodies. Figure 5 shows pachytene chromosomes from a wild-type cell stained with DAPI and anti-Bdf1 antibodies. Almost all of the DAPI-staining material stains with anti-Bdf1 antibodies. Compared with the uniform and continuous staining pattern obtained with DAPI, staining with anti-Bdf1 antibodies appears somewhat speckled and discontinuous, as if Bdf1 is more concentrated in some regions than others. To examine further the specificity of anti-Bdf1 antibodies, spread meiotic chromosomes from a diploid strain heterozygous for a *bdf1*::*lacZ* translational fusion gene were stained with anti-β-galactosidase antibodies (Fig. 5). The staining pattern observed for the fusion protein was indistinguishable from that obtained with the endogenous Bdf1 protein.

In each of the spread nuclei shown in Fig. 5, a region of chromosomal DNA that fails to stain with antibodies directed against Bdf1 can be clearly distinguished. Double staining of meiotic nuclear spreads with both antibodies to Bdf1 and antinucleolar antibodies indicates that the chromosomal segments lacking Bdf1 are in the nucleolus (Fig. 5). Bdf1 is also absent from the nucleolus in mitotic cells. In vegetative cells, the nucleolus is sometimes visible as a lightly staining subdomain of the nucleus when visualized with DAPI. In favorable preparations, it is evident that anti-Bdf1 antibodies localize exclusively to the region of the nucleus not within the nucleolar domain (data not shown).

Protein components of the synaptonemal complex show a pattern of localization similar to that of Bdf1. During pachytene, these proteins are present in synapsed chromosomes and absent from the unsynapsed nucleolar region. The synaptonemal complex is assembled during prophase of meiosis I and disassembles prior to the first meiotic division. To determine whether Bdf1 localizes to chromosomes at stages other than pachytene, spreads from late stages of meiosis were prepared and incubated with anti-Bdf1 antibodies. After pachytene, meiotic chromosomes decondense and the corresponding nuclear spreads appear diffuse when visualized with DAPI (Fig. 6). The stage of meiosis is revealed by the pattern of tubulin staining, with meiosis I and meiosis II spindles clearly visible. Meiotic chromosomes in cells undergoing meiosis I and II stain with anti-Bdf1 antibodies (Fig. 6), indicating that the Bdf1 protein is present on chromosomes at these stages.

These experiments suggest that the *BDF1* gene product is a component of chromosomes at all stages of the mitotic and meiotic cell cycles. The Bdf1 protein appears to localize to almost all DAPI-staining material, except that it is absent from the nucleolus.

A *bdf1* null mutant grows slowly and is defective in DNA damage repair. A null allele of *BDF1* (Fig. 1) was constructed and introduced into yeast by one-step gene disruption. The *bdf1*::*LEU2* null mutant is sensitive to MMS, like the original *bdf1-1* mutant. To compare the sensitivity of the *bdf1* mutant with other mutants defective in DNA damage repair, the *bdf1* null mutant and isogenic wild-type, *rad9*, and *rad52* strains were assayed for survival after exposure to MMS. After incubation in medium containing 0.004% MMS for 6 h, 34% of wild type (PC440), 18% of *bdf1*::*LEU2* (PC469), 26% of *rad9* (PC623), and less than 2% of *rad52* (PC624) cells survived. Mutants in the *RAD50* series of genes (of which *RAD52* is a member) are defective in double-strand break repair, and they are sensitive to X and γ irradiation as well as MMS (17). The *bdf1* mutant is less sensitive to MMS than mutants in the *RAD50* series, and it displays wild-type resistance to γ irradiation (data not shown).

The *bdf1* null mutant exhibits a growth defect that varies in severity in different strain backgrounds. All of the experiments described in this report were carried out in strain BR2171-7B, in which the growth defect conferred by a *bdf1* null mutation is relatively modest. In rich medium, the doubling time of the wild type (PC622) is 81 min, whereas the doubling time of the

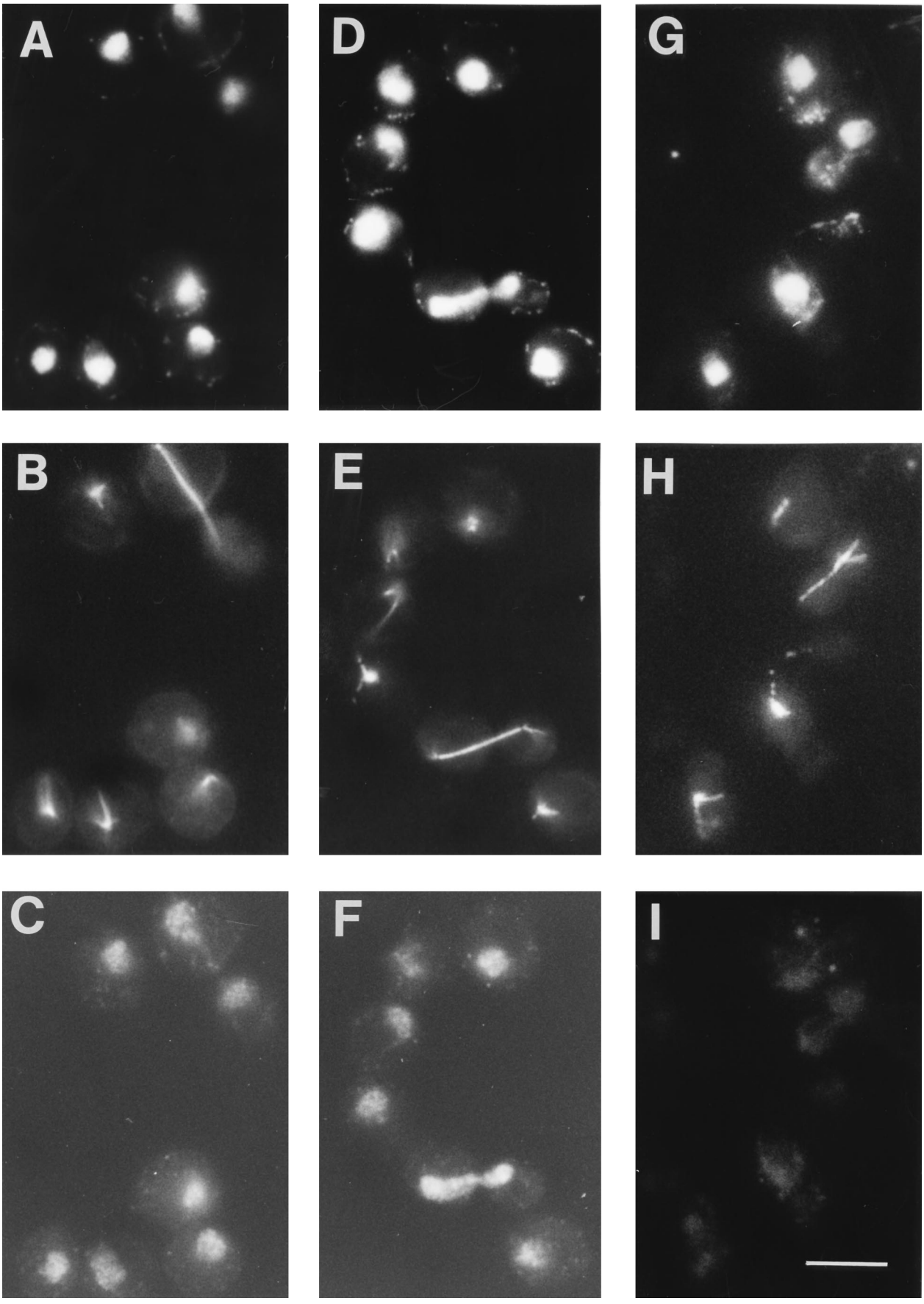

A
D
G
B
E
H
C
F
I

FIG. 4. Bdf1 localizes to the nucleus at all stages of the mitotic cell cycle. Whole cells from exponentially growing cultures were fixed and stained with DAPI (A, D, and G), antitubulin antibodies (B, E, and H), and anti-Bdf1 antibodies (C, F, and I). (A to C and D to F) Two fields of cells from the wild type (BR2171-7B); (G to I) a field of cells from the *bdf1* null mutant (PC621). Bar, 1.9 μm.

bdf1::*LEU2* mutant (PC621) is 147 min. There is no accumulation of cells at a specific stage in the cell cycle (data not shown), suggesting that the Bdf1 protein does not act at a unique point in the cell division cycle.

The *bdf1* null mutant is unable to complete meiosis. Unlike the original *bdf1-1* (presumed point) mutation, the *bdf1*::*LEU2* deletion mutation confers an absolute defect in spore formation. To assess the meiotic nuclear divisions, wild-type and mutant cells were incubated in sporulation medium and then harvested at various time points and stained with DAPI (Fig. 7). In the wild-type culture, there was a steady increase in the percentage of tetranucleate cells accompanied by a decline in the percentage of mononucleate cells. There was also a transient rise in the percentage of binucleate cells, representing the fraction of the culture that had completed meiosis I but not meiosis II. In the *bdf1* culture, the majority of cells remained mononucleate, indicating a failure to enter the meiosis I division. A subset of cells underwent the first division but then persisted as binucleate cells, indicating a failure to progress to the second division. Fewer than 5% of the cells underwent both divisions to become tetranucleate; these cells failed to make spores.

In some sporulation-defective yeast mutants, the sporulation defect can be bypassed by a mutation (such as *mei4*) that prevents the initiation of meiotic recombination and chromosome synapsis (3, 69). A *mei4 bdf1* double mutant (PC465a) does not sporulate (<1%), indicating that the *bdf1* defect is not similarly rescued. This result indicates that the *bdf1* sporulation defect is not due (at least not entirely) to the accumulation of unprocessed intermediates in the recombination pathway.

The *bdf1* null mutant undergoes nearly wild-type levels of meiotic recombination. Meiotic recombination in the *bdf1* null mutant was assayed by return-to-growth experiments, in which cells are returned to conditions of vegetative growth at various times after the initiation of meiosis (16). Figure 8 shows the result of one such experiment, in which prototroph formation at the heteroallelic *THR1* and *HIS4* loci was used to measure gene conversion. Meiotic induction of gene conversion in the null mutant occurs at the same rate and with the same kinetics as in the isogenic wild-type control.

Reciprocal exchange was assayed by a variation of the return-to-growth experiment. Prototrophs resulting from meiotic gene conversion at the *THR1* locus on chromosome VIII were selected in order to enrich for cells that had undergone commitment to meiotic recombination. These prototrophs were then assayed for crossing over in the *HIS4-MAT* interval on chromosome III. The map distances were 42.6 centimorgans for the wild type (PC608) and 21.7 centimorgans for the mutant (PC606). Thus, crossing over is reduced about twofold in the *bdf1* null mutant.

The *bdf1* null mutant is proficient in meiotic chromatin condensation and chromosome synapsis. During the pachytene stage of meiosis in *S. cerevisiae*, chromatin is maximally condensed and each pair of synapsed homologs appears as a "sausage" (11) (Fig. 5). To assess the effect of the *bdf1* mutation on meiotic chromatin condensation, chromosome spreads from the *bdf1*::*LEU2* mutant were stained with DAPI and examined in a fluorescence microscope. Individual chromosomes can be clearly distinguished, indicating that there is no gross defect in chromatin condensation in the *bdf1* null mutant (Fig. 9).

To examine the effect of the *bdf1* mutation on synaptonemal complex assembly, meiotic chromosomes were surface spread, stained with silver nitrate, and viewed in an electron microscope. Full-length synaptonemal complexes were observed in spreads from both the wild type and the mutant (Fig. 9). Synaptonemal complex morphology of the mutant is indistinguishable from that of the wild type. In most complexes, the two lateral elements, representing the proteinaceous backbones of the individual chromosomes within the pair, are evident as darkly stained parallel lines (Fig. 9). To quantitate the efficiency of chromosome synapsis in *bdf1* strains, nuclei from the wild type and the mutant were spread by the method of Loidl et al. (41) and then stained with DAPI and anti-Zip1 antibodies. The Zip1 protein is a component of the central

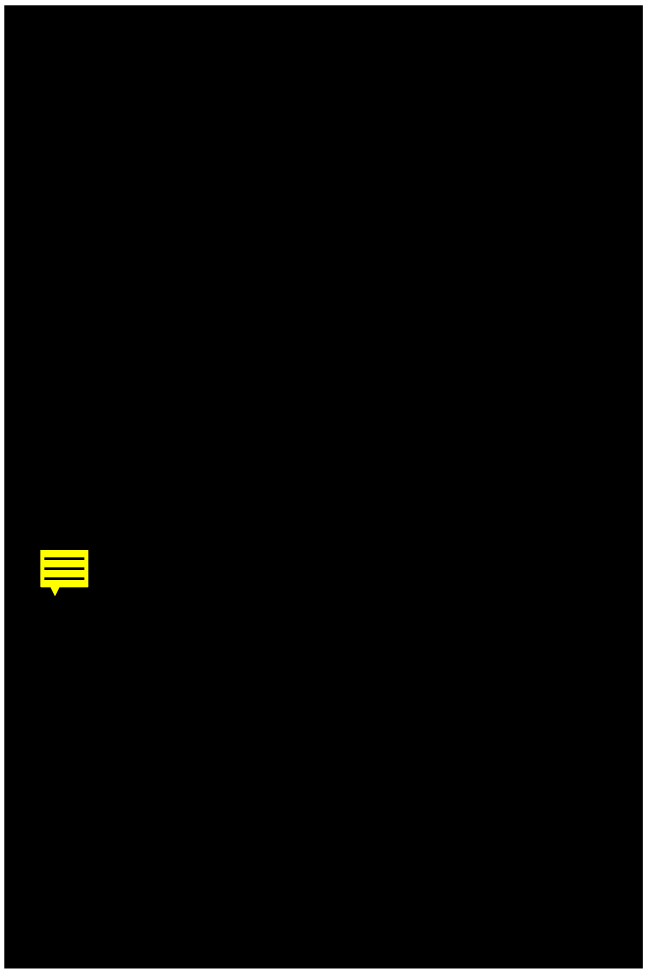


FIG. 5. Bdf1 localizes along the entire lengths of meiotic chromosomes except at the nucleolus. (A to C) A spread meiotic nucleus from the wild type (BR2171-7B); (D to F) a nucleus from a diploid (PC336) heterozygous for a *bdf1*::*lacZ* fusion gene. Chromosomes were stained with DAPI (A and D) and either anti-Bdf1 (B) or anti-β-galactosidase (E) antibodies. (C and F) Double exposures showing the Bdf1- or β-galactosidase-staining patterns (Texas red fluorescence) superimposed on the nucleolar-staining patterns (fluorescein isothiocyanate fluorescence in green). Cells were spread after 14 h in sporulation medium. Bar, 1.4 μm.

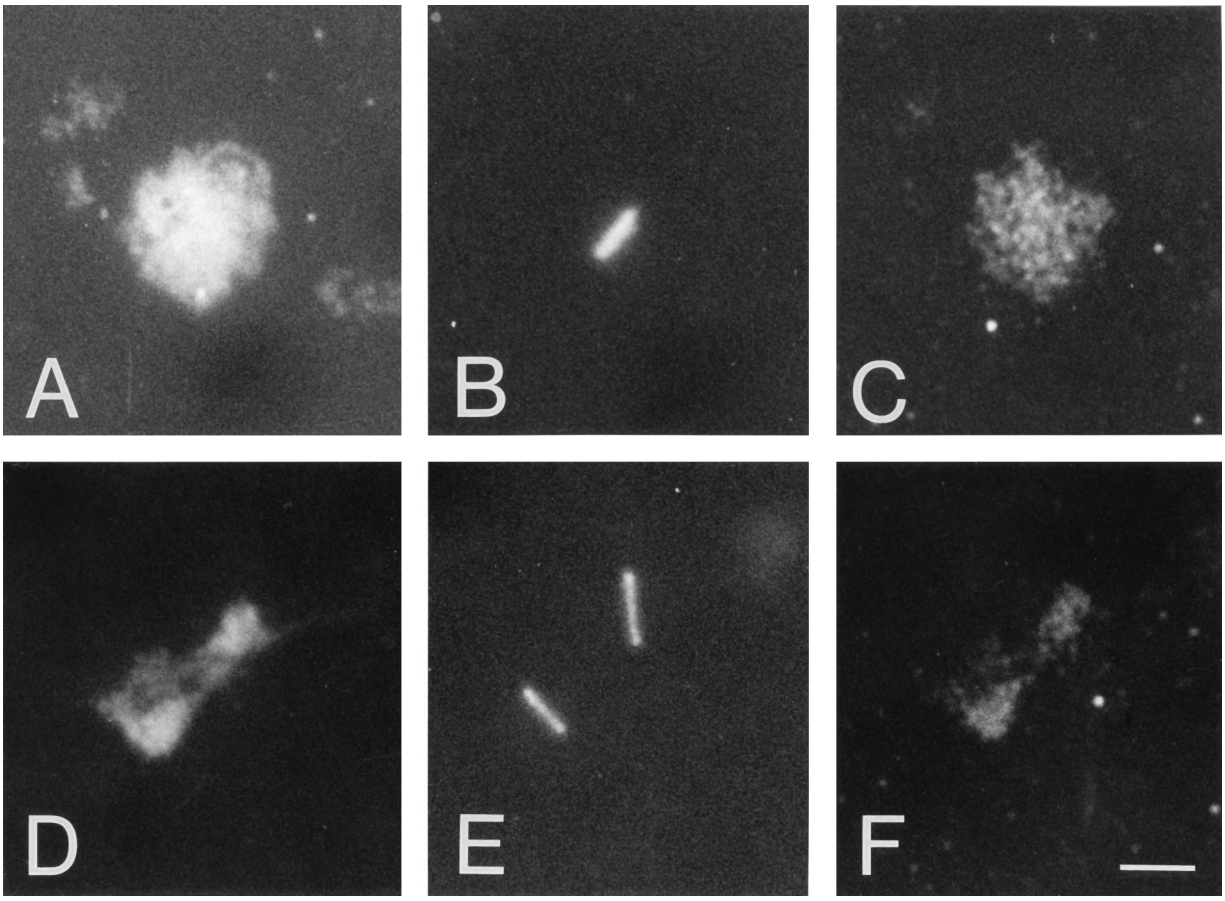


FIG. 6. Bdf1 localizes to chromatin throughout the meiotic cell cycle. After 18 h in sporulation medium, wild-type cells (BR2171-7B) were spread and stained with DAPI (A and D), antitubulin antibodies (B and E), and anti-Bdf1 antibodies (C and F). (A to C) A nucleus that has completed prophase and is undergoing the meiosis I division; (D to F) a nucleus that has completed meiosis I and is undergoing the second meiotic division. Bar, 1.6 μm.

region of the synaptonemal complex and therefore serves as a marker for synapsed chromosomes (69, 71). After 15 h in sporulation medium, when the maximum number of wild-type cells were in pachytene, 52% of spread nuclei from both the wild type (PC543) and the mutant (PC531) stained with anti-Zip1 antibodies. As meiosis progressed, chromatin decondensed and the synaptonemal complex disassembled in both the wild type and the mutant (data not shown).

Different alleles of *BDF1* confer different phenotypes. All transposon insertions in the *BDF1* coding region confer slow growth and MMS sensitivity (Fig. 1). However, only those transposon insertions that were recovered in the second bromodomain fail to sporulate, like the null mutant. Almost all other insertion mutants sporulate efficiently and produce viable spores. This observation demonstrates that the meiotic defect in *bdf1* is separable from the mitotic defect and suggests that the second bromodomain is especially important for sporulation.

The observation that a *bdf1* deletion mutation prevents sporulation, but transposon insertions near the 5′ end of the gene do not, suggests that insertion mutations do not always prevent the expression of downstream sequences. To explore this possibility, we constructed a diploid that carries a double mutation between a transposon insertion that creates an in-frame *lacZ* fusion near the carboxy terminus (at codon 661) and an out-of-frame insertion further upstream (at codon 86). The upstream transposon did not interfere with the production of β-galactosidase activity, suggesting that transcription and translation of the *BDF1* reinitiated either in the transposon or in the *BDF1* coding region. It is possible that normal meiotic expression of the *BDF1* gene depends on a promoter and initiation codon located in the coding region.

The *bdf1*::*lacZ* fusion at codon 661 is lethal in haploids, indicating that the product of the fusion gene is able to interfere with the activity of an essential protein (or proteins). However, this mutation is not dominant, suggesting that the wild-type protein effectively competes for binding of the essential gene product. Two observations indicate that most of the *BDF1* coding sequences must be present in order for the fusion gene to confer lethality. First, an in-frame *bdf1*::*lacZ* fusion in which the junction is located further upstream (at codon 418) is not lethal. Second, the double mutation described above, in which a transposon is inserted upstream of the *bdf1*::*lacZ* fusion junction, does not confer lethality.

DISCUSSION

Bdf1 localizes to chromosomes. We identified *BDF1* as a gene whose product is required for DNA damage repair and progression through meiosis. Concurrently, Lygerou et al. (42) identified *BDF1* in a screen for mutants defective in the transcription of small nuclear RNA genes. Our results demonstrate that the Bdf1 protein localizes to the nucleus at all stages of the mitotic and meiotic cell cycles. In spread preparations of

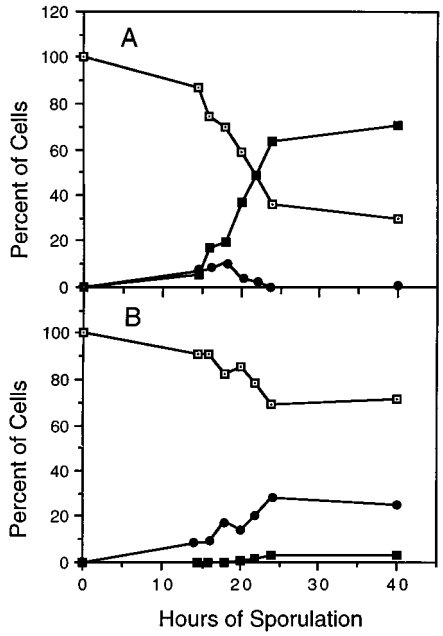


FIG. 7. Time course of meiotic nuclear divisions in the wild type and the *bdf1* mutant. Cells harvested at different time points after the introduction into sporulation medium were stained with DAPI and viewed in a fluorescence microscope. At least 100 cells from the wild type (PC543) and the mutant (PC531) were examined at each time point. (A) Wild type (PC543); (B) *bdf1*::*LEU2* mutant (PC531). Open squares, mononucleate cells; circles, binucleate cells; filled squares, tetranucleate cells.

pachytene nuclei, antibodies to Bdf1 localize to chromosomes but are excluded from the nucleolus.

Efforts to identify components of yeast chromosomes have been hindered by the fact that chromosomes in vegetative cells never condense sufficiently to permit visualization in a light microscope. This has made it difficult to determine whether any given nuclear protein is associated with chromosomes or free in solution. The only time in the yeast life cycle during which individual chromosomes can be visualized is during the pachytene stage of meiosis. Only seven other yeast proteins have been localized to chromosomes by indirect immunofluorescence. Four of these, Hop1, Zip1, Dmc1, and Msh4, are meiosis-specific gene products involved in chromosome synapsis and/or meiotic recombination (2, 31, 57, 69). The other three chromosomal proteins, Rad51, Rap1, and Top2, are present in both vegetative and meiotic cells. Like Dmc1 and Msh4, Rad51 localizes to discrete sites on meiotic chromosomes, postulated to be the sites of genetic exchange events (2, 57). Rap1 localizes primarily to chromosome ends, and Top2 is located in meiotic chromosome cores (38). The Bdf1 staining pattern is most similar to that of Top2, but Bdf1 is excluded from the nucleolus, unlike Top2 (38).

Absence from the nucleoli of both vegetative and meiotic cells is a conspicuous aspect of the Bdf1 localization pattern. In yeast cells, the nucleolar DNA consists of 100 to 200 tandem repeats of a 9.1-kb segment of DNA encoding the four rRNAs (49). The differential localization of Bdf1 is consistent with numerous studies suggesting that chromatin in the nucleolus is organized differently from chromatin elsewhere. Nucleolar chromatin is transcriptionally active and generally less condensed than the rest of the chromosomal DNA (13). Furthermore, the timing of chromatin condensation in the nucleolus appears to differ from that of nonnuclear DNA (23). During meiosis, the repeated ribosomal DNA sequences do not engage in synaptonemal complex formation (11). Studies of both mitotic and meiotic recombination indicate that recombination in the ribosomal DNA requires different gene products and is differentially regulated (6, 21, 22, 54, 79). At least one protein, nucleolin, has been shown to be associated specifically with nucleolar chromatin (15, 48). Bdf1 provides a unique example of a protein associated specifically with nonnucleolar DNA.

Bdf1 contains two bromodomains. The Bdf1 protein contains two bromodomains, a sequence motif of unknown function present in many proteins identified as transcription factors or as transcriptional regulators. Two copies of the bromodomain are present in *CCG1*, which encodes the $TAF_{II}250$ subunit of TFIID, required for basal expression of all genes transcribed by RNA polymerase II (29, 59, 62). A single bromodomain is present in each of the transcriptional regulators Snf2/Swi2, brm, hbrm, BRG1, Gcn5, Spt7, and CBP, which are regulators required for the activated transcription of specific subsets of genes (1, 7, 18, 20, 37, 47, 72). Of this set of regulators, the yeast Snf2/Swi2 protein is the best characterized; it has been shown to activate transcription by altering the conformation of chromatin at the *SUC2* promoter. This alteration of chromatin structure is independent of TFIID binding, leading to a model in which Snf2 activates transcription by causing chromatin to assume a configuration that facilitates transcriptional activation (28). Snf2 contains a predicted helicase domain postulated to play a role in chromatin alteration and has been shown biochemically to have DNA-stimulated ATPase activity (39). A role in alteration of chromatin structure has also been proposed for brm, the *Drosophila* homolog of Snf2 (72). Mutations in

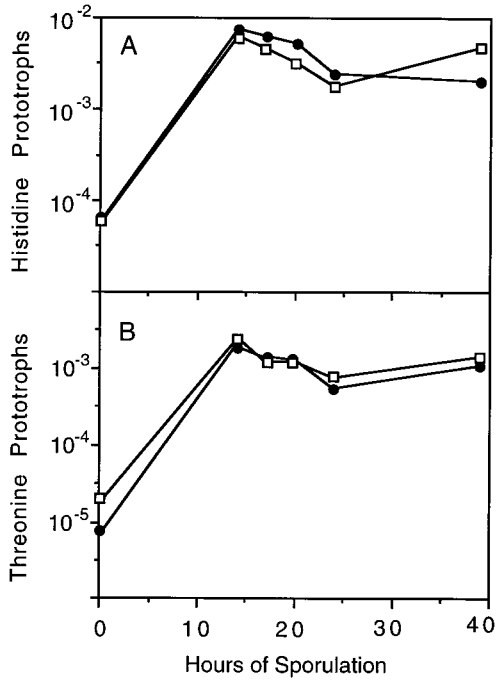


FIG. 8. Meiotic recombination in *bdf1*::*LEU2* and wild-type strains. Commitment to meiotic gene conversion was measured at two different heteroallelic loci by monitoring prototroph formation at various times after introduction into sporulation medium. Shown are the frequencies of prototroph formation as at *HIS4* (A) and *THR1* (B). Assays were performed in triplicate, and the mean frequencies are shown. Squares, wild type (PC543); circles, *bdf1*::*LEU2* mutant (PC531).

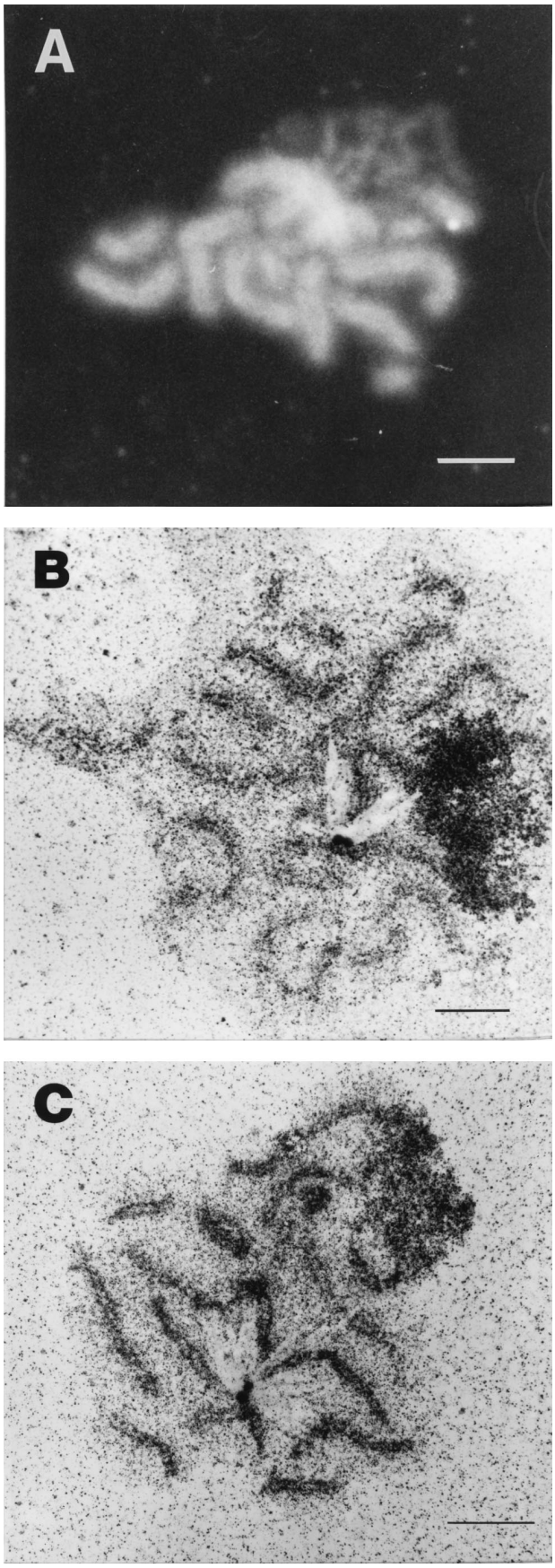


FIG. 9. Chromosome condensation and chromosome synapsis in the *bdf1* null mutant. After 14 h in sporulation medium, cells were spread and either stained with DAPI and viewed in a fluorescence microscope (A) or stained with silver nitrate and visualized in an electron microscope (B and C). (A and C) Spread nuclei from the *bdf1*::*LEU2* mutant (PC531); (B) a spread nucleus from the wild type (PC543). Bar, 1.0 μm.

brm bypass the repressive effects of the Polycomb group genes; this repression is thought to be mediated via regional chromatin compaction (52).

A number of bromodomain-containing proteins (Snf2/Swi2, hbrm, and CBP) have been demonstrated to have transcriptional activating properties (7, 40, 47). When fused to a DNA-binding domain that binds to the promoter region of a reporter gene, these hybrid proteins activate the expression of the target gene. However, a number of observations suggest that the bromodomain is dispensable for transcriptional activation. Derivatives of the *SNF2* and *SPT7* genes that lack the bromodomain are still able to complement the corresponding null mutations (18, 39), and an hbrm construct lacking the bromodomain is still able to activate transcription at target promoters (47). In the case of hbrm, it is hypothesized that the bromodomain controls protein stability and nuclear localization, since deletion of the bromodomain results in redistribution of hbrm from its normal nuclear location to a uniform localization throughout the cell (47).

Outside the bromodomains, the only extensive homology between the Bdf1 sequence and protein sequences in existing databases involves the last 165 amino acids of the *BDF1* gene product. This region of Bdf1 is homologous to sequences near the carboxy termini of the *Drosophila* Fsh1 protein and the human OrfX and Ring3 proteins. Nothing is known about the functions of the hypothetical OrfX and Ring3 proteins. The *Drosophila* Fsh1 protein participates in the establishment of segmental identify by activating certain homeotic genes (26, 32, 36). The molecular mechanism of Fsh1 action is not known.

Models for Bdf1 function. The localization pattern of the Bdf1 protein, together with the observed mitotic and meiotic defects of the *bdf1* null mutant, suggests two models for Bdf1 function. The first postulates that Bdf1 is a transcriptional regulator and that the mutant phenotype stems from a failure to activate the transcription of multiple genes. The second model proposes that Bdf1 is a component of chromatin and that the mutant phenotype is a consequence of alterations in chromatin structure. These models are not mutually exclusive, since alterations in chromatin structure obviously can result in changes in gene expression.

The hypothesis that Bdf1 is a transcriptional activator is compromised by the results of Lygerou et al. (42), who showed that the Bdf1 protein has no transcription-stimulating activity when tethered to the promoter region of a reporter gene. Lygerou et al. (42) also found that a *bdf1* null mutation reduces the expression of small nuclear RNA genes transcribed by both RNA polymerases II (U1, U2, and U4) and III (U6). Thus, if Bdf1 is a transcription factor, it must be involved in transcription by both types of polymerase. It is unlikely that Bdf1 is absolutely required for transcription by RNA polymerase III, since no Bdf1 was detected in the nucleolus where the 5S gene (a polymerase III transcription unit) is located. On the basis of these results, we suggest that Bdf1 causes a widespread alteration in chromatin structure and thereby affects gene expression to various extents from different promoters. Consistent with this hypothesis, the purification properties of the Bdf1 protein suggest that it has nonspecific, DNA-binding activity (42).

Secondary structure predictions indicate that the bromodomain consists of two amphipathic α helices followed by reverse turns; it has been suggested that the hydrophobic surfaces of these helices serve as sites of intermolecular protein-protein interactions (25). Perhaps the bromodomain interacts with one or more components of chromatin. Such a functional sequence motif might be expected to appear in transcriptional regulators as well as structural components of chromatin. The second bromodomain in Bdf1 may interact with a meiosis-specific chromatin component.

Bdf1 is required for sporulation. In meiosis, the *bdf1* null mutant exhibits a delay in progression from meiotic prophase to the first meiotic division, a further delay in advancing from meiosis I to meiosis II, and an absolute defect in spore formation. The *bdf1* sporulation defect is not bypassed by a *mei4* mutation, ruling out the possibility that the sporulation defect is due to the accumulation of intermediates in the recombination or synapsis pathways. One possible cause of the inability to sporulate is a failure to express genes required for the meiotic divisions and spore formation. Using the meiosis-specific *lacZ* fusion genes described by Burns et al. (5), we examined the effect of the *bdf1* mutation on the expression of meiotic genes induced specifically in mononucleate, binucleate, and tetranucleate cells. The *bdf1* mutation did not affect expression of these reporter genes (data not shown), indicating that any transcriptional defect must be specific to certain genes. Another explanation for the sporulation defect is that the absence of Bdf1 leads to alterations in chromatin structure that impair chromosome segregation. For example, Bdf1 might be required for proper chromatin decondensation following pachytene. Such an explanation could account for the greater severity of the *bdf1* mutant phenotype in meiosis than in mitosis, since yeast chromatin undergoes greater condensation in meiosis than during vegetative growth.

The *bdf1* mutation decreases meiotic crossing over twofold but does not reduce gene conversion. This result suggests that the Bdf1 protein affects the resolution of recombination intermediates, promoting resolution in favor of crossing over. However, the Bdf1 protein need not be directly involved in the enzymology of recombination. For example, the *bdf1* mutation might perturb the structure of chromatin in a manner that is inhibitory to the isomerization of Holliday junctions. A null mutation in the *ZIP1* gene (which encodes a structural component of the synaptonemal complex) also reduces crossing over but not conversion, indicating that the context in which resolution occurs can influence the frequency of crossing over (69–71).

The *bdf1* null mutant is sensitive to MMS but not γ irradiation, indicating a defect in the repair of some types of DNA damage. Sensitivity to MMS might be due to defects in transcription in the *bdf1* mutant, since the detection and repair of certain lesions in DNA are linked to transcription (4). Alternatively, perturbations in chromatin structure in the *bdf1* mutant might affect accessibility of the damaged DNA to the repair machinery.

Mutants with mutations in *BDF1* displays interesting similarities to *rad6* mutants. *RAD6* encodes an enzyme that conjugates ubiquitin to histones H2A and H2B (35, 68). Like the *bdf1* mutants, the *rad6* null mutant grows slowly and is defective in DNA damage repair and sporulation. Furthermore, for both *BDF1* and *RAD6*, there exist separation-of-function alleles that are able to sporulate and produce viable spores but are deficient in DNA damage repair (46, 68). The striking phenotypic similarities between *bdf1* and mutations in a gene known to be involved in modifying chromatin, together with our observations that the Bdf1 protein localizes to chromosomes, support a role for Bdf1 in the regulation of chromatin structure.

ACKNOWLEDGMENTS

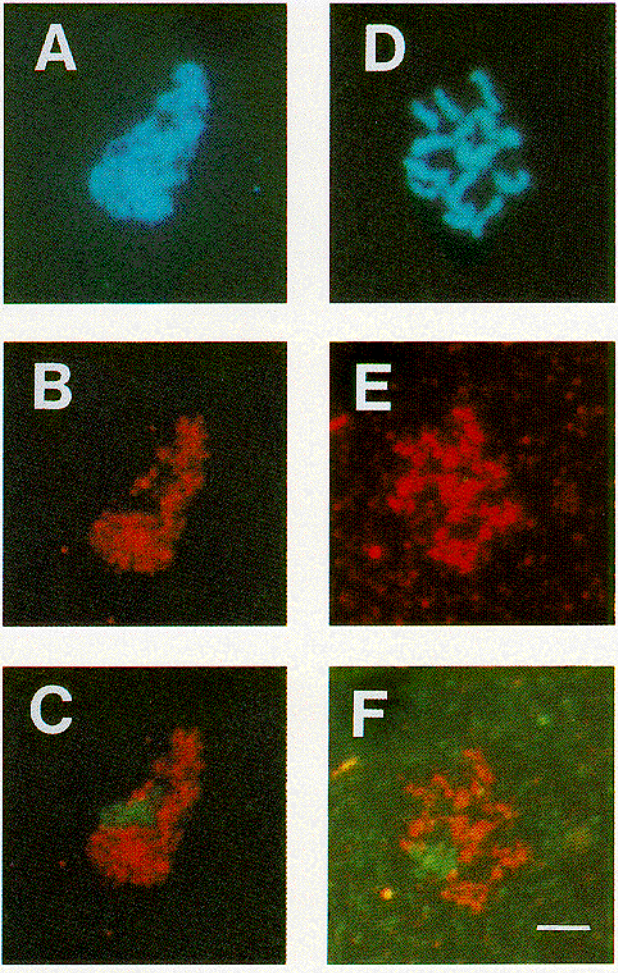
We thank Ralph Keil for performing tests of sensitivity to γ irradiation, Bertrand Séraphin for communicating unpublished results, and members of the Roeder laboratory for helpful discussion. We thank Janet Novak, Beth Rockmill, Petra Ross-Macdonald, and Albert Smith for comments on the manuscript.

This work was supported by grant GM28904 from the National Institutes of Health.

REFERENCES

1. **Abrams, E., L. Neigeborn, and M. Carlson.** 1986. Molecular analysis of *SNF2* and *SNF5*, genes required for expression of glucose-repressible genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **6:**3643–3651.
2. **Bishop, D. K.** 1994. RecA homologs Dmc1 and Rad51 interact to form multiple nuclear complexes prior to meiotic chromosome synapsis. Cell **79:**1081–1092.
3. **Bishop, D. K., D. Park, L. Xu, and N. Kleckner.** 1992. *DMC1*: a meiosis-specific yeast homolog of E. coli *recA* required for recombination, synaptonemal complex formation, and cell cycle progression. Cell **69:**439–456.
4. **Buratowski, S.** 1993. DNA repair and transcription: the helicase connection. Science **260:**37–38.
5. **Burns, N., B. Grimwade, P. B. Ross-Macdonald, E.-Y. Choi, K. Finberg, G. S. Roeder, and M. Snyder.** 1994. Large-scale analysis of gene expression, protein localization and gene disruption in *Saccharomyces cerevisiae*. Genes Dev. **8:**1087–1105.
6. **Christman, M. F., F. S. Dietrich, and G. R. Fink.** 1988. Mitotic recombination in the rDNA of *S. cerevisiae* is suppressed by the combined action of DNA topoisomerases I and II. Cell **55:**413–425.
7. **Chrivia, J. C., R. P. S. Kwok, N. Lamb, M. Hagiwara, M. R. Montminy, and R. H. Goodman.** 1993. Phosphorylated CREB binds specifically to the nuclear protein CBP. Nature (London) **365:**855–859.
8. **Chu, G., D. Vollrath, and R. W. Davis.** 1986. Separation of large DNA molecules by contour-clamped homogeneous electric fields. Science **234:** 1582–1585.
9. **Dobson, M., R. E. Pearlman, A. Karaiskakis, B. Spyropoulos, and P. B. Moens.** 1994. Synaptonemal complex proteins: occurrence, epitope mapping and chromosome disjunction. J. Cell Sci. **107:**2749–2760.
10. **Donahue, T. F., P. J. Farabaugh, and G. R. Fink.** 1982. The nucleotide sequence of the *HIS4* region of yeast. Gene **18:**47–59.
11. **Dresser, M. E., and C. N. Giroux.** 1988. Meiotic chromosome behavior in spread preparations of yeast. J. Cell Biol. **106:**567–578.
12. **Earnshaw, W. C., B. Halligan, C. A. Cooke, M. M. S. Heck, and L. F. Liu.** 1985. Topoisomerase II is a structural component of mitotic chromosome scaffolds. J. Cell Biol. **100:**1706–1715.
13. **Eissenberg, J. C., I. L. Cartwright, G. H. Thomas, and S. C. R. Elgin.** 1985. Selected topics in chromatin structure. Annu. Rev. Genet. **19:**485–536.
14. **Engebrecht, J., and G. S. Roeder.** 1990. *MER1*, a yeast gene required for chromosome pairing and genetic recombination, is induced in meiosis. Mol. Cell. Biol. **10:**2379–2389.
15. **Erard, M. S., P. Belenguer, M. Caizergues-Ferrer, A. Panaloni, and F. Amalric.** 1988. A major nucleolar protein, nucleolin, induces chromatin decondensation by binding to histone H1. Eur. J. Biochem. **175:**525–530.
16. **Esposito, R. E., and M. S. Esposito.** 1974. Genetic recombination and commitment to meiosis in *Saccharomyces*. Proc. Natl. Acad. Sci. USA **71:**3172–3176.
17. **Friedberg, E. C., W. Siede, and A. J. Cooper.** 1991. Cellular responses to DNA damage in yeast, p. 147–192. *In* J. R. Broach, J. R. Pringle, and E. W. Jones (ed.), The molecular and cellular biology of the yeast *Saccharomyces*: genome dynamics, protein synthesis, and energetics. Cold Spring Harbor Laboratory Press, Plainview, N.Y.
18. **Gansheroff, L. J., C. Dollard, P. Tan, and F. Winston.** 1995. The *Saccharomyces cerevisiae SPT7* gene encodes a very acidic protein important for transcription *in vivo*. Genetics **139:**523–536.
19. **Gasser, S. M., T. Laroche, J. Falquet, E. B. de la Tour, and U. K. Laemmli.** 1986. Metaphase chromosome structure: involvement of topoisomerase II. J. Mol. Biol. **188:**613–629.
20. **Georgakopoulos, T., and G. Thireos.** 1992. Two distinct yeast transcriptional activators require the function of the GCN5 protein to promote normal levels of transcription. EMBO J. **11:**4145–4152.
21. **Gottlieb, S., and R. E. Esposito.** 1989. A new role for a yeast transcriptional silencer gene, *SIR2*, in regulation of recombination in ribosomal DNA. Cell **56:**771–776.
22. **Gottlieb, S., J. Wagstaff, and R. E. Esposito.** 1989. Evidence for two pathways of meiotic intrachromosomal recombination in yeast. Proc. Natl. Acad. Sci. USA **86:**7072–7076.
23. **Guacci, V., E. Hogan, and D. Koshland.** 1994. Chromosome condensation

and sister chromatid pairing in budding yeast. J. Cell Biol. **125:**517–530.
24. **Guan, K., and J. E. Dixon.** 1991. Eukaryotic proteins expressed in *Escherichia coli*: an improved thrombin cleavage and purification procedure of fusion proteins with glutathione S-transferase. Anal. Biochem. **192:**262–267.
25. **Haynes, S. R., C. Dollard, F. Winston, S. Beck, J. Trowsdale, and I. B. Dawid.** 1992. The bromodomain: a conserved sequence found in human, *Drosophila* and yeast proteins. Nucleic Acids Res. **20:**2603.
26. **Haynes, S. R., B. A. Mozer, N. Bhatia-Dey, and I. B. Dawid.** 1989. The *Drosophila fsh* locus, a maternal effect homeotic gene, encodes apparent membrane proteins. Dev. Biol. **134:**246–257.
27. **Heyting, C., A. J. J. Dietrich, P. B. Moens, R. J. Dettmers, H. H. Offenberg, E. J. W. Redeker, and A. C. G. Vink.** 1989. Synaptonemal complex proteins. Genome **31:**81–87.
28. **Hirschhorn, J. N., S. A. Brown, C. D. Clark, and F. Winston.** 1992. Evidence that SNF2/SWI2 and SNF5 activate transcription in yeast by altering chromatin structure. Genes Dev. **6:**2288–2298.
29. **Hisatake, K., S. Hasegawa, R. Takada, Y. Nakatani, M. Horikoshi, and R. G. Roeder.** 1993. The p250 subunit of native TATA box-binding factor TFIID is the cell-cycle regulatory protein CCG1. Nature (London) **362:**179–181.
30. **Hoekstra, M. F., H. S. Seifert, J. Nickoloff, and F. Heffron.** 1991. Shuttle mutagenesis: bacterial transposons for genetic manipulations in yeast. Methods Enzymol. **194:**329–342.
31. **Hollingsworth, N. M., L. Goetsch, and B. Byers.** 1990. The *HOP1* gene encodes a meiosis-specific component of yeast chromosomes. Cell **61:**73–84.
32. **Huang, D.-H., and I. B. Dawid.** 1990. The maternal-effect gene *fsh* is essential for the specification of the central region of the *Drosophila* embryo. New Biol. **2:**163–170.
33. **Ito, H., Y. Fukada, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153:**163–168.
34. **Jensen, R., G. F. Sprague, and I. Herskowitz.** 1983. Regulation of yeast mating-type interconversion: feedback control of *HO* gene expression by the mating-type locus. Proc. Natl. Acad. Sci. USA **80:**3035–3039.
35. **Jentsch, S., J. P. McGrath, and A. Varshavsky.** 1987. The yeast DNA repair gene *RAD6* encodes a ubiquitin-conjugating enzyme. Nature (London) **329:** 131–134.
36. **Kennison, J. A.** 1993. Transcriptional activation of Drosophila homeotic genes from distant regulatory elements. Trends Genet. **9:**75–78.
37. **Khavari, P. A., C. L. Peterson, J. W. Tamkun, D. B. Mendel, and G. R. Crabtree.** 1993. BRG1 contains a conserved domain of the *SWI2/SNF2* family necessary for normal mitotic growth and transcription. Nature (London) **366:**170–174.
38. **Klein, F., T. Laroche, M. E. Cardenas, J. F.-X. Hofmann, D. Schweizer, and S. M. Gasser.** 1992. Localization of RAP1 and topoisomerase II in nuclei and meiotic chromosomes of yeast. J. Cell Biol. **117:**935–948.
39. **Laurent, B. C., I. Treich, and M. Carlson.** 1993. The yeast SNF2/SWI2 protein has DNA-stimulated ATPase activity required for transcriptional activation. Genes Dev. **7:**583–591.
40. **Laurent, B. C., M. A. Treitel, and M. Carlson.** 1991. Functional interdependence of the yeast SNF2, SNF5, and SNF6 proteins in transcriptional activation. Proc. Natl. Acad. Sci. USA **88:**2687–2691.
41. **Loidl, J., K. Nairz, and F. Klein.** 1991. Meiotic chromosome synapsis in a haploid yeast. Chromosoma **100:**221–228.
42. **Lygerou, Z., C. Conesa, P. Lesage, R. N. Swanson, A. Ruet, M. Carlson, A. Sentenac, and B. Seraphin.** 1994. The yeast *BDF1* gene encodes a transcription factor involved in the expression of a broad class of genes including snRNAs. Nucleic Acids Res. **22:**5332–5340.
43. **Menees, T. M., and G. S. Roeder.** 1989. *MEI4*, a yeast gene required for meiotic recombination. Genetics **123:**675–682.
44. **Meuwissen, R. L. J., H. H. Offenberg, A. J. J. Dietrich, A. Riesewijk, M. van Iersel, and C. Heyting.** 1992. A coiled-coil related protein specific for synapsed regions of meiotic prophase chromosomes. EMBO J. **11:**5091–5100.
45. **Moens, P., and W. C. Earnshaw.** 1989. Anti-topoisomerase II recognizes meiotic chromosomes cores. Chromosoma **98:**317–322.
46. **Morrison, A., E. J. Miller, and L. Prakash.** 1988. Domain structure and functional analysis of the carboxyl-terminal polyacidic sequence of the RAD6 protein of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **8:**1179–1185.
47. **Muchardt, C., and M. Yaniv.** 1993. A human homologue of *Saccharomyces cerevisiae SNF2/SWI2* and *Drosophila brm* genes potentiates transcriptional activation by the glucocorticoid receptor. EMBO J. **12:**4279–4290.
48. **Olson, M. O. J., and B. A. Thompson.** 1983. Distribution of proteins among chromatin components of nucleoli. Biochemistry **22:**3187–3193.
49. **Olson, M. V.** 1991. Genome structure and organization in *Saccharomyces cerevisiae*, p. 1–39. *In* J. R. Broach, J. R. Pringle, and E. W. Jones (ed.), The molecular and cellular biology of the yeast *Saccharomyces*: genome dynamics, protein synthesis, and energetics. Cold Spring Harbor Laboratory Press, Plainview, N.Y.
50. **Olson, M. V., J. E. Dutchik, M. Y. Graham, G. M. Brodeur, C. Helms, M. Frank, M. MacCollin, R. Scheinman, and T. Frank.** 1986. Random-clone strategy for genomic restriction mapping in yeast. Proc. Natl. Acad. Sci. USA **83:**7826–7830.
51. **Parent, S. A., C. M. Fenimore, and K. A. Bostian.** 1985. Vector systems for the expression, analysis and cloning of DNA sequences in *S. cerevisiae*. Yeast **1:**83–138.
52. **Paro, R.** 1990. Imprinting a determined state into the chromatin of Drosophila. Trends Genet. **6:**416–421.
53. **Petes, T. D., R. E. Malone, and L. S. Symington.** 1991. Recombination in yeast, p. 407–521. *In* J. R. Broach, J. R. Pringle, and E. W. Jones (ed.), The molecular and cellular biology of the yeast *Saccharomyces*: genome dynamics, protein synthesis, and energetics. Cold Spring Harbor Laboratory Press, Plainview, N.Y.
54. **Prakash, L., and P. Taillon-Miller.** 1981. Effects of the *rad52* gene on sister chromatid recombination in *Saccharomyces cerevisiae*. Curr. Genet. **3:**247–250.
55. **Pringle, J. R., A. E. Adams, D. G. Drubin, and B. K. Haarer.** 1991. Immunofluorescence methods for yeast. Methods Enzymol. **194:**565–602.
56. **Rockmill, B. M., and G. S. Roeder.** 1991. A meiosis-specific protein kinase homolog required for chromosome synapsis and recombination. Genes Dev. **5:**2392–2404.
57. **Ross-Macdonald, P., and G. S. Roeder.** 1994. Mutation of a meiosis-specific MutS homolog decreases crossing over but not mismatch correction. Cell **79:**1069–1080.
58. **Rothstein, R.** 1991. Targeting, disruption, replacement and allele rescue: integrative DNA transformation in yeast. Methods Enzymol. **194:**281–301.
59. **Ruppert, S., E. H. Wang, and R. Tjian.** 1993. Cloning and expression of human $TAF_{II}250$: a TBP-associated factor implicated in cell-cycle regulation. Nature (London) **362:**175–179.
60. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
61. **Schultes, N. P., A. D. Ellington, J. M. Cherry, and J. W. Szostak.** 1990. *Saccharomyces cerevisiae* homoserine kinase is homologous to prokaryotic homoserine kinases. Gene **96:**177–180.
62. **Sekiguchi, T., Y. Nohiro, Y. Nakamura, N. Hisamoto, and T. Nishimoto.** 1991. The human CCG1 gene, essential for progression of the G1 phase, encodes a 210-kilodalton nuclear DNA-binding protein. Mol. Cell. Biol. **11:**3317–3325.
63. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1986. Methods in yeast genetics: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
64. **Shuster, E. O., and B. Byers.** 1989. Pachytene arrest and other meiotic effects of the start mutations in *Saccharomyces cerevisiae*. Genetics **123:**29–43.
65. **Simchen, G.** 1974. Are mitotic functions required in meiosis? Genetics **76:** 745–753.
66. **Smith, D. B., and K. S. Johnson.** 1988. Single-step purification of polypeptides expressed in *Escherichia coli* as fusions with glutathione S-transferase. Gene **67:**31–40.
67. **Snyder, M.** 1989. The *SPA2* protein of yeast localizes to sites of cell growth. J. Cell Biol. **108:**1419–1429.
68. **Sung, P., S. Prakash, and L. Prakash.** 1988. The RAD6 protein of *Saccharomyces cerevisiae* polyubiquitinates histones, and its acidic domain mediates this activity. Genes Dev. **2:**1476–1485.
69. **Sym, M., J. Engebrecht, and G. S. Roeder.** 1993. ZIP1 is a synaptonemal complex protein required for meiotic chromosome synapsis. Cell **72:**365–378.
70. **Sym, M., and G. S. Roeder.** 1994. Crossover interference is abolished in the absence of a synaptonemal complex protein. Cell **79:**283–292.
71. **Sym, M., and G. S. Roeder.** 1995. Zip1-induced changes in synaptonemal complex structure and polycomplex assembly. J. Cell Biol. **128:**455–466.
72. **Tamkun, J. W., R. Deuring, M. P. Scott, M. Kissinger, A. M. Pattatucci, T. C. Kaufman, and J. A. Kennison.** 1992. brahma: a regulator of Drosophila homeotic genes structurally related to the yeast transcriptional activator SNF2/SWI2. Cell **68:**561–572.
73. **Thompson, E. A., and G. S. Roeder.** 1989. Expression and DNA sequence of *RED1*, a gene required for meiosis I chromosome segregation in yeast. Mol. Gen. Genet. **218:**293–301.
74. **Voelkel-Meiman, K., and G. S. Roeder.** 1990. A chromosome containing *HOT1* preferentially receives information during mitotic interchromosomal gene conversion. Genetics **124:**561–572.
75. **von Wettstein, D., S. W. Rasmussen, and P. B. Holm.** 1984. The synaptonemal complex in genetic segregation. Annu. Rev. Genet. **18:**331–413.
76. **Weinert, T. A., and L. H. Hartwell.** 1990. Characterization of *RAD9* of *Saccharomyces cerevisiae* and evidence that its function acts posttranslationally in cell cycle arrest after DNA damage. Mol. Cell. Biol. **10:**6554–6564.
77. **Widner, W. R., and R. B. Wickner.** 1993. Evidence that the SKI antiviral system of *Saccharomyces cerevisiae* acts by blocking expression of viral mRNA. Mol. Cell. Biol. **13:**4331–4341.
78. **Yang, C. H., E. J. Lambie, J. Hardin, J. Craft, and M. Snyder.** 1989. Higher order structure is present in the yeast nucleus: autoantibody probes demonstrate that the nucleolus lies opposite the spindle pole body. Chromosoma **98:**123–128.
79. **Zamb, T. J., and T. D. Petes.** 1981. Unequal sister-strand recombination within yeast ribosomal DNA does not require the *RAD52* gene product. Curr. Genet. **3:**125–132.


A
D
B
E
C
F