The yeast *BDF1* gene encodes a transcription factor involved in the expression of a broad class of genes including snRNAs

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Received August 22, 1994; Revised and Accepted November 9, 1994

ABSTRACT

While screening for genes that affect the synthesis of yeast snRNPs, we identified a thermosensitive mutant that abolishes the production of a reporter snRNA at the non-permissive temperature. This mutant defines a new gene, named *BDF1*. In a *bdf1-1* strain, the reporter snRNA synthesized before the temperature shift remains stable at the non-permissive temperature. This demonstrates that the *BDF1* gene affects the synthesis rather than the stability of the reporter snRNA and suggests that the *BDF1* gene encodes a transcription factor. *BDF1* is present in single copy on yeast chromosome XII, and is important for normal vegetative growth but not essential for cell viability. *bdf1* null mutants share common phenotypes with several mutants affecting general transcription and are defective in snRNA production. *BDF1* encodes a protein of 687 amino-acids containing two copies of the bromodomain, a motif also present in other transcription factors as well as a new conserved domain, the ET domain, also present in *Drosophila* and human proteins.

INTRODUCTION

The nucleus of eukaryotic cells contains small nuclear ribonucleoproteins (snRNPs), particles consisting of small nuclear RNA (snRNA) associated with proteins (1,2). The best characterized snRNPs are probably the U1, U2, U4, U5 and U6 snRNPs which are involved in pre-mRNA splicing (3). The synthesis of these snRNPs has been extensively analyzed in vertebrates (4–6). The primary polymerase II (polII) U1, U2, U4 and U5 transcripts are exported to the cytoplasm where they assemble with a group of eight Sm proteins. This binding triggers

the hypermethylation of the m7G cap initially present at the 5' end of all polII transcripts to yield a trimethyl cap structure (3mG), characteristic of most of the U snRNAs. The binding of Sm proteins and, in some cases, the presence of a 3mG cap structure are required for transport of the assembled particle back to the nucleus. Along this complex assembly pathway, snRNAs are covalently modified and assemble with several snRNP specific proteins. Although the majority of the U snRNAs are transcribed by polymerase II, the U6 snRNA is transcribed by polymerase III (polIII). Unlike its counterparts, the U6 snRNA remains in the nucleus where it gets covalently modified internally and at both its 5' and 3' ends. Finally, the U4 and U6 snRNP assemble to form a double U4/U6 snRNP where the two snRNAs are associated through an extensive base pairing interaction (3).

In vertebrates, the promoter regions of the polII and polIII transcribed snRNAs are very similar with the presence of interchangeable distal and proximal sequence elements (DSE and PSE, respectively) upstream of the coding region (4). The main difference between the polII and polIII promoters is the presence of a TATA box in the latter one. However, transcription of both classes of snRNAs requires the TATA box binding (TBP) protein (7–11). Additional proteins interacting with the DSE (12,13) and PSE (10,13,14) elements are required for transcription. As in mammals, plant U1, U2, U4 and U5 snRNAs are polII transcribed while plant U6 is a polIII product. Both polII and polIII promoters contain a TATA box element and an upstream sequence element (USE). However the spacing between these elements differs between the two classes of promoters [reviewed in (4)]. The *S.cerevisiae* U1, U2, U4 and U5 snRNAs have a 3mG cap suggesting that they are polII transcribed. Expression of the polIII transcribed yeast U6 snRNA is dependent on TBP but also TFIIIC and its cognate DNA binding sites (15–19). Although these data suggest that the mode of transcription of

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spliceosomal snRNAs is highly conserved throughout evolution, it has recently been found that the U2 snRNA from trypanosomes is a polIII product (20).

We have recently developed a screening procedure for yeast mutants that would affect snRNP synthesis (Z.Lygerou and B.Sraphin, unpublished data). Analysis of one of the mutants recovered from this screen is presented here. At the non-permissive temperature, this mutant does not accumulate a reporter U4 snRNA. This results from a transcriptional defect rather than a reduction of snRNA stability. The wild type allele has been cloned and sequenced. It encodes a protein with two bromodomains, hence the name of *BDF1* (Bromodomain Factor 1). Beside the bromodomain, *BDF1* contains a new domain (Extra Terminal domain or ET domain) that is also present in proteins from higher eukaryotes. Our experiments suggest that *BDF1* encodes a transcription factor with a broad class of target genes that include snRNAs.

MATERIAL AND METHODS

Yeast strains and genetic methods

Saccharomyces cerevisiae strains BSY295 (*MATa ade2 arg4 leu2-3,112 TRP1::SNR14* ura3-52*), BSY360 (*MAT ade2 ade5 his3-1 leu2-3,112 TRP1::SNR14* ura3-52*), YNN281 (*MATa trp1-901 his3-200 ura3-52 lys2-801 ade2-101*), YNN282 (*MAT trp1-901 his3-200 ura3-52 lys2-801 ade2-101*), MCY829 (*MAT his3-200 lys2-801 ura3-52*), MCY3015 (*MATa/MAT leu2-3,112/leu2-3,112 his3-200/HIS+ lys2-801/LYS+ ura3-52/ura3-52 ade2-101/ADE+ SUC2/SUC2*) and MCY3017 (*MAT leu2-3,112 his3-200 ura3-52 bdf12::LEU2 SUC2*) were used in this study. The ts mutants are in the BSY295 background. The *SNR14** represents the tagged U4 allele. Standard genetic methods were used.

snRNAs analysis

The screen for mutants defective in snRNP assembly will be described in detail elsewhere (Z.Lygerou and B.Sraphin, in preparation). Briefly, a GAL-regulated tagged U4 (U4*) gene was constructed by introducing a neutral tag in the U4 coding sequence (positions 76–86 were changed to CUCUUAAGCC-AUAG) and fusing the GAL-U1 promoter (21) upstream of the coding sequence as reported previously for the GAL-U2 and GAL-U5 constructs (21,22). This fusion gene uses the proper transcription initiation site (data not shown). The U1 snRNA TATA box region was used because the conservation of the TATA box region in yeast U snRNAs makes them interchangeable (21,23). The fusion construct was integrated in a single copy at the *TRP1* locus to give strain BSY295. Strain BSY295 and thermosensitive derivatives thereof were grown at 23C in YPlactate–glycerol media to OD₆₀₀ 0.5–1.0. They were shifted to 37C by quickly adding one volume of YPlactate–glycerol media pre-warmed at 51C. After incubation at 37C for 30 min, the U4* gene was induced by addition of galactose to 2% final. Two hours later, cells were collected and RNA extracted (24). RNAs were fractionated on 5% non-denaturing polyacrylamide gels, electro-transferred to nylon membrane (GeneScreen) and hybridized at 37C with 5' labeled oligonucleotide ol23 (5' ACGCTATGGCTTAAGAGAATT 3') complementary to the U4* snRNA and oligonucleotide DT168 (24) complementary to U1 snRNA. To test for the stability of snRNAs in the *bdf1* background, cells were grown at 23C in YPGal media and shifted to 37C in YPGal media for the

indicated time before RNA extraction. As a control an aliquot of the culture of *bdf1-1* strain was transferred to YPD at 23C and RNA collected at the same time points. For the analysis of snRNA production in the *bdf1* null mutant, cells were grown in YPD at 23C and shifted in YPD at 37C for the indicated time. Primer extensions were done as previously described. The following oligonucleotides were used: U1 [DT168 (24)]; U2 (DT800, 5' GCCAAAAAATGTGTATTGTAAC 3'); U4 (oligo6, 5' GGTATTCCAAAAATTCCTACATAGTC 3'); U5 (EM19, 5' GGCCACAGTCTCTGATGTTG 3') and U6 (oligo5, 5' TCATCCTTATGCAGGG 3'). Oligo5 was also used for Northern analysis. The U4 and U4* snRNA can be distinguished by primer extension using oligo6 which hybridizes at their 3' ends because they differ by three nucleotides in length. For primer extension, RNA concentration was calculated from OD_{260nm} reading while RNA quality was ensured by calculating the ratio OD_{260nm}/OD_{280nm}. Equal loading is shown by control extension on unaffected snRNAs. The faint band seen above the U4 and U4* bands can also be used for loading control. This band does not represent a spliceosomal snRNA.

Cloning the *BDF1* gene

A *bdf1-1* strain was transformed with a yeast genomic library on a LEU2-marked centromeric vector and plated on YPD at 37C. From independent transformants, three different complementing plasmids were recovered. Restriction analysis indicated that the genomic inserts of these plasmids originated from a single locus (Fig. 4). The *BDF1* gene was further localized by subcloning DNA fragments shared by the three original plasmids and assaying for complementation of the thermosensitive growth phenotype (Fig. 4). To check that the cloned fragment covers the *BDF1* gene we used it to target integration of a LEU2 marker in the genomic DNA of a wild-type strain. Southern blot confirmed that the integration occurred correctly. The resulting strain was crossed with a *bdf1-1* strain and tetrads were dissected. Each of the 10 tetrads analyzed contained two thermoresistant spores that were Leu⁺ and two thermosensitive spores that were Leu⁻.

The *BDF1* gene was also cloned independently by screening a yeast chromosomal gt11 expression library (25) using polyclonal antibodies raised against SDS/polyacrylamide gel-purified 95 kDa polypeptide from a partially purified preparation of TFIIC (see Discussion). The 4.4 kb *EcoRI* insert of the largest clone was used for further analysis.

Nucleic acid methods

The 4.4 kb *EcoRI* fragment that overlaps the *BDF1* gene was completely sequenced on both strands by subcloning fragments in appropriate vectors and using the dideoxy chain termination method (26). Sequence analyses and database searches were done using standard programs (27,28). Southern and Northern analyses were performed using standard protocols (26).

Disruption of the *BDF1* gene

The pBAC5 plasmid contains the 4.4 kb *EcoRI* fragment harboring the *BDF1* gene in a pBluescript KS backbone. pBA-C5 was cleaved with *SmaI* and *SpeI*, filled with Klenow and self ligated to give pBAC24. pBAC24 was cleaved by *BamHI* and *BglII*. The 2.1 kb *BamHI*–*BglII* fragment was replaced by a 1.8 kb *BamHI*/*BamHI* fragment that contains the yeast *HIS3* gene creating pBAC25. DNA from pBAC25 cleaved with *EcoRI* was used to transform a *his3/his3* diploid yeast strain made by

crossing strains YNN281 (a) x YNN282 (α). Correct integration was verified by Southern analysis. A second disruption, *bdf1* Δ 2::LEU2 was made in the same way in strain MCY3015 by replacing the 2.1 kb *Bam*HI–*Bgl*III fragment of pBAC24 with the 2.9 kb *Bgl*III–*Bgl*III fragment harboring the yeast *LEU2* gene.

Assaying the phenotypes of the *bdf1* disruption strain

For the YNN281xYNN282 derivatives, growth was scored on solid rich medium containing 2% glucose (YPD) or other carbon sources as indicated in the results. Hypertonic growth conditions were monitored on YPD containing 2 M ethylene glycol. With the MCY3015 derivatives, growth on different carbon sources was scored by spotting cell suspensions on solid media. Growth on rich medium containing glucose (2%), galactose (2%), sucrose (2%) or raffinose (2%) was scored anaerobically by incubating the plates in a GasPak disposable anaerobic system (BBL) or scored aerobically in presence of 1 μ g/ml of antimycin A. Antimycin A increases the dependence on these sugars by blocking respiration. Growth on rich medium containing glycerol (3%) was scored aerobically. Glucose-repressed and derepressed cells were prepared from exponentially growing cultures as described previously (29). Secreted invertase activity was assayed in whole cells (30).

Nucleotide sequence accession number

The sequence reported here has been entered into the EMBL and GenBank data bases, under the accession number Z18944.

RESULTS

Characterization of a strain defective in U4 snRNA accumulation

We have recently developed a screening procedure for yeast mutants that would affect snRNP synthesis (Z.Lygerou and B.Séráphin, in preparation). This screen makes use of a reporter U4 snRNA gene (hereafter referred to as U4*) with the following properties: i) The reporter U4 gene contains a tag that allows for its easy and specific detection. The tagged U4 snRNA is fully functional and assembles in a U4/U6 complex in the same way as the wild-type U4 snRNA. ii) A single copy of the reporter U4 gene is integrated at the TRP1 locus while a functional copy of the wild-type U4 gene is still present at the original locus. iii) The transcription of the reporter gene is driven by the GAL regulatory sequence (21,31). This promoter can be conveniently turned on to initiate synthesis of the U4* snRNA whose assembly into snRNP can then be followed. Without an inducible promoter, the identification of *trans*-acting mutants defective in snRNP synthesis would have been complicated by the extremely long half-life of the snRNAs in yeast [(32) see also refs. (21,22) and B.S. unpublished observation].

A strain carrying the U4* snRNA was mutagenized and temperature sensitive mutants were recovered, as snRNP synthesis is likely to be an essential process (3). Mutants were grown individually, shifted to the non-permissive temperature (37°C) after which the transcription of the U4* snRNA gene was turned on by addition of galactose. RNAs extracted from these strains were fractionated on native gels and analyzed by northern blotting using a probe specific for the U4* snRNA. In a wild type strain, almost all of the resulting U4* snRNA migrates as a U4*/U6 RNA hybrid (e.g., Fig. 1C, lane 2). This indicates that the U4* snRNA was transcribed and assembled into a functional U4* snRNP that is competent to associate with the

Figure 1. A *bdf1*^{ts} strain is defective in U4 snRNA accumulation. (A) Levels of U4* snRNA in the *bdf1*^{ts} strain and two temperature sensitive strains, ts22 and ts23, at the non-permissive temperature. The position of migration of U4*/U6 and free U4* snRNA are indicated. The U1 snRNA serves as a loading control. The pattern observed for strains ts22 and ts23 is identical as the pattern of the isogenic wild type strain (data not shown). (B) State of the endogenous U6 and U4 snRNA in the *bdf1*^{ts} strain and strains ts22 and ts23. The filter presented in figure A was stripped and reprobed for the U6 snRNA. [The slight reduction in the level of U6 snRNA in strain *bdf1*^{ts} might result from a reduction in U6 synthesis (see text and Figure 6)]. The presence of a U4/U6 species indicates that the endogenous U4 is associated with U6 in the *bdf1*^{ts} strain and that the probe for the U4* snRNA (Panel A) does not cross-hybridize with the endogenous U4. (C) Co-segregation of the thermosensitive phenotype and absence of U4* in two tetrads. For each of the two tetrads, the phenotypes of the four spores (labeled A–D) are shown. Growth, or absence of growth, at 37°C is indicated by + or –, respectively. Labels are as in part A.

U6 snRNP. Two main mutant phenotypes were observed. In some cases no signal corresponding to the U4* snRNA is detected. This suggests either that the U4* snRNA is not transcribed or that it is unstable in this mutant. In other mutants, the U4* snRNA accumulates as a free U4* snRNA species. This suggests that the assembly or intracellular transport of the U4* snRNP is defective. Alternatively the mutated gene might be required for the stability of the U6 snRNA or for the joining of the U4* and U6 snRNPs into the U4*/U6 particle. The exact function affected by individual mutants can then be assessed by additional experiments.

We report here the isolation and characterization of a thermosensitive strain, referred to as *bdf1*^{ts}, in which the transcription of the U4* gene is blocked at the non-permissive temperature. Figure 1A shows that in the *bdf1*^{ts} strain the U4*

snRNA does not accumulate at the non-permissive temperature. This is not a general phenotype for thermosensitive strains grown at 37°C (see control ts22 and ts23 strains in Fig. 1A). The absence of U4* snRNA in the *bdf1^{ts}* strain can not be explained by underloading or non-specific RNA degradation as the level of the U1 snRNA is close to normal in that strain (Fig. 1A). The absence of the U4* snRNA in the *bdf1^{ts}* strain is only seen at the non-permissive temperature (data not shown) indicating that the reporter gene is still present and functional. To check if the phenotype observed in the *bdf1^{ts}* strain reflects a general instability of the U4 and/or U6 snRNAs, the original probe was stripped and the filter was hybridized with an oligonucleotide complementary to U6 snRNA. The results (Fig. 1B) show that in the *bdf1^{ts}* strain a substantial amount of endogenous U4 and U6 RNAs are present as U4/U6 hybrid or as free U6 RNA. (Although the amount of free U6 snRNA is slightly reduced in the mutant strain, U6 snRNA is still in excess over U4 snRNA allowing the assembly of all of the wild-type U4 snRNA in U4/U6 snRNP.) This indicates that the newly synthesized U4 snRNA is specifically affected and rules out the possibility that the *BDF1* gene is involved in U4/U6 association during splicing.

To determine if the absence of the U4* snRNA and the temperature sensitive phenotype present in the original strain are linked, we crossed the *bdf1^{ts}* strain to a wild type strain and assayed for the segregation of these phenotypes in the meiotic progeny. The results for two representative tetrads are shown in Figure 1C. In every case the absence of U4* snRNA at the non-permissive temperature co-segregates with the temperature sensitive phenotype. Furthermore the U4* under-accumulation phenotype segregated 2:2 in three tetrads while the temperature sensitive phenotype segregated 2:2 in 6 tetrads. This indicates that a single gene, *BDF1*, is responsible for both phenotypes.

The *bdf1-1* allele might have affected the production of the U4* snRNA by interfering with the induction of the GAL regulatory sequence present upstream of the TATA box of our test gene. This was unlikely because the *bdf1^{ts}* strain grows well on galactose containing media. Nevertheless, to rule out this possibility we followed the induction of the GAL10 mRNA in the *bdf1^{ts}* strain. Additional strains were used as positive (ts33 and wild-type) or negative controls (ts5 that is defective in transcription). These strains were grown at the permissive temperature in lactate-glycerol media, switched to 37°C for 20 min and the GAL10 transcription was induced by addition of galactose. After two further hours of incubation at 37°C the cells were collected, RNAs extracted and analyzed by northern blot using a probe specific for the *GAL10* gene. The results shown in Figure 2 indicate that the GAL10 mRNA is induced in the *bdf1^{ts}* strain. We conclude that the mutant gene does not prevent induction via the galactose regulatory sequence.

The reporter U4 snRNA is stable at the non-permissive temperature

The results reported above suggest that the *bdf1^{ts}* strain is defective in the production of the U4* snRNA. One explanation for these results is that in the *bdf1-1* background this snRNA is unstable. To test this possibility, isogenic strains, carrying either the *bdf1-1* or *BDF1* allele, were grown at the permissive temperature in galactose media thereby inducing the transcription of the U4* snRNA. These strains were then transferred to the non-permissive temperature and RNAs extracted at various time points. These RNAs were then analyzed by primer extension using a primer that allows the detection of both U4 snRNAs

Figure 2. The GAL10 mRNA is induced in a *bdf1^{ts}* strain at 37°C. Induction of the GAL10 mRNA in strains ts5, *bdf1^{ts}*, ts33 and wild type (WT) was assayed by Northern blotting. Normal induction is seen for all the strains except ts5 that is defective in transcription. The small variation in the level of the GAL10 mRNA in lanes 2–4 are due to loading as shown by probing the same gel with a probe for the RP29 ribosomal protein mRNA.

Figure 3. The reporter U4 is stable in a *bdf1^{ts}* strain at 37°C. The levels of the U4*, endogenous U4 and U2 snRNAs were assayed by primer extension. The *bdf1^{ts}* strain, pre-grown in galactose media, was switched either to 37°C in galactose media or kept at 23°C in glucose media for the indicated time. As a control the wild type (WT) strain was also switched to 37°C. The two U4 snRNA species differ by three nucleotides in length.

Figure 4. Organization of the *BDF1* locus on chromosome XII. Some restriction sites are indicated. The location and direction of transcription of the *SFP1*, *BDF1* and *SKI2* genes are indicated. The presence of the 3' end of a putative open reading frame upstream of *BDF1* is depicted (ORF1, 105 amino-acids). Interestingly, the ORF1 encoded product shows significant sequence similarity with the C-terminal region of a protein of unknown function from *Bacillus subtilis* (Accession number: D26185). The extents of the genomic DNA present in the three plasmids recovered by complementation of the temperature sensitivity of the *bdf1-1* strain are indicated below the map (first three lines). The structure of the subclones used to localized the *BDF1* gene is also indicated below (four last lines). + and - signs indicate if the corresponding plasmid complemented or not a *bdf1-1* mutant. The central *EcoRI* fragment was sequenced.

owing to their size difference. As control we simultaneously used a primer specific for U2 snRNA (Fig. 3) and for U1, U5 and U6 snRNA (data not shown). The U4* snRNA, that represents about half of the starting U4 snRNA, is still present at high levels in the mutant cells even 24 h after the shift to the non-permissive temperature (lanes 1–5). In contrast, when the same strain is kept at permissive temperature but shifted to glucose media (to repress the GAL promoter) the U4* snRNA decreases to a level undetectable by 24 h (lanes 6–9). These results indicate that the U4* snRNA is stable for long periods at the non-permissive temperature in a *bdf1^{ts}* strain. Because instability of the U4* snRNA cannot explain why the newly synthesized reporter snRNA is not detected in the *bdf1^{ts}* strain at 37°C, we conclude that the *BDF1* gene is required for the transcription of the U4* snRNA. However, we cannot formally rule out that BDF1p is required for the stability of nascent transcripts. This hypothesis seems unlikely because of the presence in BDF1p of motifs characteristic of transcription factors and because our results suggest that BDF1p interacts (directly or indirectly) with DNA (see below).

Cloning and sequencing of the *BDF1* gene

The *BDF1* gene was cloned by complementation of the *bdf1-1* thermosensitive phenotype using a library of genomic fragments from a wild type yeast strain inserted in a centromeric vector. The three different plasmids that were recovered originated from a single locus (Fig. 4). The cloned DNA directed integration of a LEU2 marker to the *BDF1* locus demonstrating that the cloned fragment is allelic with the *bdf1-1* mutation. The complementing activity was further localized to a *XhoI*–*EcoRI* fragment by subcloning and testing the resulting constructs for complementation of the thermosensitive phenotype.

A 4.4 kb *EcoRI* fragment covering the *BDF1* gene was entirely sequenced on both strands (Fig. 4) uncovering a long open reading frame of 2061 base pairs. The DNA upstream of the first ATG in the open reading frame is extremely AT rich with two imperfect TATA boxes located at positions –80 and –115. Northern analysis with a probe internal to the BDF1 reading frame revealed the presence of a major mRNA of 2.4 kb and a minor mRNA of 3 kb (data not shown). Southern analyses indicates that there is a single copy of the *BDF1* gene in the yeast genome (data not shown). Comparison of the sequence obtained from one extremity of one of the original plasmids indicated that the *BDF1* gene is tightly linked to the *SFP1* gene (Fig. 4). Because the *SFP1* gene has been mapped to chromosome XII between *ura4* and *ilv5* (33), we concluded that *BDF1* lies in the same region. Consistent with this, a *BDF1* probe hybridized to one of the largest yeast chromosomes (IV or XII, data not shown) in genomic Southern blot. While this work was in progress, Widner and Wickner reported the sequence of the *SKI2* gene (34). Database search indicated that the *SKI2* gene lies immediately downstream of the *BDF1* gene (Fig. 4). Consistent with our data, the *SKI2* gene has been genetically and physically mapped to the *ura4*–*ilv5* interval on chromosome XII (34).

Analysis of the *BDF1* encoded protein

The *BDF1* encoded protein (BDF1p) is 687 amino-acids long (calculated molecular weight of 77 084 Da) and acidic (predicted pI 5.6). However, *in vitro* expression of the *BDF1* gene yielded a single aberrantly migrating polypeptide that comigrated with a 94 kDa marker protein (data not shown). BDF1p contains several domains of biased composition (proline-rich: residues

124–151, basic domain: residues 486–525 and acidic regions: residues 420–454 and 674–687). Putative nuclear localization signals can also be found in BDF1p, consistent with the result of a recent study (35) showing that fragments of BDF1p can target a fused β -galactosidase reporter protein to the nucleus.

BDF1p shows sequence similarity to Ring3, fsh and Orfx proteins, however, this similarity is limited to three domains. First, BDF1p contains two copies of a repeated sequence of 87 amino-acids (Fig. 5A and B) rich in proline and aromatic residues called bromodomain (36,37). To date, bromodomains have only been observed in proteins involved in transcription or in proteins with no known function. This motif occurs twice in BDF1p, fsh, CCG1, RING3 and YK107 proteins and once in the other proteins (Fig. 5B). The two BDF1 bromodomains share some distinctive characteristics with the bromodomains of *Drosophila* fsh and human Ring 3 as well as the bromodomains present in the Orfx product that is encoded by a randomly sequenced human cDNA. These include a 2 amino-acid insertion at positions 15–16 as well as some specifically conserved residues (e.g., D at position 10, Y at position 59, M at position 71 and P at position 87).

Besides the bromodomains, BDF1p contains a new domain named the ET domain (for Extra Terminal domain) which shows sequence similarity with the Ring3, fsh and Orfx protein sequence. The ET domain is located at the extreme C-termini of the proteins except for the long form of the fsh protein (Fig. 5A). The amino terminal part (residue 1–64, Fig. 5C) of this 167 amino-acid long domain shows significant sequence conservation [also noted by Widner and Wickner (34)]. In contrast the carboxy-terminal part of this domain is less conserved in primary sequence but is highly enriched in serine, threonine and charged residues.

Disruption of the *BDF1* gene

The *BDF1* gene was disrupted in two different diploid strains by replacing part of its coding sequence with selectable markers. Upon sporulation at 30°C, all asci analyzed gave four viable spores. Thus the *BDF1* gene is not essential for viability. However, the two spores carrying the disrupted *bdf1* allele were distinctly smaller than the wild-type spores. *bdf1 Δ* carrying haploid strains presented morphological and growth defects: they grew 2.5 times slower than the wild-type haploid at 30°C and were thermosensitive and cryosensitive at 37 and 16°C, respectively. The disruption conferred a flocculent phenotype and the mutant cells looked larger and rounder than the wild-type cells. The *bdf1 Δ* haploids showed defects in utilizing various carbon sources as well as in resistance to hypertonic media. The cells exhibited no growth on YPD + 2 M ethyleneglycol, on YP + 2% galactose, YP + 2% ethanol or YP + 2% melibiose, and very slow growth on YP + 2% glycerol and YP + 2% maltose. The *bdf1* null mutants share several common phenotypes with previously isolated mutants in glucose repression [e.g., (29)], however direct measurement of invertase levels indicated that BDF1p does not have a major role in *SUC2* expression. Thus BDF1p is probably not directly involved in glucose repression.

A *bdf1* null allele is defective in snRNA production

The conditional *bdf1-1* allele recovered from our screen is involved in the transcription of the U4* snRNA. We tested whether the *BDF1* gene is also required for transcription of the endogenous snRNAs. A strain carrying the *bdf1 Δ* null allele as well as a wild type control strain were grown at 23°C in glucose containing media. Cells were shifted to 37°C at time zero

Figure 5. Bromodomains and ET domains. (A) Structure of the BDF1, Ring3, Orfx and fsh proteins. The short and long form of the fsh protein result from alternative splicing. The long form contains all the amino acids present in the short form. Other events of alternative splicing make additional small changes in these proteins. The bromodomain and ET domain are indicated as dark and gray areas respectively. (B) Alignment of the bromodomains. The protein and species names are given. For proteins with two bromodomains, these are indicated by the protein name followed by a dash and 1 or 2. The number of amino acids preceding and following the bromodomain in a given protein are indicated before and after the protein sequence, respectively. For the fsh protein, the numbers of amino acids following the second bromodomain in the short and long form are both indicated. For proteins with two bromodomains, the number of amino acids present between the two domains is indicated after the first domain and before the second domain either followed or preceded by a dash. The consensus sequence represents the positions that are more than 60% conserved. The Swissprot, EMBL or Genebank accession number for the protein sequences, or for the nucleic acid sequences from which peptide sequences were derived, are as follows: CE0201 (T01020), SPT7 (L2537, X76294, M87651), GCN5 (Q03330), BDF1 (this study, Z18944), Ring3 (P25440), Orfx (D26362), Fsh (P13709), R10E11.1 (Z29095), p300 (U01877), CREB-BP (S66385), CCG1 Human (P21675), CCG1 Hamster (D26114), TFIID-230 (S61883), YK107 (Q02206), HBm/hSNF2 α (X72889, D26155), BRG-1/hSNF2 β (S66910/D26156), Brm (P25439), SNF2 (P22082), NPS1/STH1 (P23597), Prgm (M91585). (C) Alignment of the ET domains. Conventions are as in part B except that the consensus represents amino acids that are conserved between the four sequences. Amino acids indicated in small characters in the fsh sequence (positions 77–81) are absent in some alternative splicing variants. In that case they are replaced by a single histidine residue.

and collected at various time points. RNAs were extracted and analyzed by primer extension using primers specific for the U1, U2, U4, U5 and U6 snRNAs. The results, depicted in Figure 6, indicate that the U2 and U6 snRNAs were the most affected by the *bdf1Δ1* null mutation but levels of the U1 and U4 snRNA decreased also significantly. In contrast, the level of the U5 snRNA was barely affected. No effect of the temperature shift on snRNA levels could be detected in the wild type control strain. Comparison of the level of U2 snRNA (quantified using a PhosphorImager) and of the growth of cells (followed by optical density) after the temperature shift indicates that the decrease in the level of U2 snRNA is strictly accounted for by dilution of the pool of snRNAs present at the time of the temperature shift by cell division (data not shown). Given the very long half life of snRNAs in yeast [(32), see also Fig. 3] this strongly suggests that the *BDF1* gene is involved in the transcription of the U2 snRNA and possibly also of the U1, U4 and U6 genes. The *bdf1-1* allele recovered from our screen affected the transcription of the U4* but not the transcription of the endogenous U2 and U4 snRNAs (see Fig. 3 and data not shown). This probably results from an incomplete block of *BDF1* function with this allele. This is supported by the fact that strains carrying the *bdf1-1* allele grow well on galactose and do not display a cryosensitive phenotype.

Figure 6. snRNA levels in a *bdf1Δ1* strain. The levels of the U1, U2, U4, U5 and U6 snRNA in a strain carrying a disruption of the *BDF1* locus (*bdf1Δ1*) and a wild type (*BDF1*) control were assayed by primer extension. The strain were grown in YPD and switched to 37°C for the indicated time.

DISCUSSION

We have shown that the wild-type *BDF1* function is required for the production of a reporter U4 snRNA. Furthermore our data demonstrate that the absence of this RNA in the *bdf1-1* strain after shift to galactose is not due to its instability. This indicates that *BDF1* is required for the transcription of our reporter gene. In wild type cells, BDF1p is probably involved in the transcription of the U2 snRNA and possibly also U1, U4 and U6 snRNAs. However, the multiple phenotypes (thermosensitive and cryosensitive growth, flocculation and absence of, or reduced growth on several carbon sources) observed with the *bdf1* deletion mutants suggest that BDF1 affects the transcription of many genes. These phenotypes are consistent with BDF1p being a transcription factor as similar pleiotropic phenotypes have been reported for mutations affecting general transcription factors (38). For example mutations in *SPT7* also result in clumpiness (39) while deletion of *SNF2/SWI2* results in defective growth on several carbon sources (29,40). How does *BDF1* affect transcription in yeast? We have shown that the galactose induction pathway is functional in a *bdf1-1* strain by analyzing the induction of the *GAL10* gene. However the activation signal is not transmitted from the upstream GAL4 binding site to the downstream gene in the case of the U4* snRNA reporter, even though it contains an identical GAL UAS. The main difference between these two genes resides in the TATA box and transcription initiation region. As for other snRNAs, the TATA box present upstream of the U4 reporter gene is highly conserved in sequence and position (23). This is rather unusual for yeast promoters. One might thus speculate that BDF1p is specifically required for bridging upstream activators and the TFIID complex present on the snRNA TATA box. In that context, it is noteworthy that two other bromodomain containing proteins (CCG1 and SPT7, Fig. 5 and see below) have been shown to interact physically or genetically with the TBP subunit of the TFIID transcription factor (39,41–43). An alternative possibility is that BDF1p affects chromatin structure. The specific effect of the *bdf1-1* mutation on our reporter gene would then result from its chromosomal location, that differs from the GAL locus. An effect of BDF1 on chromatin could also explain why the different snRNAs (e.g., U2 and U5) are affected to a different extent by the disruption of the *BDF1* locus. This hypothesis is not unreasonable since the bromodomain containing protein encoded by the *SNF2/SWI2* gene (SNF2p) has been shown to affect chromatin structure (44). Both of these models are consistent with the nuclear location of BDF1p (35).

The product of the *BDF1* gene was fortuitously found to copurify with a preparation of the TFIIC factor (45). When polyclonal antibodies raised against the gel purified 95 kDa TFIIC subunit were used for screening a yeast chromosomal λ gt11 expression library, the only clones recovered contained the *BDF1* gene. Recombinant BDF1 protein was shown to be recognized by antibodies raised against the 95 kDa component of yeast TFIIC (data not shown). These antibodies alter the migration of TFIIC–DNA complexes in mobility-shift assays and to inhibit tRNA synthesis in an *in vitro* transcription system (45). However, fragments of peptide sequences that have been obtained for the 95 kDa subunit of TFIIC were not found in the polypeptide encoded by the *BDF1* gene (46). These data indicate that the *BDF1* gene encodes an immunodominant contaminant that comigrates with the 95 kDa subunit of TFIIC.

These data suggest that BDF1p is a DNA binding protein since it copurifies with the yeast TFIIC transcription factor during four chromatographic steps including a DNA affinity column. Alternatively, BDF1p might interact indirectly with DNA through a tight association with a DNA binding protein. Both of these possibilities are consistent with the involvement of BDF1p in transcription.

To test whether *BDF1* could function directly in transcriptional activation, we constructed LexA-BDF1 and GAL4-DNA binding domain-BDF1 (G_{BD} -BDF1) fusion constructs under the control of the constitutively active *ADHI* promoter. Complementation analyses indicate that the LexA-BDF1 fusion provides full *BDF1* function while the G_{BD} -BDF1 is only partially functional. The LexA-BDF1 or G_{BD} -BDF1 protein fusions were unable to activate the transcription of reporters carrying the corresponding DNA binding sequences including the U4* snRNA (data not shown). Thus, we were unable to detect transactivation at promoters to which BDF1p was targeted by fusion to two specific DNA binding domains. Although these negative results could be explained by the nature of the fusions made, this strategy has often been successful. This suggests that some bromodomain containing proteins, including BDF1p, might not be able to directly activate transcription (44).

Two bromodomain motifs are found in the *BDF1* product. Database search reveals that bromodomains are widespread, being present in proteins from yeast, animals and plants (the partial sequence of a plant bromodomain can be found in a randomly sequenced expressed sequence tag from rice, accession number OS1690A). We recovered 20 bromodomain containing proteins making a total of 28 bromodomains as one or two bromodomains are present in individual proteins. The location of this motif within individual proteins is highly variable (Fig. 5B). The functional significance of the bromodomain is not known. However all proteins of known function that harbor a bromodomain are involved in some aspect of transcription. This includes the SPT7 protein that has been shown to genetically interact with the *SPT15* gene encoding the TBP subunit (39). *GCN5* is necessary for the activity of at least two sequence specific transcriptional regulators (47). The two *Drosophila* proteins encoded by the maternally expressed and alternatively spliced *fsh* transcripts are necessary for the activation of the *Ubx* gene (48,49). The product of the human CCG1 gene is a TFIID subunit, as are its homologues in *Drosophila* and hamster (41–43). The murine CREB-binding protein (CREB-BP) is probably involved in the normal delivery of a cAMP-dependant transcriptional activation signal (50,51). CREB-BP is highly related, but not homologous, to the E1A-associated protein p300, a putative TBP-associated polypeptide (51) that also affects transcription (52). *Drosophila* brm protein is required for the transcriptional activation of several *ANT-C* and *BX-C* genes (37) while its human counterparts (hbrm/hSNF2 α /BRG-1/hSNF2 β) affect *in vivo* transcription and potentiate transcriptional activation by the glucocorticoid, oestrogen and retinoic acid receptors (53–55). In yeast, SNF2p is part of a large complex (56,57) required for the transcriptional activation of numerous endogenous genes (29,58–61) as well as transcriptional enhancement by steroid receptors (62). CCG1 and STH1/NPS1 proteins have been shown to affect cell cycle progression. In the former case, it has been shown that the CCG1 protein is required for transcriptional activation of genes whose products are required at a specific step in the cell cycle (63). We have now shown that BDF1p, a nuclear protein that contains

two bromodomains, is required for expression of many yeast genes and in particular for the transcription of snRNA genes. Of the six remaining proteins that contain bromodomains, four have been identified through random sequencing projects [CE0201 (64), Orfx (65), R10e11 (66) (related to CREP-BP and p300 (51)) and YK107 (67)] while one (human Ring3) was identified as a homologue of the *Drosophila* fsh protein (68). The last one, human peregrin (69), is a nuclear protein containing a zing finger making it likely to be also involved in transcription. It has been proposed that the bromodomain could serve as a surface for intermolecular protein-protein interactions influencing the assembly or activity of multicomponent complexes involved in transcription activation (36).

Beside the bromodomain, BDF1p contains a new conserved domain at its C terminus. This ET domain is also present at the C-terminus of the Ring3, Orfx and the short form of the fsh protein and in the middle of the long form of the fsh protein. Data base search indicates that the ET domain is restricted to this four proteins. Interestingly these four proteins contain two bromodomains. Furthermore the bromodomains of these proteins show more sequence similarity to each other than to other bromodomains (see Results and Figure 6). Even greater conservation is observed if the four N-terminal and C-terminal bromodomains are aligned separately. The particular conservation of the two bromodomains and of the presence of the ET domain makes it likely that *BDF1* represents the yeast homologue of the *Drosophila* fsh and human Ring3 and Orfx even though sequence similarity between these proteins is mostly restricted to these three regions. Further studies will be required to pin-point the role of *BDF1* in transcription as well as the function of bromodomains in transcription factors.

ACKNOWLEDGEMENTS

Z.Lygerou, C.Conesa and P.Lesage contributed equally to this work. We thank C.Marck, T.Gibson and C.Ouzounis for help with biocomputing, M.Snyder for the gift of the lamdba expression library and P.Thuriaux for providing yeast strains and plasmids. We are also grateful to G.Dujardin, F.Forquignon, B.Lebianc, I.Mattaj and S.Taraviras for comments and/or discussions. This work was supported by EMBL (Z.L. and B.S.) and NIH grant GM47259 to M.C. P.L. was supported by an 'Association pour la Recherche contre le Cancer' post-doctoral fellowship. B.S. is on leave from CNRS.

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