Essential Roles of Bdp1, a Subunit of RNA Polymerase III Initiation Factor TFIIIB, in Transcription and tRNA Processing

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The essential *Saccharomyces cerevisiae* gene *BDP1* encodes a subunit of RNA polymerase III (Pol III) transcription factor (TFIIIB); TATA box binding protein (TBP) and Brf1 are the other subunits of this three-protein complex. Deletion analysis defined three segments of Bdp1 that are essential for viability. A central segment, comprising amino acids 327 to 353, was found to be dispensable, and cells making Bdp1 that was split within this segment, at amino acid 352, are viable. Suppression of *bdp1* conditional viability by overexpressing *SPT15* and *BDF1* identified functional interactions of specific Bdp1 segments with TBP and Brf1, respectively. A Bdp1 deletion near essential segment I was synthetically lethal with overexpression of *PCF11*, a dominant gain-of-function mutation in the second tetracopeptide repeat motif (out of 11) of the Tfc4 (π131) subunit of TFIIIC. The analysis also identifies a connection between Bdp1 and posttranscriptional processing of Pol III transcripts. Yeast genomic library screening identified *RPR1* as the specific overexpression suppressor of very slow growth at 37°C due to deletion of Bdp1 amino acids 253 to 269. *RPR1* RNA, a Pol III transcript, is the RNA subunit of RNase P, which trims pre-tRNA transcript 5' ends. Maturation of tRNA was found to be aberrant in *bdp1-D253-269* cells, and *RPR1* transcription with the highly resolved Pol III transcription system in vitro was also diminished when recombinant Bdp1Δ253-269 replaced wild-type Bdp1. Physical interaction of RNase P with Bdp1 was demonstrated by coimmunoprecipitation and pull-down assays.

RNA polymerase III (Pol III) transcribes genes encoding tRNAs, SS rRNA, U6 snRNA, and other small RNAs. Accurate initiation of this transcription requires basal transcription factor IIIA (TFIIMA), TFIIIB, and TFIIIC. In the yeast *Saccharomyces cerevisiae*, TFIIIB is required for all Pol III transcription in vitro and in vivo, TFIIIC is required for all Pol III transcription in vivo, and TFIIIA is required only for SS tRNA gene transcription. TFIIIB is composed of three subunits, TATA-binding protein (TBP), Brf1 (the TFIIIB-related factor), and Bdp1; all three are also essential for Pol III transcription in vitro (7, 12, 14, 21, 27, 28, 33, 40, 51, 52, 65). In vivo analysis defines multiple interactions of TFIIIB with the rest of the *S. cerevisiae* Pol III transcription machinery; Brf1 and Bdp1 interact with Tfc4 (the second largest subunit of TFIIIC), and Brf1 also interacts with the RPC34 and RPC17 subunits of Pol III (3, 5, 19, 20, 39, 47, 52, 64).

In human cells, two TFIIIB-related assemblies have been identified (46, 60). TFIIIBα, which contains TBP, Bdp1 (previously called hTFIIIB50), and Brf2 (a hBrf1 parologue previously called hTFIIIB50), is required for transcription of Pol III genes with upstream promoter elements, such as 7SK and U6 (53, 61). TFIIIBβ, containing TBP, Bdp1, and Brf1, is required for transcription of genes with internal promoters (53). Alternatively spliced variants of hBrf1 have also been noted (44). Human TFIIIB interacts with a subcomplex of Pol III-specific subunits—hRPC32, hRPC39, and hRPC62 (homologues of yeast Rrpc31, Rrpc34, and Rrpc82, respectively)—through direct interactions hBrf1 and hTBP with hRPC39 (63).

The conservation of interactions of yeast and human Brf1 and yRPC34/hRcp39 implies a conservation of TFIIIB functions between yeast and higher eukaryotes. Functional domains of the subunits of yeast TFIIIB have been analyzed by in vitro transcription, gel shift assay and DNA footprinting (3, 13, 24, 26, 29, 30, 36, 55, 56). Although TFIIIB can bind directly to genes with strong TATA boxes (43), most Pol III-transcribed genes of *S. cerevisiae*, including most tRNA genes, require prior TFIIIC binding to boxA and boxB promoter sites (6, 34). TATA box-directed binding of TFIIIB is mediated by TBP. TFIIIC-dependent recruitment of TFIIIB to Pol III promoters is mediated through interaction with Brf1 and potentially with Bdp1 and TBP (5, 15, 20, 42). The fully assembled TFIIIB-DNA complex is very stable against dissociation by high concentrations of simple electrolytes and polyanions.

The ability to recruit Pol III to the transcription start site is a key property of TFIIIB, but its Brf1 and Bdp1 subunits also play an essential role in postrecruitment steps of transcriptional initiation in vitro (22, 29, 31, 32). Less is known about functions in vivo (22), particularly in regard to Bdp1. The work that is presented here is intended to fill this gap.

Recent studies of RNA polymerase II (Pol II) focused on the relationship between transcription and mRNA maturation. mRNA processing factors, capping enzyme, splicing factors, and polyadenylation factors interact with general transcription initiation factors or with Pol II itself (reviewed in reference 49). Pol III transcripts also undergo processing. In particular, three processing steps are required for tRNA maturation: 5' end processing by RNase P, 3' end processing involving La (yeast Lhp1) and intron excision by endonuclease- and ligase-mediated splicing (2, 4, 17, 45, 67, 69). La is not essential for yeast viability (68); all genes encoding subunits of RNase P and splicing endonuclease are essential (8, 62). Direct interactions
of transcript-processing enzymes with the Pol III transcription apparatus of yeast have not been examined. Human La, a phosphoprotein, is involved in 5'-end and 3'-end processing of tRNA (18, 25), but little is known about its interaction with the relevant endonucleases.

In this work, we present the results of an analysis that identified the parts of Bdp1 that are essential to its functioning in vivo. The effects of deleting segments of Bdp1 on viability were examined, and core essential segments of Bdp1 were identified. We also report on suppressors and enhancers of conditional viability that indicate interactions of particular segments of Bdp1 with other components of Pol III transcription and identify a relationship between Bdp1 and posttranscriptional processing of Pol III transcripts.

MATERIALS AND METHODS

Media and strains. Cells were grown in synthetic dextrose (SD) medium (2% dextrose, 0.5% ammonium sulfate, and 0.17% yeast nitrogen base without amino acids and ammonium sulfate) containing required amino acids or YPD (1% yeast extract, 2% peptone, and 2% dextrose). 5-FOA plates contained 0.5% 5-fluoroorotic acid in SD medium. The strain used for the genetic analysis is a haploid BDP1 disruptant (MATa bdp1::TRP1 ura3-52 leu2-3,112 rta1-regl-501) that was replaced by the CEN plasmid pRS316. 

RPR1 expression plasmids. Rat1p expression plasmids with a centromere (CEN6/ARS4) or 2μm origin were constructed by PCR cloning. The BDP1 expression cassette plasmid pRS315UD was constructed by inserting the flanking segments of the RPR1 open reading frame, 0.5 kb upstream from its ATG codon and 0.5 kb downstream from its stop codon, as appropriately cleaved PCR products, using primers AIP003, AIP004, AIP005, and AIP006. The upstream and downstream fragments were inserted between the XhoI and PstI sites of the plasmids pRS315 and pRS316BDP1W (constructed as specified below) in two steps. First, the strain was transformed with pRS315BDP1WT and transformants were grown on 5-FOA medium to eliminate YEp24. BDP1. Second, pRS315BDP1WT was replaced by freshly introduced pRS316BDP1W by selection on SD plates for Leu·Ura growth.

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Yeast transformation. Yeast total cell extracts were prepared from 50 g of S. cerevisiae (strain YBS334; MATA pep4-3 hph1-122 ura3-52 leu2-3,112 regl-501) (23) grown in YPD medium. Cells were broken in 100 ml of disruption buffer (75 mM Tris HCl [pH 8.0], 6% [vol/vol] glycerol, 200 mM ammonium sulfate, 1.5 mM DTT, 0.15 mM EDTA, 1.0 mM phenylmethylsulfonyl fluoride [PMSF], and 0.5% benzamidine HCl) with glass beads. After centrifugation (10,000 × g, 1 h), the supernatant was dialyzed against buffer containing 50 mM Tris HCl (pH 8.0), 10% [vol/vol] glycerol, 100 mM NaCl, 0.5 mM DTT, 0.1 mM EDTA, and 0.01% Tween 20. Rabbit antibodies to E. coli-produced recombinant yeast tRNA (full length), Brfl (full length), and Bdp1 (amino acids 40 to 487) were prepared. Protein A affinity beads (protein A-Sepharose CL-4B; 25 μl) were saturated with incubation with each antisera (250 μl) and used for immunoprecipitation. Recombinant Bdp1, Bdp1Δ253-269, or BSA (50 μg each) was immobilized on cyogen bromide-activated Sepharose 4B beads (10 μl) in 1 ml of buffer containing 50 mM HEPES (pH 7.8), 10% [vol/vol] glycerol, 100 mM NaCl, 0.5 mM DTT, 0.1 mM EDTA, and 0.01% [vol/vol] Tween 20, 0.2 M NaCl, 0.5 M glycerol, and 1% dextran sulfate (SDS) (see Fig. 7A) or in 200 μl of buffer containing 100 mM NaCl and 1% (wt/vol) sodium dodecyl sulfate (SDS) (see Fig. 7A). Yeast total cell extracts were separated by 7 M urea–8% polyacrylamide gel electrophoresis (PAGE) and analyzed with autoradiography.

Immunoprecipitation and pull-down assays. Total yeast cell extracts were prepared from 50 g of S. cerevisiae (strain YBS334; MATA pep4-3 hph1-122 ura3-52 leu2-3,112 regl-501) (23) grown in YPD medium. Cells were broken in 100 ml of disruption buffer (75 mM Tris HCl [pH 8.0], 6% [vol/vol] glycerol, 200 mM ammonium sulfate, 1.5 mM DTT, 0.15 mM EDTA, 1.0 mM phenylmethylsulfonyl fluoride [PMSF], and 0.5% benzamidine HCl) with glass beads. After centrifugation (10,000 × g, 1 h), the supernatant was dialyzed against buffer containing 50 mM Tris HCl (pH 8.0), 10% [vol/vol] glycerol, 100 mM NaCl, 0.5 mM DTT, 0.1 mM EDTA, and 0.01% Tween 20. Rabbit antibodies to E. coli-produced recombinant yeast tRNA (full length), Brfl (full length), and Bdp1 (amino acids 40 to 487) were prepared. Protein A affinity beads (protein A-Sepharose CL-4B; 25 μl) were saturated with incubation with each antisera (250 μl) and used for immunoprecipitation. Recombinant Bdp1, Bdp1Δ253-269, or BSA (50 μg each) was immobilized on cyogen bromide-activated Sepharose 4B beads (10 μl) in 1 ml of buffer containing 50 mM HEPES (pH 7.8), 10% [vol/vol] glycerol, 100 mM NaCl, 0.5 mM DTT, 0.1 mM EDTA, and 0.01% [vol/vol] Tween 20, 0.2 M NaCl, 0.5 M glycerol, and 1% dextran sulfate (SDS) (see Fig. 7A) or in 200 μl of buffer containing 100 mM NaCl and 1% (wt/vol) sodium dodecyl sulfate (SDS) (see Fig. 7A).

Northern blotting. BDP1 wild-type and bdp1Δ253-269 mutant cells were grown at 30°C in YPD containing 20 μg of adenine, 15 μg of l-lysine, 10 μg of l-histidine, and 10 μg of uracil per ml and harvested just before and at 30, 60, 120, and 240 min after a temperature shift to 37°C. Harvested cells were frozen, disrupted with glass beads in disruption buffer (100 mM NaCl, 10 mM EDTA, 50 mM Tris HCl [pH 7.5], 5% SDS), and extracted with an equal amount of phenol-chloroform-isooamyl alcohol (50:10:1). After precipitation with ethanol, aliquots of extracted total RNA (10 μg) were separated by 7 M urea–6% PAGE, and analyzed by Northern blotting.

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RESULTS

Regions of Bdp1 that are required in vivo. In order to identify the essential core of Bdp1, yeast expression plasmids carrying 16 terminally truncated and internal deletion mutants of *BDP1* were constructed in centromeric and multicopy (2µ/H9262m) versions. All plasmids were introduced into a haploid strain with a disrupted chromosomal copy of *BDP1* (*bdp1::TRP1*) and resident wild-type *BDP1* expression plasmid pRS316 *BDP1* WT (*CEN URA3*). Viability was tested after selection for plasmid shuffling on 5-FOA-containing plates. Viable mutants were maintained on 5-FOA plates and checked for elimination of pRS316 *BDP1* WT by their uracil requirement. High temperature sensitivity (Ts) was monitored at 37°C and cold sensitivity (Cs) at 18°C. In the CEN plasmid series, nine mutants (*bdp1-(1-487), bdp1-(40-594), bdp1-(40-487), bdp1-(138-594), bdp1-Δ253-269, bdp1-Δ321-325, bdp1-Δ327-338, bdp1-Δ340-353, and bdp1-Δ355-372 mutants*) were viable and five of these (*bdp1-(1-487), bdp1-(40-487), bdp1-Δ253-269, bdp1-Δ321-325, and bdp1-Δ355-372 mutants*) showed different growth phenotypes (Fig. 1). In the multicopy (2µ) plasmid series, three additional mutants (*bdp1-(138-487), bdp1-(158-594), and bdp1-(158-487) mutants*) were viable, and only one mutant that was viable in the CEN series, the *bdp1-Δ355-372* strain, was inviable in the multicopy series (Fig. 1). These results identified three separate regions of Bdp1 (amino acids 158 to 252, 269 to 312, and 372 to 487; regions III, II, and I, respectively) that are required in vivo. Regions II and I overlap with domains of Bdp1 that interact with other components of the TFIIIB-DNA complex, as determined by protein footprinting (36), and region I overlaps with the relatively conserved SANT structural domain (amino acids 415 to 472) of Bdp1 (Fig. 1). In general, the requirements for Bdp1 in vivo are more restrictive than for function in the resolved Pol III transcription system in vitro (36; unpublished results) (Fig. 1, right).

Multicopy suppression. It was anticipated that the viability or temperature sensitivity (Ts or Cs) of some *bdp1* mutants might be affected by overproduction of proteins that interact with Bdp1. Recent reports show that TBP, Brf1, and Tfc4 (also called r131, TFIIIC131, and Pcf1) interact directly with Bdp1 (5, 9, 13, 35, 47, 52). To detect suppression by plasmid shuffling, strains carrying pRS316 *BDP1*WT and mutant genes on CEN plasmids were transformed by multicopy pRS423 plasmids carrying the SPT15 (encoding TBP), *BRF1*, or *TFC4* gene or the *TFC4* dominant mutant *PCF1-1* gene with a mutation in the second tetracopeptide repeat (originally isolated as a suppressor of negative effect of a tRNA gene boxA promoter mutation [50]), and also the wild-type *BDP1* gene as a control. None of the *bdp1* deletion mutants that were inviable when

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**FIG. 1.** Viability of cells producing truncated and internal-deletion Bdp1. Bars show the extent of the retained *BDP1* open reading frame (594 amino acids) in expression cassette plasmids pRS315UD (CEN LEU2) and pRS423UD (2µm HIS3), both of which contain flanking 0.5-kbp promoter- and terminator-proximal segments of *BDP1*. Shading indicates growth at 30°C after 5 days. Open bars, completely defective; black bars, viable; gray bars, viable but showing Ts and/or Cs conditional growth with a CEN or multicopy plasmid, as indicated on the right. Growth of strains in the CEN series was observed on SD plates lacking tryptophan and leucine but containing 5-FOA. Growth of strains in the multicopy plasmid series was observed on SD plates lacking histidine and leucine but containing 5-FOA. The abilities of the corresponding proteins to support TFIIIC-dependent transcription of the SUP4 rRNA*^Tyr* gene and TFIIIC-independent transcription of the U6 snRNA gene are shown on the right (data from reference 36 and unpublished data). The locations of the SANT domain and of two segments of Bdp1(*Δ*) that are protected from hydroxyl radical-mediated cleavage upon assembly into a TFIIIB-DNA complex (*Δ*) are indicated at the bottom. nd, not determined.
TABLE 1. Effects of overexpression of Bdp1-interacting proteins on temperature phenotypes of Bdp1 mutant strains

<table>
<thead>
<tr>
<th>Growth conditions</th>
<th>Bdp1 mutation</th>
<th>Growth of strain overexpressing:</th>
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<tr>
<td></td>
<td></td>
<td>None (vector)</td>
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<tr>
<td>18°C, 5 days</td>
<td>1-594</td>
<td>++</td>
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<tr>
<td></td>
<td>1-487</td>
<td>+</td>
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<tr>
<td>40-594</td>
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<td>Δ253-269</td>
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<td>Δ312-325</td>
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<td>Δ327-338</td>
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<td>Δ340-353</td>
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<tr>
<td>Δ355-372</td>
<td>+/−</td>
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<tr>
<td>30°C, 2 days</td>
<td>1-594</td>
<td>++</td>
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<td></td>
<td>1-487</td>
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<tr>
<td>40-594</td>
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<td>Δ253-269</td>
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<td>Δ340-353</td>
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<td>Δ355-372</td>
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<td>+/−</td>
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<tr>
<td>37°C, 4 days</td>
<td>1-594</td>
<td>++</td>
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<tr>
<td></td>
<td>1-487</td>
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<td>40-594</td>
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<td>Δ253-269</td>
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<td>Δ312-325</td>
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<td>Δ327-338</td>
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<td>Δ340-353</td>
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<td>++</td>
</tr>
<tr>
<td>Δ355-372</td>
<td>−</td>
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</table>

*ND*, not determined.

harbored on CEN plasmids in Fig. 1 were rescued by TBP, Brf1, or Tfc4 overexpression. One bdp1 deletion strain, bdp1(40-487), which was Ts and Cs in the initial screen (Fig. 1), failed to grow in the somewhat more complex multicopy suppression screen in attempts with two different CEN vectors (data not shown). The remaining eight bdp1 mutant strains that retained viability after loss of pRS316 BDP1 WT (by 5-FOA counterselection followed by verification of uracil auxotrophy) were examined for growth phenotype at 18, 30, and 37°C in the multicopy suppression strains. The amino acid 355 to 372 deletion proved to be lethal at 30°C in cells overexpressing PCF1-1, the dominant gain-of-function variant of Tfc4 (data not shown), and was not examined at other temperatures. The Cs phenotype of bdp1(1-487) was weakly suppressed by SPT15 (TBP) overexpression (Table 1; Fig. 2A, left). The completely Ts phenotype of bdp1-D312-325 was suppressed by overexpression of BRF1 (Table 1; Fig. 2C, right). The Ts phenotype of another internal deletion mutant, the bdp1-Δ253-269 mutant, was evidently dominant, as it was not suppressed by wild-type BDP1 (Fig. 2B, right). The normal growth phenotype of the bdp1-(1-352) strain at 37°C was somewhat impaired by overexpression of PCF1-1 both at 18°C and at 37°C (Table 1). Curiously, the bdp1-(138-594) strain, with the more extensive N-terminal deletion, did not show any influence of PCF1-1 overexpression.

A *split Bdp1 is functional*. Because plasmid shuffling experiments identified amino acids 327 to 353 of Bdp1 as dispensable (Fig. 1), one might anticipate that severing the N- and C-proximal halves of Bdp1 in this region would not destroy function in vivo. Split Bdp1 functions in vitro (55). To examine the in vivo counterpart of this result, the BDP1-disruptant strain carrying pRS316 BDP1 WT was transformed with plasmid pair pRS315 bdp1-(1-352)/pRS423 bdp1-(352-594) or pRS315 bdp1-(352-594)/pRS423 bdp1-(1-352), and viability was tested on 5-FOA plates (Fig. 3). As controls, the empty cassette plasmids and wild-type Bdp1 plasmids were also substituted for the four plasmids encoding Bdp1(1-352) and Bdp1(352-594) in all screenable combinations (Fig. 3). The strain carrying the pRS315 bdp1-(1-352)/pRS423 bdp1-(352-594) combination was indistinguishable from the wild type, and the pRS315 bdp1-(352-594)/pRS423 bdp1-(1-352) combination resulted in a slower-growth phenotype. No high or low temperature sensitivity was noted (data not shown). Bdp1(1-352) is stable, and large amounts accumulate in vivo (data not shown). We have not tried to identify localization. However, these results suggest that the C-terminal fragment Bdp1(352-594) may be less stable or not normally distributed in the cell.

**Suppressor gene of Bdp1 Δ253-269.** Cells producing Bdp1-Δ253-269 in place of wild-type Bdp1 grow very slowly at 37°C but normally at 30 and 18°C. The Ts phenotype conferred by this deletion was not suppressed by overexpression of TBP, Brf1, or Tfc4 (Table 1). As noted above, even the BDP1 wild-type gene on plasmid pRS423 did not rescue temperature sensitivity (Fig. 2B). In order to isolate suppressors, a yeast genomic library in the multicopy plasmid Yep352(URA4) was screened. From 10,000 transformants, 16 candidates were isolated (Dm1SR#1 to -16) at 37°C as cells that grew well. Seven of these were 5-FOA sensitive at 37°C (Dm1SR#3, -4, -8, -9, -10, -12, and -14). The correspond-
The suppressor is RPR1. All five non-BDP1 suppressor clones harbor three genes: TIM9 (a mitochondrial inner membrane protein), YELC (a transposon Ty4 long terminal repeat), and RPR1 (the RNA subunit of RNase P). The suppressor gene was anticipated to be RPR1, for three reasons. First, RNase P is one of the tRNA maturation enzymes, processing the 5' ends of pre-tRNAs. Yeast RNase P is composed of one RNA subunit (RPR1) and nine protein subunits; eight of the nine proteins are common to RNase P and MRP (the mitochondrial rRNA processing enzyme, which also processes nuclear rRNAs). Although the function of RNase P has been characterized, the possibility of a relationship between tRNA recognition and Pol III transcription has not been examined. Second, RPR1 has been isolated as a suppressor gene of a mutation in TFC3, the gene encoding r138, the largest subunit of TFIIIC (39). Third, RPR1 appears to be transcribed by RNA Pol III in vivo, as its transcription is impaired by both a temperature-sensitive lesion in RNA Pol III and point mutations in putative boxA and boxB TFIIIC recognition elements (37). To confirm this expectation, RPR1 with its promoter region (see below) was cloned into the multicopy vector YEp352 (2μm, URA3) and used to transform cells producing Bdp1Δ253-269 (off pRS315). YEp352 RPR1 (Fig. 4A) restored normal growth at 37°C (Fig. 4B).

The Ts phenotype of cells producing Bdp1Δ253-269 and its suppression by overexpression of RPR1 might reflect three possible situations. (i) Assembly of TFIIIB with Bdp1Δ253-269 in vivo may lead to defective transcription of RPR1, which has a tRNA-like promoter with a boxA and a suboptimally placed, imperfect (nonconsensus) boxB (38). (ii) Interaction between RNase P and Bdp1 in the Pol III initiation complex may be required for effective RNA processing; the deletion of amino acids 253 to 269 may weaken that interaction. (iii) Globally lower Pol III transcription levels due to Bdp1Δ253-269 may generate a deficit of RPR1 RNA that is critical at high temperatures. However, TFIIIBs assembled with Bdp1Δ253-269 and wild-type Bdp1 have approximately the same in vitro transcription activity, DNA-binding activity, and DNase I footprint as TFIIIB assembled with wild-type Bdp1 (36), arguing against the probability of a global effect on all Pol III transcription in vivo.

RPR1 was also tested for suppression of other Bdp1 deletion

FIG. 3. Viability of cells producing split Bdp1. Twelve combinations of plasmids were tested by plasmid shuffling. Three-plasmid transformant cell lines carrying pRS315 BDP1/WT and two other plasmids in various pairwise combinations were constructed. Neither of the terminally truncated bdp1-(1-352) nor bdp1-(352-594) mutants confers viability separately after elimination of the wild-type BDP1 gene, but they do so in combinations of plasmids pRS315 bdp1-(1-352) with pRS423 bdp1-(352-594) and pRS315 bdp1-(352-594) with pRS423 bdp1-(1-352) after elimination of the wild-type gene (bold).
cursors were abnormal in cells producing Bdp1 transcripts. Specific probes for RPR1 RNA, 5S rRNA, U6 snRNA, and tRNA\(^{\text{th}}\) (UAAU) were used, and a U4 snRNA (a Pol II transcript) probe served as an internal control. Levels of SS rRNA, U6 snRNA, and U4 RNA in cells producing wild-type Bdp1 and Bdp1\(\Delta 253-269\) were comparable at 30°C and after a shift to 37°C (Fig. 5C). RPR1 RNA and tRNA\(^{\text{th}}\) precursors were abnormal in cells producing Bdp1\(\Delta 253-269\) at both temperatures (Fig. 5C, lanes 1 and 6). The accumulation of RPR1 RNA was also deficient in mutant cells and the proportion of mature to pre-RPR1 RNA was very different (Fig. 5D). Although tRNA\(^{\text{th}}\) levels were approximately normal, the pools of tRNA\(^{\text{th}}\) processing intermediates were elevated. Maturation of tRNA can proceed along two pathways: either the 5’ and 3’ ends of the primary transcript are processed first and the intron is excised subsequently, or both termini are processed after splicing. In wild-type Bdp1-producing cells, intron-retaining intermediates of tRNA\(^{\text{th}}\) maturation accumulated to the same levels at 30 and 37°C and the +5'/3' form was not detected (Fig. 5C, bottom). In mutant cells, transcripts retaining the 5' leader (reflecting absence of cleavage by RNase P) were much more abundant than in wild-type cells. Thus, very clearly, 5'-end processing of the tRNA\(^{\text{th}}\) Pol III transcript is defective in cells producing Bdp1\(\Delta 253-269\). These results eliminate globally defective Pol III transcription in vivo as a primary cause of temperature sensitivity in cells producing Bdp1\(\Delta 253-269\) and argue in favor of an RPR1-specific effect, possibly involving a direct interaction of a Bdp1 and RNase P.

**RPR1 transcription in vitro.** RPR1 transcription with recombinant TFIIHB containing wild-type and mutant Bdp1 was analyzed in vitro (Fig. 6). Accurately initiating transcription of RPR1, like tRNA gene transcription (SUP4), was TFIIIC-dependent (Fig. 6A, lanes 1 and 6) and also required Bdp1 and Brf1 (data not shown). Surprisingly, although there were no apparent differences of SUP4 transcription between wild-type Bdp1 and Bdp1\(\Delta 253-269\) (Fig. 6A, bottom, compare lanes 2 to 5 with lanes 7 to 10) the yield of RPR1 transcripts with Bdp1\(\Delta 253-269\) was reduced to approximately one-half to one-third of the wild-type level (Fig. 6A, top, compare lanes 2 to 5 with lanes 7 to 10, and 6B), comparable with what was observed in vivo (Fig. 5C and 5D). The transcriptional defect of the RPR1 promoter with Bdp1\(\Delta 253-269\) was not compensated for by doubling its concentration or the concentrations of TFIIIC (Fig. 6B). Doubling the concentrations of both Brf1 and TBP approximately doubled RPR1 transcription with both wild-type Bdp1 and Bdp1\(\Delta 253-269\) (Fig. 6A and data not shown) and did not compensate for the Bdp1\(\Delta 253-269\) defect (Fig. 6B, 2x'B'). These results indicate that the RPR1 and SUP4 tRNA promoters require the same complement of initiation factors but suggest a greater sensitivity of transcription of the RPR1 gene, with its variant promoter, to deletion of amino acids 253 to 269 in Bdp1.

**Association of Bdp1 with RNase P.** Physical interactions between Bdp1 (or TFIIHB) and RNase P have also been explored by communoprecipitation and pull-down assays. A total cell extract (yeast strain YBS334; BDP1 wild type) was reacted with polyclonal antibody to TBP, Brf1, and Bdp1, and immune complexes were adsorbed to protein A affinity beads. Coimmobilizing material was eluted with detergent (SDS)-containing buffer and probed for RPR1 RNA (Fig. 7A, top), which was seen to have been captured with anti-Bdp1 antibody (lane 5) but not significantly (above background) with anti-TBP or anti-Brf1 antibody (lanes 3 and 4). An essentially identical result was obtained when coimmobilizing material was eluted with high-salt buffer (data not shown). A control probing for tRNA\(^{\text{th}}\) (cf. Fig. 5C) showed essentially no signal above background (Fig. 7A, bottom). Anti-Bdp1 pulled down 3% of the input RPR1 RNA (upper panel) but, in contrast, less
than 0.01% of input tRNA\textsuperscript{Ile} (lower panel) under conditions of direct competition by all RNA species in the cell extract for binding to Bdp1. An interaction with Bdp1 was also detected by direct pull-down assay, using recombinant wild-type Bdp1 immobilized on beads (Fig. 7B, lane 3). RPR1 RNA was also bound by immobilized Bdp1\textsubscript{253-269} with somewhat lower efficiency (compare lanes 3 and 6), but the dependence of RPR1 capture by wild-type Bdp1 and Bdp1\textsubscript{253-269} on electrolyte concentration in the binding buffer was comparable (lanes 3 to 8). These experiments point to a physical interaction (though not necessarily a direct one) between Bdp1 and RNase P, although they do not account directly or entirely for the multicopy suppression by RPR1 of the temperature sensitivity conferred by bdp1\textsubscript{253-269}. The interaction that associates RPR1 RNA with Bdp1 is not especially strong, since it can be dissociated with neutral salt.

**DISCUSSION**

We have identified three separate segments of Bdp1 that are required for viability (Fig. 8). Regions I, II, and III correspond to parts of Bdp1 that have already been assigned some significance in the context of other analysis. Regions I and II overlap with segments of Bdp1 that were found to become less reactive...
to cleavage by hydroxyl radicals upon assembly of Bdp1 into a TFIIIB-DNA complex (36), implying at least partial burial as a consequence of changing protein-protein and/or protein-DNA interactions. Region III overlaps with a segment of Bdp1 that was found to become more reactive to hydroxyl radicals, implying possible displacement from an internal protein-protein interaction upon formation of the TFIIIB-DNA complex. Region I is also associated with other features of Bdp1: (i) it encompasses the SANT domain, a motif associated with pro-
tein-protein interaction (1) and is the segment that is most conserved among Bdp1 genes (Fig. 8), and (ii) it also comes to notice in connection with analysis of in vitro transcription. Deletions between amino acids 424 and 464 in region I make Bdp1 defective for TFIIIC-dependent transcription of supercoiled DNA (36). Deletions of 13- to 22-amino-acid segments between amino acids 355 and 421 of Bdp1 make TFIIIB defective for TFIIIC-independent transcription of linear DNA. Further analysis of this defect has revealed that TFIIIB plays a role in Pol III transcription extending beyond polymerase recruitment to the promoter (29). Region II encompasses a segment of Bdp1 that cross-links efficiently to DNA in a TFIIIB-DNA complex –10 bp upstream of the TATA box (55). Deleting amino acids 272 to 292 in region II also abolishes TFIIIC-dependent transcription in vitro (36). However, in general, the global requirements for Bdp1 function in vivo are more demanding and restrictive than requirements for function in the defined Pol III in vitro transcription system (36).

Deletions of several segments of Bdp1 generate high- or low-temperature sensitivity. Some of these defects are partly rescued by overexpression of other components of Pol III transcription. Thus, bdpl-(1-487) partial Cs and bdpl-312-325 Ts phenotypes were suppressed by overexpression of TBP and Brf1. In contrast, the bdpl-355-372 deletion, which confers Ts phenotype on its own, was seen to be synthetically lethal with overexpression of the dominant gain-of-function variant PCF1-1 of the TFC4 gene. These effects suggest that specific segments of Bdp1 are required for (normal) interactions with other components of TFIIIB and with TFIIIC. Although the lethality of deletions covering regions I, II, and III was not rescued by overproducing Brf1, TBP, or Tfc4, this does not exclude these regions as potential sites of such interaction. In

![Functional map of Bdp1](http://mcb.asm.org/)

FIG. 8. Functional map of Bdp1. (A) Summary of this work. Solid bars show absolutely required regions I, II, and III. Lightly shaded segments cover abnormal but viable alleles. Open segments are not necessary for normal growth at 18, 30, or 37°C. Sites associated with suppression by RPR1, Brf1, and TBP and synthetic lethality with Tfc4 (Pcf1-1) are marked. (B) Regions of Bdp1 required in vitro (29, 36). U6 gene transcription requires one of the two darkly shaded segments, and both segments are required for rRNA (SUP4) gene transcription. The lightly shaded segment is required for transcription of linear but not supercoiled DNA. (C) Alignment of fungal Bdp1 homologues. Region I and II consensus residues in at least three species are boxed and shaded. Region I is the most conserved and includes the SANT domain. Region II is also conserved. Region III is divergent and not required for in vitro transcription. The preliminary sequences of Bdp1 from C. albicans, N. crassa, and A. fumigatus were obtained from Stanford Genome Technology Center Contig6-2237 (http://www-sequence.stanford.edu/group/candida), WIMCCR Contig2.668 assembly 2 (http://www-genome.wi.mit.edu), and TIGR_5085 (http://www.tigr.org), respectively. The GenBank accession numbers for Bdp1 from S. cerevisiae and Schizosaccharomyces pombe are AAC49073 and CAA22645, respectively.
fact, the rescue of conditional phenotypes generated by deletions at the boundaries of these Bdp1 segments suggests their involvement also. On the other hand, the ability to split Bdp1 at amino acid 352 is consistent with the nonessential nature of the surrounding 325-to-355 segment of Bdp1.

Relationships between transcription and RNA processing have remained largely unexplored for yeast Pol III. The finding that yeast genomic library screening captures RPR1 as a suppressor of the partial temperature sensitivity of the bdp1-Δ253-269 strain therefore holds particular interest. The product of the RPR1 gene is the RNA subunit of RNase P. RNase P trims 5' ends of pre-tRNAs to generate mature tRNA, and this function is universally conserved. Bacterial, archaeal, and eucaryal RNase Ps are composed of one RNA subunit and several protein subunits, and their catalytic activity is contained in the RNA subunit (67). Yeast RNase P is also composed of one RNA subunit (RPR1 RNA) and nine protein subunits that are all essential for cell viability (8, 11, 16, 38, 41, 59). Bacterial RNase P recognizes pre-tRNA structure directly (48). This is evidently not the case for the yeast enzyme (67), and how it is brought into contact with its substrate is not known. Finding RPR1 as a suppressor of the temperature sensitivity of the bdp1-Δ253-269 strain suggests a possible involvement of TFIIIB in posttranscriptional processing of tRNA but could also reflect defective transcription of RPR1 itself, due to the bdp1-Δ253-269 deletion. RPR1 has a relatively weak promoter with a nonconsensus and suboptimally placed boxB, and RPR1 transcription in vitro by Pol III in conjunction with wild-type TFIIIC and TFIIHB is relatively weak compared to that of the standard SUP4 tRNA\(^{3\text{\prime}}\) gene with its near-consensus and optimally configured boxA and boxB promoter sites (Fig. 6A). Under the conditions of analysis, transcription of RPR1 with TFIIHB (Bdp1Δ253-269) is reduced ~2 to 3-fold relative to transcription with wild-type TFIIHB when activities are normalized to SUP4 transcription (Fig. 6). When DNA templates are transcribed under favorable conditions in vitro (using genes with strong promoters, and with transcription factors in excess), Bdp1Δ253-269 does not generate quantitative defects of transcript yield or qualitative changes of DNA footprint (36, 55), but RPR1 and other genes with weaker promoters might be more sensitive indicators of defects in the transcription apparatus, particularly when transcribed in competition with all Pol III genes in the cell (Fig. 5B and C).

However, this is not the sole defect generated by bdp1-Δ253-269. Maturation of tRNA [as assayed with tRNA\(^{3\text{\prime}}\)(UAU)] is also aberrant at 30 and 37°C (Fig. 5C), and the relative accumulation of pre-RPR1 and mature RPR1 RNA is strongly affected (Fig. 5D). It remains conceivable that Bdp1, separately or in conjunction with promoter-bound TFIIIB, interacts directly with RNase P. Pol II processing factors, capping enzyme, and splicing and polyadenylation complexes interact with Pol II transcription through the C-terminal domain of the largest Pol II subunit (49). It is plausible to consider a comparable coupling between Pol III transcription and posttranscriptional processing. Because Pol III transcription units are characteristically short, it is also reasonable to consider the possibility that this coupling could be effected by a transcription initiation factor instead of the elongating RNA polymerase complex.

It might be thought that RPR1 suppression of temperature sensitivity conferred by the bdp1-Δ253-269 deletion is merely due to nonspecific relief of a quantitative defect of Pol III transcription. If this were the case it would point to RNA processing by RNase P as the Pol III transcript-supported cellular process that is quantitatively most limiting. A similar inference was made when RPR1, along with the genes encoding all three subunits of TFIIIB, the TFC1 and TFC4 subunits of TFIIIC, and the RPC10 subunit of Pol III turned up as multicyclic suppressors of a temperature-sensitive mutation in TFC3, the gene encoding the largest subunit of TFIIIC (39, 52). However, we surmise that the connection between RNase P and Bdp1/TFIIIB has a wider significance. First, the temperature sensitivity of the bdp1-Δ253-269 mutant was not suppressed by overproduction of Brf1, TBP, or Tfc4 (Fig. 2; Table 1). Second, RPR1 multicyclic suppression is specific to the 253-to-269 deletion in BDP1. If a mere two- to threefold reduction of RPR1 transcription in vivo were responsible for temperature sensitivity, then RPR1 multicyclic suppression should be general to conditions and mutations that depress Pol III transcription. Instead, the RPR1 multicyclic suppression is specific to a single BDP1 deletion. Other BDP1 mutations, which presumably also lower transcription in vivo, are specifically suppressed by overproducing TBP and Brf1, for example, but not RPR1 RNA. Third, the specificity of action of yeast RNase P in pre-tRNA processing evidently does not reside in direct substrate recognition. A mediator of specificity is called for; presumably, this additional component is required for efficient catalysis. Finding RPR1 multicyclic suppression has, in fact, led to experiments demonstrating that Bdp1 interacts with RNase P/RPR1 RNA with a high degree of specificity and moderate affinity.

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REFERENCES


