Characterization of a Nuclear Protein Conferring Brefeldin A Resistance in Schizosaccharomyces pombe*

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The fungal metabolite brefeldin A disrupts protein secretion and causes the redistribution of the Golgi complex to the endoplasmic reticulum. Previously we isolated six genes that, when present in multiple copies, confer brefeldin A resistance to wild type Schizosaccharomyces pombe. Here we describe the characterization of one of these genes, hba1. This gene encodes an essential protein that shares homology with the mammalian protein RanBP1 and the protein encoded by the Saccharomyces cerevisiae gene YRB1 and contains a peptide motif present in several proteins found within the nuclear pore complex. The protein encoded by hba1 is localized to the nucleus, and it was determined that this protein is phosphorylated in vivo. The characterization of hba1 thus demonstrates a novel mechanism of drug resistance in S. pombe.

A number of pharmacological agents have been used as probes to examine the processes and mechanisms responsible for intracellular protein transport and secretion. One of these compounds, brefeldin A (BFA),† has been used extensively to investigate the underlying mechanisms of both protein transport and also maintenance of intracellular organelles. Addition of BFA to cultured cells results in the rapid inhibition of protein secretion and the redistribution of Golgi proteins and membranes into the endoplasmic reticulum (Lippincott-Schwartz et al., 1989; Doms et al., 1989; Fujiiwara et al., 1988). BFA is thought to exert its effects on protein transport and Golgi structure by inhibiting the GDP to GTP exchange on ADP-ribosylation factor (ARF) found on Golgi membranes (Donaldson et al., 1992; Helms and Rothman, 1992). However, more recent data using partially purified ARF guanine nucleotide exchange factor demonstrated that BFA did not inhibit the GDP-GTP exchange reaction (Tsai et al., 1994). Therefore, it is unclear whether the nucleotide exchange on ARF is affected by BFA directly.

We have utilized the fission yeast Schizosaccharomyces pombe in order to identify the target molecule of BFA as well as other proteins capable of conferring BFA resistance. Our previous work has demonstrated that wild type S. pombe is sensitive to the effects of BFA in an analogous manner to that seen in mammalian cells, i.e. inhibition of protein secretion and the disassembly and redistribution of the Golgi complex (Turi et al., 1994). Mutant S. pombe strains that are resistant to BFA were isolated and characterized and found to contain a mutation in either CRM1, a gene required for maintaining chromosomal structure, or a second locus termed BAR2. In addition, six genes that confer resistance to BFA when present in high copy were also identified (Turi et al., 1994). One of these six genes encoded a homologue of the mammalian transcription factor AP1 termed pap1, while yet another of the six genes encoded a novel multidrug resistance transporter (Turi and Rose, 1995). Here we describe a third gene conferring BFA resistance. The protein encoded by this gene is an essential nuclear phosphoprotein that contains a conserved sequence motif present in proteins that interact with a nuclear GTP-binding protein known as Ran in mammalian cells, or Sp1 and Gsp1 in fission and budding yeast, respectively.

MATERIALS AND METHODS

Media, Yeast Strains, and Genetic Methods—S. pombe strain FWP1 (h ura4) was grown in YES or in EMM (Mitchinson, 1970). The antibiotic brefeldin A was purchased from Sigma and dissolved in ethanol at a concentration of 10 mg/ml. For genetic analysis, standard methods were followed. S. pombe was transformed by the lithium acetate method (Okazaki et al., 1991).

Restriction Mapping and Subcloning of the hba1 Gene—For restriction mapping, plasmid pBAR2–1 was digested with HindII, EcoRI, BglII, or pairwise combinations of these enzymes. The DNA restriction fragments were separated on a 1% agarose gel and processed for Southern analysis. The resulting filters were probed with restriction fragments derived from the pBAR2–1 insert that had been radiolabeled via random primer labeling (Feinberg and Vogelstein, 1983).

The hba1 gene on plasmid pBAR2–1 was localized by subcloning restriction fragments corresponding to the insert into plasmid pFL20 (Lesson and Lacroute, 1983). Recombinant plasmids were used to transform wild type S. pombe and BFA resistance examined using a growth or a secretion assay previously described (Turi et al., 1994). Plasmid pBAR2–1H contained a 4.2-kb HindII fragment that conferred BFA resistance in both assays. To further map the hba1 gene, a 2.7-kb HindII–EcoRI subfragment of the pBAR2–1 insert was cloned into pFL20 and assayed for BFA resistance.

DNA Sequence Analysis—The nucleotide sequence of the entire 4.2-kb insert of pBAR2–1H was determined using the dideoxy chain termination method using a modified T7 DNA polymerase (Sequenase, U. S. Biochemical Corp.) and synthetic oligonucleotide primers. Additional synthetic oligonucleotide primers were used to extend the sequence of the adjacent open reading frame present within pBAR2–1H. For these sequencing reactions, the original pBAR2–1 plasmid was used as a template. The sequences were assembled and analyzed using Lasergene software (DNASTar, Madison, WI). The hba1 gene present on plasmid pBAR1–2 (Turi et al., 1994) was sequenced utilizing the synthetic primers synthesized for analysis of pBAR2–1H. DNA and protein homology searches were conducted on the EMBL and GenBank data bases.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank/EMBL Data Bank with accession number(s) U38783.

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The abbreviations used are: BFA, brefeldin A; ARF, ADP-ribosylation factor; kb, kilobase(s); PCR, polymerase chain reaction; ORF, open reading frame; PAGE, polyacrylamide gel electrophoresis; RanBP, Ran-binding protein.
Epitope Tagging—The DNA sequence encoding the human c-Myc epitope was appended onto the 3′ terminus of the hba1 gene by the polymerase chain reaction using plasmid pBAR2-1H as a template. To facilitate cloning, a Smal restriction site was incorporated upstream of the hba1 start site using the primers ATGCCCGGAATTCGACGCAAAATGGAAAAAATAT and CGATGCGCAGTCATTTTTCATC. The c-Myc epitope was introduced onto the 3′ terminus with primers GAGGCTTAAAGATAGCCTATTTAA and GATCGGATCCTCACAAGCTCTTCCACCCTAGGAAT. The first PCR product was digested with Smal and PstI and the second PCR product was digested with PstI and BamHI. The two fragments were ligated into pREP4 (Maundrell, 1990) that had been digested with MscI and BamHI. The resulting fusion was confirmed by DNA sequence analysis.

The wild type hba1 gene was also cloned into pREP4 using the 5′ PCR product described above digested with Smal and PstI and mixed with a PstI to EcoRI (made blunt with Klenow fragment of DNA polymerase) restriction fragment of pBAR2-1H containing the 3′ half of the hba1 gene. These two fragments were then ligated into the MscI site of pREP4.

Metabolic Labeling and Immunoprecipitation— Cultures were grown for 18 h in EMMP containing 1 mM of phosphate (Moreno et al., 1991). The overnight culture was diluted in 35 ml of fresh medium to a density of 1 × 10^6 cells/ml and allowed to grow to a density of 5 × 10^6 cells/ml. The cells were harvested by centrifugation and resuspended in 2 ml of EMMP containing 50 μM of phosphate and 1.5 mCi of [32P]H3PO4. The cultures were labeled for 3 h at 30°C.

For immunoprecipitation, the labeled cells were disrupted with glass beads in detergent lysis buffer (Rose and Bergmann, 1983) and immunoprecipitates produced using 100 μl of conditioned medium from 9E10 hybridoma (Evans et al., 1985) followed by 1 μl of a rabbit anti-mouse polyclonal antiseraum and fixed Staphylococcus aureus. The immune complexes were washed five times with RIPA (Rose and Bergmann, 1983), one time with 50 mM Tris-HCl, pH 8.0, and one time with Tris containing 100 μg/ml RNase. The samples were subjected to electrophoresis on a 10% polyacrylamide-SDS gel. The gel was soaked in water containing 10 mM ATP for 10 min, dried, and exposed to film.

Indirect Immunofluorescence—Wild type cells containing pREP4, pREP-hba1, or pREP-hba1-Myc were grown overnight in EMM in the presence or absence of 1 mM thiamine. Cells were fixed in formaldehyde and processed for immunofluorescence as described (Hagan and Hymes, 1988), except the cells were digested with 1 mg/ml Zymolyase 100T for 5 min. Culture supernatant from the 9E10 hybridoma was used as the primary antibody. An anti-mouse fluoroscein isothiocyanate-labeled antibdy (Pierce) was used as a secondary antibody.

Gene Disruption—For gene disruption, plasmid pBS(KS)-2-1H (pBS-5(KS)) containing the 4.2-kb HindIII fragment from pBAR2-1, was digested with Smal and BglII to excise the coding region of hba1, the termini were made blunt with Klenow fragment of DNA polymerase, and ligated with a 1.8-kb fragment containing the S. pombe ura4 gene from pREP4. The resulting plasmid, pBS2-1Hura, was digested with BamHI and ligated to a 1-kb HpaI-BstXI fragment that had been made blunt and ligated to BamHI linkers. The final plasmid, pBS2-1KO, was digested with XbaI and HindIII to release a 4.8-kb fragment containing the disrupted hba1 gene. This fragment was isolated and used to transform wild type diploid S. pombe. Genomic Southern analysis was performed to determine homologous recombination.

RNA Analysis—RNA isolation and Northern blotting were performed as described previously (Turii et al., 1994). Radiolabeled probes corresponding to the coding regions of hba1, crml, pap1, leu1a, and hba2 were prepared by the random primer labeling method (Feinberg and Vogelstein, 1983), and the membrane was hybridized overnight with each of these probes. Analysis and quantitation was performed on a Molecular Dynamics PhosphorImager (Sunnyvale, CA).

RESULTS

Isolation of hba1—The isolation of the three Group II plasmids, pBAR1-2, pBAR2-1, and pBAR2-16, capable of conferring brefeldin A resistance has been described previously (Turii et al., 1994). These plasmids contained overlapping genomic DNA inserts that were between 8 and 15 kb. Because plasmid pBAR2-1 contained the smallest insert, it was chosen for further characterization. To localize the gene on pBAR2-1 conferring BFA resistance, this plasmid was digested with several enzymes and the fragments corresponding to the genomic insert were subcloned into pFL20. The resulting plasmids were used to transform wild type S. pombe and BFA resistance assessed in each of the transformants using both a growth and secretion assays. A 4.2-kb HindIII fragment common to all of the original Group II plasmids conferred BFA resistance (Fig. 1).

The DNA sequence of the 4.2-kb fragment was determined. Two long open reading frames were present within this fragment (diagrammed in Fig. 1). The upstream ORF was complete, while the downstream ORF was truncated at the distal HindIII site. The remaining portion of this second ORF was determined by sequencing directly from plasmid pBAR2-1. To determine which of the two ORFs was capable of conferring the BFA resistance, each gene was individually subcloned into pREP4, transformed into wild type S. pombe, and transformants analyzed as before. Only the complete upstream ORF present on the original 4.2-kb HindIII fragment conferred BFA resistance (data not shown). The nucleotide and amino acid sequence of this ORF, which we refer to as hba1 for hyperresistance to brefeldin A, was shown in Fig. 2. The hba1 coding sequence begins at nucleotide 612 and terminates at nucleotide 1809. The second ORF, which is referred to as dbh1 for downstream of hba1, begins at nucleotide 1268 and extends downstream of the hba1 ORF (sequence not shown, but is included in GenBank accession no. U38783). Comparison of the dbh1 ORF to the GenBank data base failed to detect any significant sequence similarity to any other known protein.

The library used to isolate hba1 on pBAR2-1 was constructed from a S. pombe strain possessing a dominant mutation within the bar2 gene, which itself confers BFA resistance. It was possible that the hba1 allele isolated may have contained a mutation. We therefore determined the sequence of hba1 from plasmid pBAR1-2. This plasmid was isolated from a library constructed from a strain harboring a bar1/crm1 mutation in a wild type background, and it would be expected that the hba1 allele isolated from this strain be wild type. Comparison of the hba1 sequence from pBAR1-2 and pBAR2-1 determined that the two sequences were identical, demonstrating that the gene isolated from pBAR2-1 was wild type.

Primary Structure of hba1—The ORF of hba1 potentially encodes a protein of 399 amino acids. Using this deduced amino acid sequence, a search of the GenBank and EMBL data bases showed that this ORF encoded a novel protein. The proteins within the data bases showing the greatest degree of sequence similarity to Hba1p were the Schizosaccharomyces cerevisiae CST20 gene product (Ouspenski et al., 1995), subsequently renamed...
YRB1 (Dingwall et al., 1995), and human RanBP1. These proteins interact with the yeast and mammalian Ras-like nuclear proteins GSP1 and Ran in their GTP-bound state. Both proteins possessed 29% identity (54% overall similarity) to \( hba1p \) (Fig. 3).

Several proteins in addition to RanBP1 and YRB1 were also found to have significant similarity to the COOH-terminal half of \( hba1p \). These proteins included the \( S. \) cerevisiae genes \( NUP2 \) and an ORF present on Chromosome IX with sequence similarity to \( NUP2 \) (\( NUP2 \)-like), a \( C. \) elegans ORF (Fig. 4) and three human proteins, two of which were identified by screening a hippocampal expression library with \( 32P \)-GTP-Ran (Beddow et al., 1995) and the other by hybridization with murine RanBP1 (Bischoff et al., 1995). The latter of the three human RanBP homologues, subsequently identified and renamed RanBP2 (Yokayama et al., 1995), contains two homology domains and is similar in structure to the \( C. \) elegans ORF. The region of similarity between all these proteins is centered around two potential leucine zippers that have been predicted in RanBP1 (Coutavas et al., 1993).

Nuclear Localization of \( hba1p \)—Of the previously identified proteins that are similar to \( hba1p \) and contain the RanBP domain, several are localized to the nuclear membrane and the others are cytosolic. To determine the subcellular localization of \( hba1p \), we performed indirect immunofluorescence using a \( hba1 \) gene, which had an epitope of the human c-Myc protein appended to the COOH terminus. This construct and the wild type \( hba1 \) gene were expressed in wild type \( S. \) pombe under the control of the thiamine repressible \( nmt1 \) promoter (Maundrell, 1990). With cells grown in the presence of thiamine, no immunofluorescence could be detected in \( hba1 \), or \( hba1 \)-myc transformed cells (data not shown) using a monoclonal antibody specific for the c-Myc epitope. In contrast, cells transformed with the \( hba1 \)-myc construct and grown in the absence of thiamine showed intense nuclear staining using the anti-c-Myc mAb, while \( hpa4 \) and \( hba1 \) transformed showed around two potential leucine zippers that have been predicted in RanBP1 (Coutavas et al., 1993).
no nuclear staining (Fig. 5). The Hba1-Myc protein conferred BFA resistance to wild type S. pombe only in the absence of thiamine, suggesting that the fusion protein was functionally active and transported to the correct cellular organelle.

To exclude mislocalization by the Myc epitope, we appended the Myc epitope onto the carboxyl terminus of the cytoplasmic protein Obr1 and expressed this construct using the nmt1 promoter (Toda et al., 1994). The Obr1-Myc fusion was localized throughout the cytoplasm with no evidence of nuclear staining (data not shown). These results demonstrate that the nuclear localization of Hba1p is not due to the presence of the Myc tag. Examination of the deduced Hba1p sequence identified a possible bipartite nuclear localization signal located between residues 191 and 214, which contains the sequence KKFAAGTAVETESGSGKEKENDKK (Figs. 2 and 3).

Hba1p is a Phosphoprotein—To examine the biochemical properties of Hba1p, we utilized the c-Myc epitope-tagged construct under the control of the nmt1 promoter. Wild type cells transformed with pREP4, pREP4-hba1, or pREP4-hba1-Myc were grown for 18 h either in the presence of thiamine to repress expression or in the absence of the vitamin to induce expression. Equivalent numbers of cells from each overnight culture were transferred to fresh media and metabolically labeled with [35S]methionine for 30 min. After the labeling period, the cells were disrupted, immunoprecipitated with the anti-c-Myc monoclonal antibody 9E10, and the precipitates analyzed by SDS-polyacrylamide gel electrophoresis. In the absence of thiamine, no proteins were precipitated in either vector alone or in the wild type Hba1-transformed strain. In the strain possessing the Hba1-c-Myc construct a broad, diffuse band migrating at approximately 70 kDa was observed in addition to two lower molecular mass bands of 28 and 30 kDa (Fig. 6A). The predicted molecular mass of Hba1 with the appended c-Myc epitope was 41 kDa. Thus, Hba1 migrates aberrantly. A likely explanation for the aberrant migration pattern of Hba1 is this protein contains a large number of charged residues. The lower molecular weight proteins are most likely proteolytic cleavage products of the native Hba1 protein, which are detectable on immunoblots by anti-c-Myc antibody (data not shown).

To further investigate the nature of the Hba1p doublet, we repeated the metabolic labeling experiment with [32P]orthophosphate to determine whether the observed heterogeneity was due to post-translational modification such as phosphorylation. Derepressed strains transformed with vector alone, Hba1, or Hba1-c-Myc were metabolically labeled for 3 h with [32P]orthophosphate, immunoprecipitated, and analyzed by SDS-PAGE as before. In vector alone and Hba1-transformed cells, no signal was detected. In contrast, a single phosphoprotein of approximately 70 kDa was detected in addition to the two lower molecular mass labeled proteolytic products of 28 and 30 kDa (Fig. 6B). These results demonstrate that Hba1 is a nuclear phosphoprotein.

hba1 Is Essential for Viability—To determine if hba1 was essential, we constructed strains deleted for hba1. Null mutants were created by deleting the entire hba1 coding region and replacing it with ura4. The deletion construct was used to transform diploid S. pombe and targeted replacements were verified by Southern analysis. After conversion of hba1 into hba1Δ, the diploid was induced to sporulate. Very few of the disrupted heterozygous diploids successfully completed meiosis, yielding four spore asci compared to the untransformed parental strain. Because of the poor sporulation of the hba1 disrupted diploid, random spore analysis was performed rather than tetrad dissection. Of the germinated spores no ura4 segregants were obtained. These results indicate that hba1 is required for viability.

BFA Resistance Conferred by hba1 Is Independent of the crm1 Regulatory Pathway—Previously we have determined that resistance to BFA was dependent upon alteration in the crm1 regulatory pathway (Turi et al., 1994). A mutation within the negative regulatory protein crm1 showed an increase in expression in the crm1 mutant (data not shown). Mutations within crm1 or overexpression of hba2 from a multicopy plasmid was sufficient to confer BFA resistance. To determine if crm1 participated in regulation of hba1, we examined expression of hba1 in isogenic wild type and crm1 mutant strains. Total RNA was isolated from each strain, fractionated and transferred to nitrocellulose, and hybridized with either crm1, hba1, pap1, or leu1a as a control for loading. Of the genes examined, only pap1 showed an increase in expression in the crm1 mutant (data not shown). Furthermore, hba1 was undetectable even with prolonged exposure.

To determine if overexpression of hba1 affected message levels of any of the known genes conferring BFA resistance, Northern analysis was performed using total RNA isolated from a wild type strain transformed with a multicopy plasmid containing hba1 (pBAR2-1H) or vector alone. The resulting blot was hybridized with probes for hba1, hba2, crm1, pap1,
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**DISCUSSION**

In this report we describe the characterization of \( hba1 \), a \( S. pombe \) gene conferring brefeldin A resistance. The region of sequence similarity between Hba1p and Ran-binding proteins is present in Hba1p. The protein sequences were aligned with the Megalign program of DNAstar. Sequences used were \( S. pombe \) hba1, \( S. cerevisiae \) proteins encoded by the CTS20, NUP2, and NUP2-like genes, the mouse RanBP-1 (L25255), the dog open reading frame F59A2 (which contains both a NH\(_2\)-terminal and COOH-terminal motif), the human gene RanBPX (which also contains two motifs: a and b) (X83617), and the human AB1 (U19240) and AB2 (U19248) genes. Closed circles denote amino acid residues that are identical in all the aligned proteins; open circles denote residues that are highly conserved between the aligned proteins.

**FIG. 4.** A conserved motif found in Ran-binding proteins is present in Hba1p. The protein sequences were aligned with the Megalign program of DNAstar. Sequences used were \( S. pombe \) hba1, \( S. cerevisiae \) proteins encoded by the CTS20, NUP2, and NUP2-like genes, the mouse RanBP-1 (L25255), the dog open reading frame F59A2 (which contains both a NH\(_2\)-terminal and COOH-terminal motif), the human gene RanBPX (which also contains two motifs: a and b) (X83617), and the human AB1 (U19240) and AB2 (U19248) genes. Closed circles denote amino acid residues that are identical in all the aligned proteins; open circles denote residues that are highly conserved between the aligned proteins.

**FIG. 5.** Localization of Hba1p in \( S. pombe \). Indirect immunofluorescence was performed on wild type \( S. pombe \) transformed with vector control (pREP4) or with a vector containing the \( hba1 \) gene (pREP4-HBA1) or the \( hba1 \) gene with the human c-Myc epitope appended to the carboxyl terminus (pREP4-HBA1-Myc) under the control of the nmt1 promoter. Panels on the left correspond to 4',6-diamidino-2-phenylindole, dihydrochloride-stained nuclei. Panels on the right correspond to fluorescence observed when the cells are stained with the anti-c-Myc monoclonal antibody 9E10 and a fluorescein isothiocyanate-labeled secondary antibody. Immunofluorescence was performed as described by Hagan and Hyams (1988), except that spheroplasting was terminated when approximately 50% of the cells became refractory.

**FIG. 6.** In vivo metabolic labeling of Hba1p. A, immunoprecipitates of \( S. pombe \) extracts that had been labeled with \(^{[35S]}\)methionine. B, immunoprecipitates of \( S. pombe \) extracts that had been labeled with \(^{[32P]}\)orthophosphate determined Hba1p to be a phosphoprotein.

The \( hba1 \) gene encodes a nuclear protein essential for \( S. pombe \) viability. Initial characterization of \( hba1 \) yielded several unexpected results. Although the \( hba1 \)-c-Myc fusion protein was expected to migrate with a mobility of 41 kDa, the observed mobility for this protein was approximately 70 kDa. The aberrant mobility of Hba1p is posttranslational modification. Immunoprecipitation of the c-Myc epitope-tagged Hba1p after \(^{[35S]}\)methionine labeling yielded a broad heterogeneous band. Labeling with \(^{[32P]}\)orthophosphate determined Hba1p to be a phosphoprotein.

**DISCUSSION**

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ment that is highly conserved in Ran-binding proteins (Ran-BPs), as well as a protein of the nuclear pore complex, and several other proteins of unknown function (Hartmann and Görlich, 1995). RanBP1 has been demonstrated to maintain Ran in the GTP-bound state or to act as a costimulator of GTP hydrolysis depending on the absence or presence of Ran-GTPase activating protein (Bischoff et al., 1995). Mutation of the first conserved glutamine, analogous to residue 266 of RanBP1, has been shown to reduce the binding of RanBP1 to Ski1p, suggesting a critical role for this domain in protein-protein interaction (Beddow et al., 1995).

The S. cerevisiae genes Gsp1p, Prp20, and Yrb1p encode homologues of Ran, RCC1, and RanBP1. In the fission yeast S. pombe, sp1 and pim1 encode homologues of Ran and RCC1. A Sp1-binding protein (Sbp1) with homology to RanBP1 has recently been identified.2,3 This leaves the possibility that Hba1p encodes a second Sp1-binding protein. However, using a yeast two-hybrid system with Hba1p and Sp1p, we have not been able to detect interaction between the two proteins.4 Additional evidence that Hba1p may not interact with Sp1p is supported by the results of an experiment in which Sp1p[35S]GTP was used to probe yeast cell extracts. No interacting proteins were identified, which was more similar to that predicted for Hba1p (Coutavas et al., 1993),5 yet this protein overlay assay was able to detect an interaction with S. cerevisiae RanBP (Yrb1) (Oupsenski et al. 1995). It is possible that Hba1p interacts with a different as yet unidentified S. pombe nuclear GTPase or that the interaction with Sp1p may not have been detected using the two-hybrid system for technical reasons or by the protein overlay due to low level expression or the inability of the protein to renature into its active conformation.

Previously we have identified two additional S. pombe nuclear proteins, encoded by crm1 and pap1, which are capable of conferring BFA resistance (Turi et al., 1994). The pap1 gene encodes the S. pombe homologue of the mammalian AP1 transcription factor (Toda et al., 1991). Transcriptional regulation by pap1 is negatively regulated by the gene product of crm1 (Toda et al., 1992; Shimakura et al., 1995). Because these two proteins regulate transcription of other genes responsible for conferring drug resistance, we determined if these genes affected hba1 transcription and also if overexpression of hba1 affected transcription of known genes involved in drug resistance. Neither crm1 or pap1 effected hba1 expression. Of the known genes capable of conferring BFA resistance when overexpressed, expression of these markers was not increased in the presence of elevated levels of hba1. Indeed, expression of hba2, a multidrug resistance transporter capable of conferring BFA resistance, was decreased in cells containing elevated levels of hba1. These results suggest that hba1 mediates BFA resistance via an undefined pathway. While the exact mechanism by which hba1 confers BFA resistance is unclear, the isolation of this gene which encodes a nuclear protein belonging to the RanBP family of proteins, has potentially expanded the role of RanBP-like proteins. Thus, through a combined genetic and biochemical approach, one may identify both the cellular function of hba1 and determine its role in BFA resistance.

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Fig. 7. Overexpression expression of hba1 and its effects on other BFA resistance conferring genes. Ten micrograms of total RNA isolated from wild type S. pombe transformed with either pH120 or pHBA1 (pBAR2-1H). RNA was fractionated on a 1% agarose gel and transferred to nitrocellulose. The filter was hybridized with a probe specific for hba1, hba2, crm1, pap1, and leu1a.