The Identification of cDNAs That Affect the Mitosis-to-Interphase Transition in *Schizosaccharomyces pombe*, Including *sbp1*, Which Encodes a spi1p-GTP-Binding Protein

Xiangwei He,* Naoyuki Hayashi,[†] Nathan G. Walcott,* Yoshiaki Azuma,[†] Thomas E. Patterson,* F. Ralf Bischoff,[‡] Takeharu Nishimoto[†] and Shelley Sazer*

* Verna and Marrs McLean Department of Biochemistry, Baylor College of Medicine, Houston, Texas 77030, [†] Department of Molecular Biology, Graduate School of Medical Science, Kyushu University, Fukuoka 812-82, Japan, and [‡] German Cancer Research Center, Division for Molecular Biology of Mitosis, Heidelberg D-69120, Germany

> Manuscript received June 10, 1997 Accepted for publication November 6, 1997

ABSTRACT

Perturbations of the spi1p GTPase system in fission yeast, caused by mutation or overexpression of several regulatory proteins, result in a unique terminal phenotype that includes condensed chromosomes, a wide medial septum, and a fragmented nuclear envelope. To identify potential regulators or targets of the spi1p GTPase system, a screen for cDNAs whose overexpression results in this terminal phenotype was conducted, and seven clones that represent three genes, named *med1*, *med2*, and *med3* (<u>mitotic exit defect</u>), were identified. Their genetic interaction with the spi1p GTPase system was established by showing that the spi1p guanine nucleotide exchange factor mutant pim1-d1^{ts} was hypersensitive to their overexpression. *med1* encodes a homologue of the human Ran-binding protein, RanBP1, and has been renamed *sbp1* (spi1binding protein). sbp1p binds to spi1p-GTP and costimulates the GTPase-activating protein (GAP)catalyzed GTPase activity. Cells in which sbp1p is depleted or overproduced phenocopy cells in which the balance between spi1p-GTP and spi1p-GDP is perturbed by other means. Therefore, sbp1p mediates and/ or regulates the essential functions of the spi1p GTPase system. *med2* and *med3* encode novel fission yeast proteins that, based on our phenotypic analyses, are likely to identify additional regulators or effectors of the spi1p GTPase system.

[▼]TPases are molecular switches that adopt differold G ent conformations and interact with different regulatory and effector proteins depending upon whether they are bound to GTP or to GDP. Ran-GTPases lack consensus membrane attachment sequences, are predominantly nuclear localized (Bischoff and Ponstingl 1991b), and are very abundant, with an estimated 107 molecules per HeLa cell (Bischoff and Ponstingl 1991b). Structural and functional homologs have been identified in eukaryotes, ranging from yeast to man, of the three core components of the Ran GTPase system: the GTPase; the guanine nucleotide exchange factor (GEF), which stimulates the conversion of Ran from the GDP- to GTP-bound state (Bischoff and Ponstingl 1991a); and the GTPase-activating protein (GAP), which stimulates GTP hydrolysis and thus the conversion of Ran from the GTP- to GDPbound state (Bischoff et al. 1994). Studies in vivo and in vitro have demonstrated that Ran and its regulators influence a variety of nuclear events: cell cycle progression; condensation, decondensation, and transmission

of chromosomes; and nucleocytoplasmic transport of protein and RNA (Sazer 1996). The terminal phenotypes that result from perturbation of this system vary among experimental systems (Sazer 1996).

The primary role(s) of the Ran-GTPase system has not been elucidated. Within a particular organism, Ran may have one primary function that indirectly affects a variety of cellular processes. Alternatively, Ran may have multiple independent functions that are mediated by its interaction with a variety of downstream effectors. Consistent with the latter possibility is the discovery of multiple Ran-binding proteins (RanBPs) in both budding yeast and mammalian cells (Beddow et al. 1995; Dingwall et al. 1995; Hartmann and Gorlich 1995; Lounsbury et al. 1994). Like effectors of other GTPases, RanBPs and isolated Ran-binding domains (RanBDs) bind specifically to the GTP-bound form of the GTPase (Beddow et al. 1995; Coutavas et al. 1993; Lounsbury et al. 1994; Schlenstedt et al. 1995). Several RanBPs and isolated RanBDs have also been shown to costimulate the GTPase activity of Ran in vitro in the presence of the GAP, but to stabilize the GTP-bound form of the GTPase in the absence of GAP (Beddow et al. 1995; Bischoff et al. 1995; Lounsbury et al. 1994; Schlenstedt et al. 1995). In mammalian cells, RanBP1 is cyto-

Corresponding author: Shelley Sazer, Verna and Marrs McLean Department of Biochemistry, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030. E-mail: ssazer@bcm.tmc.edu

plasmic (Lounsbury *et al.* 1994; Schlenstedt *et al.* 1995), whereas RanBP2/Nup358 is localized to the cytoplasmic fibrils emanating from the nuclear pore complex (Wu *et al.* 1995; Yokoyama *et al.* 1995). Their different intracellular localizations suggest that these binding proteins are likely to mediate different Ran functions, although both are required for nuclear import (Chi *et al.* 1996; Hayashi *et al.* 1995; Lounsbury *et al.* 1996; Schlenstedt *et al.* 1995; Yokoyama *et al.* 1995).

Additional evidence that the Ran-GTPase may have several independent functions is the recent discovery in mammalian cells that there are two populations of the Ran-GAP: one that is soluble and cytoplasmic, as well as another insoluble, post-translationally modified form that is associated with the nuclear envelope (Mahajan et al. 1996; Matunis et al. 1996). One clearly established role for the Ran-GTPase and its associated proteins is the nuclear import of proteins; however, there is mounting evidence of additional roles for this GTPase and its associated proteins: (1) a dominant lethal mutant of human Ran inhibits cell growth but not nuclear protein import (Carey et al. 1996), (2) depletion of Xenopus RCC1 or addition of a dominant mutant of Ran inhibits nuclear envelope growth in vitro but not nuclear protein import (Dasso et al. 1994; Kornbluth et al. 1994), (3) the post-translationally modified form of Ran-GAP associates with mitotic spindles at metaphase (Matunis et al. 1996), (4) a dominant mutant of Xenopus Ran affects cell cycle progression in vitro in the absence of nuclei (Kornbluth et al. 1994), and (5) protein import is normal in budding yeast in which the RanBP YRB2 is deleted (Noguchi et al. 1997; Taura et al. 1997).

spi1p, the *S. pombe* member of the Ran-GTPase family, is 82% identical and 90% similar to human Ran (Matsumoto and Beach 1991). pim1p, the spi1p GEF, is a structural (Matsumoto and Beach 1991) and functional (H. Seino, X. He, R. Bischoff, N. Ong, H. Ponstingl, T. Nishimoto and S. Sazer, unpublished results) homolog of the mammalian protein RCC1 that catalyzes the exchange of GDP-bound to spi1p for GTP. Inactivation of pim1p by a temperature-sensitive mutation in fission yeast results in a failure of cell cycle progression at the mitosis-to-interphase transition (Sazer and Nurse 1994). Several easily identifiable characteristics distinguish this terminal phenotype: (1) after a normal mitosis, the separated, postanaphase chromosomes are hypercondensed; (2) the nuclear envelope, which normally remains intact during the entire yeast cell cycle, loses its integrity; and (3) the cells have an abnormally wide medial septum (Demeter et al. 1995; Matynia et al. 1996; Sazer and Nurse 1994). Other defects include disruption of the nucleolus (Tani et al. 1995) and accumulation of poly(A) + RNA in the nucleus (Kadowaki et al. 1993). Taken together, these studies establish that multiple processes depend on the proper functioning of the spi1p GTPase system in fission yeast. Still unanswered is the question of whether there is one primary function whose perturbation has pleiotropic effects on multiple nuclear processes and structures, or whether these functions are independently regulated.

The loss of pim1p nucleotide exchange activity would be expected to result in an increase in the abundance of spi1p-GDP relative to spi1p-GTP. Consistent with this prediction is the finding that the lethality and terminal phenotype of cells in which pim1p function is compromised by a temperature-sensitive mutation are also observed in wild-type cells expressing a mutant form of spi1p stabilized in the GDP-bound form (J. Demeter and S. Sazer, unpublished results) and in wild-type cells overproducing the spi1p GAP, rna1p (Matynia et al. 1996). Consistent with a model in which both decreased pim1p GEF activity and increased rna1p GAP activity independently increase the spi1p-GDP pool, pim1-d1^{ts} mutant cells display an increased sensitivity to rna1 overproduction compared to wild-type cells (Matynia et al. 1996).

Cells overproducing rna1p die with a terminal phenotype indistinguishable from that of pim1-d1^{ts} cells incubated at the restrictive temperature. Several of these characteristics, including cell cycle arrest with condensed postmitotic chromosomes and a wide medial septum, are also seen in germinated spores in which the *rna1* gene has been disrupted. Taken together, these experiments suggest that increasing or decreasing the ratio between spi1p-GTP and spi1p-GDP by means other than pim1p inactivation causes the same lethal effect, indicating that maintenance of the proper balance between the two forms of the GTPase is necessary for cell viability (Matynia *et al.* 1996).

The mechanism by which an imbalance between spi1p-GTP and spi1p-GDP might cause a failure of the mitosis-to-interphase transition in fission yeast remains to be determined. To elucidate this pathway, we devised a strategy to identify both regulators and effectors of this GTPase system. A cDNA library screen was carried out to isolate clones that are toxic when expressed from the regulatable *nmt1* promoter (Maundrell 1990) and interfere with the ability of cells to properly complete mitosis. Several classes of cDNAs might be identified using this approach: (1) regulators of spi1p that directly or indirectly perturb the normal balance between spi1p-GDP and spi1p-GTP, (2) downstream components of the spi1p-GTPase pathway that do not directly affect the spi1p-GTPase but link its functioning to other cellular processes, and (3) components of parallel pathways that regulate the mitosis to interphase transition independently of the spi1p system.

MATERIALS AND METHODS

Yeast strains and cell culture: Schizosaccharomyces pombe strains used were a haploid strain (h^- leu1-32 ura4-D18 ade6-m216), a

diploid strain $(h^-/h^+ leu1-32/leu1-32 ura4-D18/ura4-D18 ade6$ m210/ade6-m216), and a mutant haploid strain pim1-d1^{is} $(h^- leu1-32 ura4-D18 pim1-d1^{is}; Sazer and Nurse 1994), all of$ which are derived from strain 972 (Leupold 1970). Cell culture conditions, media composition, and genetic analyseshave been described previously (Moreno*et al.*1991).

nmt1 **promoter regulation:** Gene expression under the control of the *nmt1* promoter (Maundrell 1990) in pREP3X or pREP41X (Forsburg 1993) was repressed by the inclusion of 5 μ g/ml thiamine in the Edinburgh Minimal Media (EMM; Moreno *et al.* 1991). To derepress expression, cells were washed three times with thiamine-free EMM and grown in fresh thiamine-free EMM.

cDNA library screen and DNA manipulations: An S. pombe cDNA library (a gift from Bruce Edgar and Chris Norbury) in the pREP3X vector (Forsburg 1993) was transformed into wild-type cells, and transformants were grown on EMM plates with appropriate supplements and thiamine to repress cDNA expression. After 5 days of incubation at 32°, the 60,000 transformants were replica plated onto thiamine-free plates to derepress cDNA expression. Phloxine B dye (Sigma, St. Louis, MO), which specifically accumulates in dead cells, was also included in the medium at a final concentration of 5 $\mu g/ml.$ After a 24-hr incubation at 32°, the 4500 dark red colonies that were preferentially stained by phloxine B were examined by light microscopy. The 150 transformants that were enriched in septated cells were picked from the replica plates plus thiamine, inoculated into liquid EMM media with supplements plus thiamine, and grown at 32° for 2 days. These precultures were washed and transferred to thiamine-free EMM medium with supplements at an initial concentration of $\sim 10^5$ cells/ml. After 13 hr at 32°, the cells were fixed in 70% ethanol and stained with 4',6-diamidino-2-phenylindole (DAPI; Moreno et al. 1991) and examined under the microscope to identify transformants enriched in cells with condensed chromosomes.

Plasmids from the transformants were recovered by standard procedures (Moreno *et al.* 1991) and amplified in *Escherichia coli.* The cDNA inserts and their restriction fragments were subcloned into Bluescript KS– (Stratagene, La Jolla, CA) and sequenced using Sequenase version 2.0 (United States Biochemical, Cleveland, OH). pREP41X-*med1*, pREP41X-*med2*, and pREP41X-*med3* were constructed by subcloning the *XhoI* / *Bam*HI fragments from the pREP3X cDNA library into pREP41X to achieve a lower level of overexpression (Forsburg 1993).

Two-hybrid screen: Two-hybrid screening using human Ran as bait was performed as described previously (Yokoyama *et al.* 1995). The *S. pombe* Matchmaker cDNA library was purchased from Clontech (Palo Alto, CA). Transformants were selected on 25 mm 3-amino triazole (3-AT) and transferred to fresh 3-AT plates to confirm drug resistance. Plasmids were isolated from the 3-AT-resistant cells and sequenced using standard protocols.

Cosmid hybridization and mapping: The *S. pombe* cosmid library filter 60-0-0, provided by Reference Library Database (RLDB) Max Planck Institute for Molecular Genetics, was probed with the *Xhol/Bam*HI fragment of the *sbp1* cDNA insert. The coordinates of the positive spots were used to map the *sbp1* gene in the *S. pombe* genome (Hoheisel *et al.* 1993; Lehrach *et al.* 1990).

Construction of the *sbp1* **deletion strain** *sbp1*- Δ 1: A 4.0-kb *Sad/Sad* genomic DNA fragment containing the *sbp1* gene was subcloned from cosmid ICRFc60D0115D (provided by Resource Center/Primary Database of German Human Genome Project, Max Planck Institute for Molecular Genetics) into Bluescript KS–, and the 1.4-kb *Eco*RV/*Pst*I fragment, which encompasses the N-terminal two-thirds of the open reading frame, was replaced with the *ura4*⁺ gene. The *sbp1* de-

letion strain $(h^-/h^+ sbp1::ura4^+/sbp1 leu1-32/leu1-32 ura4-D18/ura4-D18 ade6-m210/ade6-m216)$ was generated by transforming diploid cells $(h^-/h^+ leu1-32/leu1-32 ura4-D18/ura4-D18 ade6-m210/ade6-m216)$ with the 4.4-kb Sacl/Sacl deletion fragment, and it was confirmed by Southern blot analysis using standard techniques.

Fluorescence microscopy: Cells fixed with ethanol were stained with DAPI (Moreno *et al.* 1991). Living cells were simultaneously stained with Hoechst 33342 and 3,3'-dihexylox-acarbocyanine (DiOC_{6}) using a previously described procedure (Demeter *et al.* 1995). Stained cells were examined using an Axioskop fluorescence microscope (Carl Zeiss, Thornwood, NY).

FACS analysis: Cell samples were fixed with ethanol, treated with RNase, stained with propidium iodide, and their DNA content was measured by flow cytometry (Sazer and Sherwood 1990). To determine the DNA content of each half of the septated cells, cells were briefly digested with Novozyme (0.7 mg/ml) and Zymolyase (0.2 mg/ml) for 15 min at 37° immediately before analysis.

sbp1- Δ **1 phenotype characterization:** The sbp1- Δ 1 heterozygous diploid cells were transformed with pREP41X-*sbp1*, and the transformants were sporulated. Haploid sbp1- Δ 1 cells containing the pREP41X-*sbp1* were recovered by germinating the spores on EMM plates lacking uracil to select for the *sbp1* disruption, lacking leucine to select for the plasmid, and lacking thiamine to derepress transcription of *sbp1*. *sbp1* cDNA expression from pREP41X was sufficient to rescue the *sbp1*- Δ 1 lethality. To monitor the phenotype of cells depleted of sbp1p, thiamine was added to repress *sbp1* cDNA expression, and cells were periodically fixed in ethanol and stained with DAPI (Moreno *et al.* 1991) to determine the percentage of septated cells, or living cells were stained with DiOC₆ and Hoechst 33342 to visualize the nuclear envelope and DNA (Demeter *et al.* 1995).

GTPase assay: Ran-GTP was incubated at 25° for 30 min with fourfold excess of $[\gamma^{-32}P]GTP$ (30 Ci/mmol) in 20 mm Hepes-NaOH (pH 7.5), 20 mm EDTA, and 2 mm DTT, then MgCl₂ was added to a final concentration of 50 mm. The buffer was changed to 20 mm Hepes-NaOH (pH 7.5), 5 mm MgCl₂, 0.05% hydrolyzed gelatin, and 2 mm DTT (reaction buffer) on a Nap 5 column (Pharmacia, Piscataway, NJ). The resulting $[\gamma^{-32}P]$ GTP-bound Ran was diluted to 3.3 μ M with reaction buffer, and 30-µl aliquots were mixed with various concentrations of purified glutathione S-transferase (GST) or GST-sbp1p. The mixture was preincubated at 25° for 5 min, then 10 µl of 5 nM S. pombe rna1p was added to start GTPase reaction. After a 3-min incubation, the reaction was stopped by addition of ice-cold stop buffer containing 20 mm Tris-HCl (pH 7.5), 25 mm MgCl₂, and 100 mm NaCl, and was subsequently filtered through nitrocellulose (0.45 mm, BA85; Schleicher & Schuell, Keene, NH). The filters were dried, and the radioactivity remaining with the proteins on the filter was counted in a liquid scintillation counter.

sbp1p-GTPase binding assay: *E. coli*–produced GST-sbp1p or GST (1 μg) was separated by 10% SDS-PAGE and either transferred to polyvinyldifluoride membrane filters or stained with Coomassie brilliant blue. The filters were incubated with either ³⁵S-labeled Ran-GTP_γS or ³⁵S-labeled Ran-GDP_βS, as described previously (Noguchi *et al.* 1996; Yokoyama *et al.* 1995). Protein of crude extracts (66 μg) prepared from wild-type fission yeast or an equal amount of extract incubated with beads bound with either *E. coli*-produced GST-sbp1p fusion protein or glutathione *S*-transferase was electrophoresed in SDS-PAGE and immunoblotted with anti-spi1p antiserum (Matynia *et al.* 1996).

Amino acid sequence analysis: A BLAST (Altschul *et al.* 1990) search in March 1997 using the National Center for

Biotechnology Information's BLAST WWW Server and postprocessed by the Human Genome Center, Baylor College of Medicine, using Beauty (Worley *et al.* 1995) identified 13 proteins that show similarity with the prototypical RanBP, mouse RanBP1 (Coutavas *et al.* 1993). The predicted protein product of *sbp1* was aligned with these 13 proteins using Clustal W version 1.6 (Thompson *et al.* 1994).

RESULTS

Screen for cDNAs that cause a mitotic exit defect when expressed in wild-type cells: Overproduction of rna1p, the GAP for the spi1p GTPase, results in a terminal phenotype similar to that of the pim1-d1^{ts} mutant (Matynia *et al.* 1996). Based on this observation, to identify new genes involved in the mitotic exit pathway, a cDNA library expression screen (see materials and methods for details) was initiated to isolate cDNAs that produce effects similar to those of *rna1* overexpression. The primary screen was for genes that showed transcription-dependent toxicity, the secondary screen was for overexpression strains that accumulated septated cells, and the tertiary screen was for septated overexpression strains that had condensed chromatin.

Overexpression of one cDNA resulted in cells with multiple septa but without condensed chromatin. This cDNA, renamed *sid3*, encodes the fission yeast homolog of the budding yeast GTPase Tem1p (Shirayama *et al.* 1994) and is the subject of another manuscript (M.K. Subramanian, D. McCollum, K.C.Y. Wong, X. He, S. Sazer, L. Chang and K. Gould, unpublished results). *sid3* was independently identified as *spg1*, a high copy suppressor of the septation mutant *cdc7* (Schmidt *et al.* 1997).

In three transformants, cells accumulated with condensed metaphase chromosomes and short mitotic spindles after 13 hr of cDNA transcription. Upon longer transcriptional induction, septation occurred in the absence of nuclear division and spindle elongation, resulting in one anucleate daughter cell and one daughter cell that contained a nucleus with a short mitotic spindle. These three genes, which include components of the spindle checkpoint system, are the subject of another manuscript (He *et al.* 1997).

The seven other septated strains with hypercondensed chromosomes were binucleate, indicating that they had undergone an apparently normal mitosis but were blocked at the mitosis-to-interphase transition. The cDNA-containing plasmid from each of these seven strains was isolated and amplified in *E. coli*. The purified plasmids were transformed back into fission yeast cells to confirm the lethality and terminal phenotype resulting from their overproduction. This collection of cDNAs was temporarily named *med* (mitotic exit defect). Partial DNA sequence analysis of these seven cDNA clones revealed that they represented three different genes: *med1* and *med2* were cloned three times each, and *med3* was cloned once. Cells overexpressing *med* genes arrest with terminal phenotypes similar to *rna1* overexpressing cells and pim1^{ts} mutants: The three strains overexpressing *med1*, *med2*, or *med3* were grown in the absence of thiamine for 13 hr to derepress cDNA transcription, and these living cells were then stained with Hoechst 33342 to visualize the DNA and with $DiOC_6$ to visualize the nuclear envelope, as described previously (Demeter *et al.* 1995; Figure 1). All three cDNAs caused cells to arrest after mitosis as binucleated cells with highly condensed chromosomes and a medial septum (Figure 1), the width of which increased with time of expression (data not shown).

In wild-type cells (Demeter *et al.* 1995) and vectortransformed wild-type cells (Figure 1A), the circular



Figure 1.—*med* overexpression arrests cells with a medial septum, condensed chromosomes, and an abnormal nuclear envelope. Live cells were double stained with Hoechst 33342 to visualize the DNA and DiOC₆ to visualize the nuclear envelope. Micrographs of (A) wild-type vector-transformed cells at different stages of the cell cycle, as well as cells arrested by (B) *med1*, (C) *med2*, or (D) *med3* overproduction 20 hr after transcriptional derepression. *med3*-overexpressing cells have a unique accumulation of cytoplasmic DiOC₆ staining material (arrows, panels 1–3).

nuclear envelope surrounds the DNA at all stages of the cell cycle. The nuclear envelope surrounding the chromatin in cells in which transcription of *med1*, *med2*, or *med3* genes was repressed by thiamine was also clearly delineated (data not shown). When transcription was derepressed for 13 hr, however, the nuclear envelope could no longer be detected surrounding the hypercondensed chromatin in 18–20% of the cells (Figure 1, B–D), and after 18 hr, this percentage increased to 50%. The percentage of pim1^{ts}-like cells increased to 50% when transcription of *med1*, *med2*, or *med3* was derepressed for 18 hr. Expression of *med3* also caused an accumulation of DiOC₆ staining material in the cytoplasm (Figure 1D, arrow).

med cDNA overproduction also caused cells to arrest with an unreplicated genome. An asynchronous population of wild-type fission yeast has a 2C DNA content because daughter cells in G1, each with a 1C DNA content, remain attached to one another after mitosis and because DNA replication is virtually completed by the time of cell separation. The binucleated, *med*-arrested cells also displayed a 2C DNA content, indicating that the genome of each daughter cell was unreplicated (Figure 2, undigested). Partial digestion of the septum with a mixture of Novozyme and Zymolyase resulted in an accumulation of mononucleated cells with a 1C DNA content, confirming that the genomes of the septated cells are unreplicated in the *med1*, *med2*, and *med3* overexpression samples (Figure 2, digested).

pim1^{ts} is hypersensitive to overexpression of *med1*, *med2*, or *med3*. Expression of *rna1*, which encodes a known regulator of the spi1p GTPase system, is synthetically lethal with the *pim1-d1*^{ts} mutation (Matynia *et al.* 1996). To determine whether the *med* genes are likely to encode additional components of this system, we compared the sensitivity of wild-type and pim1-d1^{ts} mutant cells to overexpression of *med1*, *med2*, or *med3*. At the *pim1-d1*^{ts} permissive temperature of 25°, wild-type



Figure 2.—med overexpression arrests cells with an unreplicated genome. FACS analysis of the DNA content of septated, binucleated med1, med2, or med3 overexpressing cells (undigested) is 2C (1C per nucleus). Separation of the two daughter cells by treatment with a mixture of Novozyme and Zymolyase (digested) results in the accumulation of mononucleated cells with a 1C DNA Nitrogen-starved, content. wild-type haploid cells were used as a control (ctrl) to determine the position of 1C and 2C DNA peaks (arrows).

or mutant cells transformed with *med1*, *med2*, *med3*, or the vector control grew equally well when transcription from the intermediate version of the *nmt1* promoter in plasmid pREP41X (Forsburg 1993) was repressed (data not shown). When transcription was derepressed at 25°, however, the pim1-d1^{ts} mutant cells showed growth inhibition caused by expression of *med1* but not the vector when compared to wild-type cells (Figure 3). Wild-type and pim1-d1^{ts} cells grew equally well at 25° when either *med2* or *med3* were expressed (Figure 3); however, the growth inhibition of pim1-d1^{ts} with *med2* or *med3* overexpression was seen when cells were incubated at the semipermissive temperature of 33° (Figure 3). Expression of all three *med* genes was more toxic to pim1-d1^{ts} mutant cells than to wild-type cells, but overexpression of *med1* was more toxic than that of either med2 or med3.

med1 encodes the fission yeast structural homolog of RanBP1: Three *med1* cDNA-containing clones, which resulted in identical phenotypes when overexpressed, were identified in the screen. DNA sequencing revealed that two of the clones, *med1-1* and *med1-2*, contained the same 0.7-kb full-length cDNA (GenBank accession number D76431) encoding a predicted protein product of 215 amino acids (Figure 4A). A third cDNA, *med1-3*, contained the same open reading frame interrupted by a putative intron of 172 bp that was flanked by consensus 3' and 5' splice sites and had stop codons in all three reading frames.

The *med1* gene was cloned by colony hybridization, and from the DNA sequence (GenBank accession number D86381), two introns were identified (Figure 4A) based on the presence of consensus splice sites and comparison with the sequences of the *med1-1* and *med1-2* cDNAs. The first intron was 172 bp and was retained in



Figure 3.—pim1-d1^{ts} cells are hypersensitive to sublethal *med* overexpression. Wild-type cells or pim1-d1^{ts} cells, transformed with pREP41X vector, pREP41X-*med1*, pREP41X-*med2*, or pREP41X-*med3* plasmids, were grown to midlog phase in thiamine-free liquid media to derepress transcription. Fivefold serial dilutions of equal numbers of cells were spotted on thiamine-free minimal media plates and incubated at either 25°, the permissive temperature, or 33°, the semipermissive temperature of *pim1-d1*^{ts}.

med1-3 cDNA, which was presumably derived from an RNA splicing intermediate. The second intron was 86 bp and was not retained in any of the cDNA clones.

The *med1-1* cDNA insert was used as a probe to screen an ordered fission yeast cosmid library (Lehrach *et al.* 1990) to determine the chromosomal location of the *med1* gene. The five positive cosmids ICRF c60E087D, ICRF c60D0115D, ICRF c60C048D, ICRF c60B0335D, and ICRF c60A052D mapped to chromosome II between *rad11* and *mei3*.

The predicted med1p amino acid sequence is 54% identical and 67% similar to human RanBP1, and 57% identical and 70% similar to the budding yeast RanBP1 homolog *YRB1* (Figure 4B). The highest region of similarity lies in the RanBD (Figure 4A, underlined), which is highly conserved among a large family of proteins (Figure 4B). Several of these proteins have been shown to bind to the Ran family of GTPases (Figure 4B). Based on these sequence similarities, we have renamed *med1* according to the mammalian nomenclature. It will subsequently be referred to as *shp1* (spi1-binding protein 1).

Fission yeast *sbp1* encodes a functional homolog of **RanBP1:** The first indication that sbp1p was a functional homolog of RanBP1 was its identification in a two-hybrid screen for fission yeast proteins that bind to human Ran. The Y190 strain, which harbors pAS-Ran and has previously been used for two-hybrid screening of a human cDNA library (Yokoyama et al. 1995), was transformed with an *S. pombe* cDNA library. From $\sim 10^5$ transformants, 56 colonies survived selection on 25 mm 3-AT, and 31 of these retained this resistance when transferred to fresh 3-AT plates. cDNA-containing plasmids were isolated from 18 of these colonies and sequenced. Six were found to encode a protein identical to sbp1p. When assayed in quadruplicate for β -galactosidase activity, a representative *sbp1*-containing clone (the transformant bearing pFTBP112 and subsequently renamed Y190Ran) had 37.8 ± 8.7 units/mg protein of enzyme activity, a level 15 times greater than the 2.6 \pm 0.6 units/mg protein of the transformant bearing only the vector pGAD-DH. These results are consistent with there being a direct protein-protein interaction between sbp1p and human Ran. To test whether this interaction was specific for the GTP-bound form of the GTPase, as has been shown for other RanBD-containing proteins (Beddow et al. 1995; Coutavas et al. 1993; Lounsbury et al. 1994; Schlenstedt et al. 1995), GSTsbp1p immobilized on filters bound to Ran- $[\gamma^{-35}S]$ GTP but not to Ran-[β-35S] GDP (Noguchi et al. 1996; Yokoyama et al. 1995), whereas the GST control bound to neither (Figure 5A).

The ability of sbp1p to bind to spi1p, the fission yeast Ran homolog, was tested by incubating *S. pombe* cell extracts with either GST-sbp1p or GST immobilized on beads. The proteins that bound to the beads were separated by electrophoresis, transferred to a mem-

																								-1	58	ac	tag	tag	ctt	taa	ıggc	aaa	gat	ttt	.att	agc	aaa	tga
ttt	ttat	aga	ttco	cga	acta	aca	caç	cad	cca	attt	cca	ata	tgt	tttg	Itto	gaa	atac	cga	agtt	act	gta	tat	tat	aat	ttt	ttc	gtg	gta	cac	tct	tcc	tto	cat	cag	cac	aat	acc	aca
GT	CCGC	AGA	ACAA	GAA	AAA	AAG	ACT	CA	AGG	ACT	ACT	TAAA	GA	GAG	CAG	AAA	TCT	TC	TTTT	GCT	AGT	GAA	GAC	GTT	GCI	AGC	AAG	CAA	ACA	GAA	GAA	GCC	AAA	GCT	GTT	ттт	GGT	GAC
M :	5 A	E	0	E	K	K	т	0	G	т	т	K	E	E	0	ĸ	S	S	F	A	S	E	D	v	A	S	K	0	T	F	F	Δ	K	A	v	F	G	D
			-					-			-	-	-	-			-	-	-			-	-					×	-	-	-	n		n		•	9	D
TG	rggc	CAA	ACAA	GAZ	AAC	AAG	TCI	GGI	rgco	TCC	ACC	AAC	GAT	GAG	AAG	AAG	CCA	GC	FGA	GGZ	GAT	GAG	GAT	GCT	GAA	CCA	GCT	TCC	CCT	GAG	GTT	CAC	TTT	GAG	CCT	АТТ	GTC	AAA
	IA	K	0	E	N	ĸ	S	G	A	S	т	N	D	E	K	ĸ	P	A	E	G	D	E	D	A	F	P	A	S	P	F	v	н	F	F	D	т.	V	v
							-	-					-	1			<u> </u>		-	~	-	~	-		-			5		-	•	-	-	-	-	-		A
GAG	TGC	AGTO	GAG	ACI		ACT	TAA	GAI	GAZ	GAG	CAC	ACT	GTO	CAR	-		ATC	CO	PGCT		CTT	mme	INC.N		CAC				200		maa	220		000	000		0.00	
	2 7	V	F	T	v	m	N	P	P	P	P	m		P		v	MIC	nco.	1901	~~~~~		110	AGA		GAC	nnu	GCA	GCA	AGC	GAA	199	AAG	GAA	CGI	GGA	ACT	GGT	GAT
_		, v			-			- 10	- 6		-	-	<u> </u>		<u>r</u>	-			A	•	-	R		R	0	Δ.	A	A	5	E	_w	ĸ	E	R	G	T	G	D
ac	yttg	aaa	agac	att	atg	cta	icgt	gtt	gtt	tgaa	ata	actt	ttt	ctt	aat	aca	itta	aag	gcto	act	gtc	ctt	tcg	tgt	ctt	gac	tat	ttc	ttt	tct	ctg	gtt	tct	cag	jaat	gta	igta	tct
ata	icta	acti	tgtt	ttt	agī	GAT	GCC	CGI	GAT	GAA	ATT	GAC	TCC	CAA	TGI	TGO	AAG	CGI	ATCO	TAC	TTG	GGT	TTG	GAC	CGT	TGC	TGC	GGA	TGI	TAG	TGA	AGG	AGA	ACC	TAC	TGC	AGA	GAC
					T		P		C M	1 K	т			N	I U							v	7 W		U	h	n	D			F	0					P	m
							-				-	-	-		_	-		_	-		<u> </u>	¥		-	¥				¥						-		<u> </u>	
-	CTA	TTC	2777	TGC		CTC	100		acc	rat	a++	++ =	+ ~ =	ato		++-		++.				aat		+ ~ ~	2.04	a+ a	a+ -	a+ -	-		aat		+			ima	mam	
	2			100			non	-	acc	gat	gui	····a	LLCO	all	cay	LLY	ala	LLC	adua	aau	aac	CCC	all	LCa	act	yry	yta	CLa	acc	aaa	CCL	cgt	tag	ATG	CCA	ATC	TUT	TTA
-	A		<u> </u>				-	-																										<u>N</u>	A	N	L	<u>e</u>
GA	AAC	TTTC	AAA	AAT	ACC	AAG	AAG	AGA	ATG	CCA	AAT	TCT	CAA			ATT	AG																					
F	N	F	F	K	v	0	F	F	M	A	v	T	T	v	v	NT +	**																					
	- 14		~	*	*	×	-	-	14	•	L/	+		n	n	14 .																						

в

Spsbpl	1	H	FE	PI	κŪ	SA	- 1	ЕТ	KI	NE	DE	T V	ER	км	RA	(LF	RF	DKA	AS		ΕŴ	KE	RG	GD	ARI	LLK	HK	ETG	K		-тБ	LV	MRR	D -	KT	KV	CAN	HLL
SCYRB1	1	B	FE	PV	HI	EK	- 1	DV	KT	MD	EDI	EV	LY	κv	RA	LE	RF	DAD	AK		EW	KE	RG	G	CK	LK	NK	KTN	K		VI	TL	MRR	D -	KT	KI	CAN	HIT
MmRan BP1	1	0	FE	PIT	SV	PID	- 0	DET	KT	L 13	ED	EF	LE	KM	RA	LE	RF	ASE	ND	LP	EN	KE	RG	GD	VKI	LIK	HK	EKG	T		- I R	LL	MRR	D -	KT	KI	CAN	HYI
Hs Ran BP1	1	ā	FE	PT	sli	PE	- 6	ET	K D	T. 15	EDI	DE E		KM	RA		10 12	ASE	ND	T. P	EO	KE	RG	G	VK	TR	HK	EKG	A		- 212	TAT	MRR	D -	KT	KT	CAN	HVT
He Ran BP2 D1	1		FF			DI				GE	EDI			CN	DA		DP	NV B	CV		TRA	I I F	PC		17 12	TID	HW	TEC	¥		2 8	TT	MPP	÷	0 1		CAN	
Wabaa BB2 D1	î		PP							CE	EDI				DA				OV		-	VE	R C				UV						MDD	9	č v		CAN	
Nanau DF2D1					55					GEI				CN					DA		-	NE	R G				n A	1 8 6			- 21		MAR	B			CAN	
ABRANBP2D2	-		FE		Q M	1.10		191	V II	GEI	EDI	PKV	LY	sy	RV		KF	A	vs		2	KE	RG.	9.0			NE	VNG	K			ML	MRR	8-1	2 1		CAN	H."H
Amkan BP2D2	1	H	FE	P V	QM	P E	K	DL	VII	GE	EDI	PKV	LY	sQ	RV	(LF	RF	DAE	IS		QW	KE	R G	GI	LK.	IIK	NE	VNG	K - ·		- 44	ML	MRR	E -	QVL	KV	CAN	EL W L
HsRan BP2D4	1	H	FE	PI	SI	PB	-1	EV	KS	GEI	EDI	EI	LF	ΚE	RA	K L Y	RW	DRD	vs		QW	KE	RG	7 G D	IK	ILW	HTI	MKN	¥ - ·		- Y :	IL	MRR	D -	QVI	ΚV	CAN	HVI
HsRan BP2D3	1	Y	FE	PV	PI	PD	LV	EV	SS	GEI	BN	ΞQV	VF	SH	RA	K L Y	RY	DKD	VG		QW	KE	RG	IGD	IK	ΙĽQ	NY	DNK	Q		- V R	VIS	MRR	D -	QV	KL	CAN	HRI
MmRan BP2D3	1	Y	FE	PV	PI	PD	L	EV	SS	GEI	DN	9 Q V	VF	SH	RA	(LY	RY	DKD	VG		QW	KE	RG	IGD	IK	ΙĽQ	NY	DNK	Q - ·		- V 🖪	IV	MRR	D -	QVI	KL	CAN	HRI
XlRan BP1	1	Q	FE	PI	SI	PB	- 0	EI	KT	LD	EDI	EE	LF	KM	RAI	(LF	RF	ASE	ND	PP	EW	KE	RG	GD	VKI	LLK	HK	ERG	T		- I K	LL	MRR	D -	КТ	KI	CAN	HYI
BtspvarD2	1	H	FE	PI	SI	PD	-1	EV	KS	GEI	EDI	EI	LF	KE	RA	LY	RW	DRE	AS		OW	KD	RG	7 G I	IK	IW	HT	VKN	¥ - ·		- F 5	IL	MRR	D -	OVE	ΚV	CAN	HVI
Btppcti	1																																MRR	D -	ÕVI	κv	CAN	HVI
At Ran BP1	1		VA	DIT	RI	RE	-5	AW	TT	GEI	EDI		WT.	D T.	KS	V M	RE	NK B	GN		0.0	KE	RG	AGT	VKI	TR	HK	ETG	K		-VE	L.V	MRO	S-	KT	KI	CAN	HI.I
Bt any arD 1	ĩ	ž	12 12	D.V.		DD		EU		CEI	E MI	0.1	11	c =	DA		DV		20	22	č	KE	PC	CI	-	THO	NV	PNV	0-	_	v	TV	MRR	D -	0 1		CAN	E R I
Cortegazol	1							E 17		CEL									A.G		2.	K E	RO			×	NW		2			W.W.	MDD	5	õ w		CAN	P D
C	-						-	EV		GEI	9	221	m		K D				TA		E M	K E	R G				ON N		a				MDD	¥			CAN	5.5
Cersyazbz	1	E	FK	P V .	1.6	PD	L L		KI	GEI	EDI	DE V	MP	SA	RC	CL Y	KX	ISL	LK		EN	KE	R G	GI		- DK	DN.	DND		:	1	1.4	PIKK	8	Q VI	N I	CAN	IL VI
Sphbai	1							EI	I	GEI	DE	PES	IF	s v	RA	ST X	vv.	ADE	KK		TW	KE	RG	2 G 1	LK	VNV	PK	2 R G	SG.	2	SGR	L L	MRN	<u>n</u> -				
SCYRB2	1	-					- 9	EV	KS	GEI	S	EC	IY	Q V	NA	(LY	QL	SNI	KE	G -	-12	ΚE	RG	V G I	IK	INK	SK	DDV	EK.		- TR	UV	MRS	R -	GIL	KV	ILR	IQL
SCNUP2	1	-					-1	NL	QN	GEI	EDI	DVA	LF	SQ	KAI	A DIM	TR	N 8 1	1 m 17		SY	DS	RG	7 G E	MKI	LLK	KK	DDP	SK-		- V R	LL	CRS	D –	GMO	NV	LLN	ATV
HSHHCP045	1	-	-								_					× 20 11		N N E	TU		_													-		RT.	T T 53	Im v T
							- N	EV	IΠ	GEI	A	SN	VL	QM	QC	LF	VF	DKI	SQ		SW	VD	RG	RGI	LRI	LND	MA	STD	DG	гь	QSE	LV	MRT	Q -	0.00		1 1 1	TVP
							- 1	EV	IT	GEI	A	SN	VL	QM	QC	LF	VF	DKT	sQ		SW	VE	RG	RGI	LRI	LND	MA	STD	DG	гLĢ	Q S 🖸	LV	MRT	Q -	Gol		1 1 1	TINE
							- 1	EV	IT	GEI	PA	SN	VL	QМ	QC	LF	VF	KI	SQ		SW	VĐ	RG	RGI	LRI	LND	MA	STD	DG	r L (Q S E	LV	MRT	Q -	Gog		1 10	ar v P
Spsbp1	78	м	PE	IK	TE	N V	Gs	DR	IT	GEI	TV	AAD	VL	Q M E G	QC	TA	VF	FAI	SQ	 A N	SU	VE	RGI	RGI	LRI	EK	MA	STD	DG	ΓLĢ	QS <u>E</u>	LV	MRT	Q -	0 50		1 10	11 1 1
Spsbp 1 ScyrB 1	78 78	M	PE	K	TP	N V	GS	DR	SW	G E I	TVI	AAD	VS	EGEG	QC	TA	VF	FAI	RF	A NGS	SB	VE NA	R GI	RGI	LRI	EK	MA	STD	DG	r L (Q S K	AL A	MRT	Q -	6 5 5		1 10	11 K 11
Spsbpl ScYRB1 MmRanBP1	78 78 80	MA	PE	KI	TP	N V N V	Gas		SW SW AW		TVI	AAD	VS	EGEG	QC	TA	VF	FAI	RF	A N G S L N	SEKE	VE NA NA	R GI	RGI			MA	STD	DG	ΓLĢ	Q S L	LV	MRT	Q -	6.96		1 14	JI KL
Spsbp1 ScYRB1 MmRanBP1 HsRanBP1	78 78 80	MAIT	PEPM	KI	TPKP	N V N V N A	GGGGG		SW SW AW		TVI	AAD	VS		QC	TA	VF	FAI	RF	A N G S L N	SEKEAE	VE NA NA	R GI N L D K Q K	FKE			MA	STD	DG	ΓLĢ	QSE	LV	MRT	Q-	6 3		1.77	JI KL
Spsbpl ScyrBl MmRanBPl HsRanBPl HsPspBP2D1	78 78 80 80	MATI	PE PE PM PM	M K I Y T I M E I	TPKPKP	N V N V N A N A	GSSS	DR	I T SW SW AW AW		DA TVI AC NTI	ESN AAD FAD HAD	VS IA FA		QC	TA EA PKP	VF	FAILAI	RF	A N G S L N L N	SEKEAE	VE NA NA NA	R GI N L D K Q K	FKE			MA	STD	DG	r L (QSE	LV	MRT	Q-	6.96		1 24	
Spsbp1 ScYRB1 MmRanBP1 HsRanBP2D1 HsRanBP2D1	78 78 80 80 79	MATIS	PE PM PM PD	M K I M E I M E I	TPKPP	N V N V N A N A	GGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	DR DR DR DR	SW SW AW SF		DA TVI AC NTI HAI	A A D F A D H A D H A D L D Y	VL VS IA FA A-		QC E- E- C- L-	TA EA EA EA EA EA EA EA EA EA EA EA EA EA	VF	FAI FAI LAI LAI	RRFFFF	A N G S L N L N K T	SEKEAE	VE NA NA NA	R GI D KI Q KI A LI	FKEFKI			MA	STD	DG	r L (Q S E		MRT	Q-	6.96		1 24	11.4
Spsbpl ScYRBl MmRanBPl HsRanBP2 HsRanBP2Dl MmRanBP2Dl	78 78 80 79 79	MATTSS	PE PM PM PD PD	M K I M E I M E I M K I	TKPP	N V N V N A N A N A		DR DR DR DR DR	SW SW AW SF SF		DA TV AC NTI HAI	A A D F A D H A D H A D L D Y	VL IA FA A-	EGEE	QC E - : C - : L - : F - :	KLF TA EA KP KP KP	VF	FAI FAI LAI LAI	RFRFRFRFRFRF	A N G S L N K T Q T	SEKAEPE	VE NA NA NA EA	RGI DKI QKI ALI	FKEFKT	LR NF EF KF KF KF	LND	MA	STD	DG	r L (õ s E	LV	MRT	ō-				11.4
Spsbpl ScyRBl MmRanBP1 HsRanBP1 HsRanBP2D1 MmRanBP2D1 HsRanBP2D2	78 78 80 79 79 79	MATTSST	PE PM PD TT		TKPP	N V N V N A N A N A L S		DR DR DR DR DR DR	SW SW AW SF SF AW		DA TVI AC NTI HAI HAI	A A D F A D H A D H A D L D Y L D Y S D F	VS IA FA A- A- S-		QC E - 1 E - 1 C - 1 L - 1 F - 1 D - 1	KLF TAEA PKP KP KP	VF FTLLQQQ	FAI LAI LAI LAI	RFFRFRF	A N G S L N L N K T Q T	SEEAPPE	VE NAA NA EA EA LA	RGI NLI DKI QKI ALI EE	F K E F K E F K T F K T F K T	L R I N F I K F I K F I K F I	LND	MA	STD	DG	r L (δ 2 F	JL V	MRT	ō-				1.42
Spsbp1 ScYRB1 MmRanBP1 HsRanBP2 HsRanBP2D1 MmRanBP2D2 HsRanBP2D2 MmRanBP2D2	78 78 80 79 79 79 79	MATISSIT	PEPMPD	M K I M E I M K I M K I M N I	TKKFPP	N V N V N A N A N A L S L S		DR DR DR DR DR DR	SW SW AW AW SF SF AW AW		DAL TVI AC NTI HAI HAI	A A D F A D H A D H A D H A D H A D H A D H A D H A D H A D H A D H A D H A D H A D	VL IA FA A- S- S-		Q C E - 1 E - 1 C - 1 L - 1 F - 1 D - 1	A E A P K P K P K P K P K P K P K P K P K P	VF FTLLQQQQ	FAI FAI LAI LAI LAI	RRFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF	A N G S L N L N T T K T	S KEEPPE	VE NAA NAA EAA LA	RGI NLI DKI QKI ALI EEI	RGI FKE FKE FKC FKC	LR NF EF KF KF KF KF		MA	STD	DG	r L (δ z Ē	JL V	MRT	Q-				
S p s b p 1 S c Y R B 1 M R a n B P 1 H s R a n B P 2 H s R a n B P 2 D 1 H s R a n B P 2 D 2 M m R a n B P 2 D 2 M m R a n B P 2 D 4	78 80 89 79 79 79 79 79 78	MATTSSTTT	PEMMDDTTT		TKKKTTKKK	N V N V N A N A N A L S L S L N		DR DR DR DR DR DR DR	SW SW AW SF SF AW AW		TVI AC NTI HAI LAI	A A D A A D H A D	VL IA FA A- S- S-		QC E - 1 C - 1 C - 1 L - 1 F - 1 D - 1 E - 1	A E A P K P K P K P K P K P K P K P K P K P	VF FFLLQQQQQ	FAI FAI LAI LAI LAI LAI	REFEFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF	A G S N N T T T T T	S S S S S S S S S S S S S S S S S S S	VE NAAA NAA EEAA LLAA	R GI N LI Q KI Q KI A LI E E D C	RGI FKE FKE FKE FKC FKC FKC	LRI NFI EFI KFI KFI KFI	LND EKKEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE	MA	STD	DG	r L (δ z Ē	3L V	MRT	Q-				
S p s b p 1 S c x R 1 M m R a n B P 1 H s R a n B P 2 D 1 H m R a n B P 2 D 1 H m R a n B P 2 D 2 M m R a n B P 2 D 2 H s R a n B P 2 D 4 H s R a n B P 2 D 4	78 80 79 79 79 78 79 78 79	MATTSSTHTT	PEEMMDDTTTD		TKKKTPPPP	N V N A N A N A L S L S L S L S		DR DR DR DR DR DR DR R DR	I SW SW AW SF SF AW AV VW		TVI ACS NTI HAI LAS	A A D F A D F A D H A D L D Y L D Y S D F S D F S D Y C D F	VL IA FA A- S- A- AD		QC E - 1 C - 1 C - 1 L - 1 F - 1 D - 1 E - 1 R - 1		VF FFLLQQQQH	FAI LAI LAI LAI LAI	SO FFFFFFFFFFFFFFFFF	A G S N N T T T T T T	SKAAPPPEED	VE NAAANAA EEAA LLAA VA	R GI N LI O K O K I O K I O K I O K I E E E I D S	RG L FKEFFKI FFKG FFKG FFKG FFKK	LRI NFI EFI KFI KFI KFI	L N D KKEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE	MA	STD	DG	r L (õ s E	<u>a</u> r v	MRT	Q-				1.17
S p s b p 1 S c x R b 1 M m R a n B P 1 H s R a n B P 2 H s R a n B P 2 D 1 M m R a n B P 2 D 2 H m R a n B P 2 D 2 H s R a n B P 2 D 2 H s R a n B P 2 D 3 H s R a n B P 2 D 3	78 80 79 79 79 79 79 79 79 79 79 79	MATISSITIT	PERMADDITID		TKKKTTKKKQQ	N V N N A N A N A L S L S K M K		DR DR DR DR DR DR DR ER	I W SW AW SF AW SF AW AU VW VW		TVI ACS NTI HAI LAS	A A D F A D H A D	VL IA FA A- S- A- AD		QC E		VF FTLLQQQQ EQQQH		SO RFFFFFFFFFFF	AGSINTTTL	SKAEPPPEED	VE NAA NAA EEAA LLAA VA	R GI N LI D K Q K L Q K L L E E C I D S I	RGI FKEFFKI FFKC FFKC FFKC	LRI NFI EFF KFF KFF KFF	L N D EKKEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE	MA	STD	DG	r L (õ s 🗗	JL V	MRT	Q-				
S p s b p 1 S c x B 1 Mm R a n B P 1 H s R a n B P 2 D 1 Mm R a n B P 2 D 1 H m R a n B P 2 D 2 Mm R a n B P 2 D 2 H s R a n B P 2 D 3 Mm R a n B P 1 3 Mm R a n B P 1 3 Mm R a n B P 1 3	78 80 79 79 79 79 79 79 79 80	MATTSSTTTTT	PPPPPTTKPPL		TKKKTTKKKQQK	N V N N A N A N A L S L N K M A		DR DR DR DR DR DR DR DR DR DR	I SWWAWSFFAWALVWWAW		TVI ACS NTI HAI LAS TAC	A A D F A D H A D	VL VS IA FA A- S- S- A- AD 		QC E		VF FTLLQQQQH FLLQQQQH		REFEFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF	AGLLKQKKKK -L	SKAEPPPED A	VE NAAAAA EEAAA VA	RGI NLK QQLL ALEEDS - K		LRI NFI KFI KFI KFI	L ND EKEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE	MA	STD	DG	r L (õ s E	JL V	MRT	Q-				
S p s b p 1 S c x R b 1 M m R a n B P 1 H s R a n B P 2 D 1 H s R a n B P 2 D 2 H m R a n B P 2 D 2 H m R a n B P 2 D 2 H s R a n B P 2 D 2 H s R a n B P 2 D 3 H m R a n B P 2 D 3 X 1 R a n B P 1 B t s p v a T 2	78 80 799 779 779 80 779 88 78 87 80	MATTSSTUTTT	PPPPPTTKPPK		TKKKTTKKKQQKK	N V N N A A A A A A A A A A A A A A A A		DR DR DR DR DR DR DR DR DR DR DR DR	I SWWAASFFAWAALVWWAAL		TVI ACINTI HAI LAI TACINTI		VI FA FA A- S- A- A- S- A- A- A- A- A- A-		QC E - 1 E - 1 C - 1 F - 1 D - 1 E - 1 D - 1 E - 1 S -		VF FFLLQQQQQH FLLQQQQQH		RREFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF		SW SE AE PE PE RE QD AE	VE NAAAA EAA LLAAA NAA	RGI NLKKKLLEECS - KC		L R I N F I K F I	L ND KKEEEEEEEDE	MA	STD	DG	r L (õ s E	JL V	MRT	Q-				
S p s b p 1 S c x B 1 Mm R a n B P 1 H s R a n B P 2 D 1 Mm R a n B P 2 D 1 H m R a n B P 2 D 2 H m R a n B P 2 D 2 H s R a n B P 2 D 3 Mm R a n B P 2 D 3 Mm R a n B P 1 B t s p v a r D 2 B t p p c t i	788099977998 77999899 7798716	MAHISSHHHHHHH	PPPPPTTKPPLTT		T K F P P P P P P P P P P P P P P P P P P	N V N V N A N A N A L S L S L S L N K K M K L N L N L N		DR DR DR DR DR DR DR DR DR NN	I SWWAWSFFWWAAL		EA TV: AC: NTI HAI LA: TTA: TTA:		VL VSAFA- S- AD- YA- A-		QC E - 1 E - 1 C - 1 F - 1 D - 1 E -		VF FFLLQQQQQH FLLQQQQQH		RREFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF	AGSNNTTL - NTTL - NTT	SW SE AE PE PE QD AE KE	VE NAAAA EEAAA LLVV NMAA	RGI NLKKKLLEECS - KCC		L R I N F I K F I	L ND KKEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE	MA	STD	DG	r L (õ s E	3L V	MRT	Q-				
Spsbp1 ScTRB1 MmRanBP1 HsRanBP2D1 HsRanBP2D1 HsRanBP2D2 HsRanBP2D2 HsRanBP2D2 HsRanBP2D3 MmRanBP2D3 X1RanBP1 Btspvcti AtRanBP1	7880999988877777888717889998868	MAITSSTTTTTTT	PPPPDTTKPPLTKG		TKK TTKKKQQKKKQ	NVAAANN NAASSNAN NILLS		DR DR DR DR DR ER DR	I SW SW AW SF AW AW SF AW AU AU AU AL		EA TV: TACT: TACT: TACT: TACT: TAC: TACT: TAC: TAC	SNAAD HAD HAD LDY LDY SDF F SDY SDY SDY F DF	VL VSAFFA-SS-ADSA-YA-AS		Q C =		TT ELLEQQEE EQQEE EQQEE EQQEE EQQEE EQQEE EQQEE EQQEE EQQEE EQQEE EQQEE EQQEE EQQEE EQQEE EQQEE EXT	FAI FAI LAI LAI LAI LAI LAI LAI LAI LAI LAI L	RFF RFF RFF RFF RFF RFF RFF	NSNNTTTTL - NTTS	SW SE AE PE PE KE QD 	VE NAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	RGI NDQQAAEECS-KCCCT		L R NEFI KKFF KKFF I F KKFF	L ND KKEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE	MA	STD	DG	r L (õ s 🗗	3L V	MRT	0-				
Spsbp1 Spsbp1 MmRanBP1 HsRanBP1 HsRanBP2D1 HmRanBP2D2 HmRanBP2D2 HmRanBP2D2 HsRanBP2D3 HmRanBP2D3 KanBP1 BtspvarD2 StspvarD1 StspvarD1 HtspvarD1	7780099998990868777777777777777777777777	MAINSSTHTTTTS	PPPPPTTKPPKKSP			NVAAANN NAA SSUUMKANN LLNK		DR DR DR DR DR DR DR DR DR DR DR DR DR D	I T SW SW AW SF AW AW SF AW AU VW AW AL SC		EA TV: TACT: TACT: TACT: TACT: TAC: TAC: TAC:		VL VS FA A- S- A- S- A- S- A- S- A- S- A- S- A- S- A- S- S- A- S- S- S- S- S- S- S- S- S- S- S- S- S-		Q C =			FAI FAI LAI LAI LAI LAI LAI LAI LAI LAI LAI L	SQ RFFFFFFFFFFF RRRRR	NSNNTTTL - NTTS	SW SEE AE PPE PPE SC C C C C C C C C C C C C C C C C C C	VE NA NA EA LA VA VA MA NC	R G N L D K Q K L L E E E C D S - Q C C T S		L R NEFI KKFF KKFF IF	L KKEEEEEEEDE EEEEEE	MA	STD	DG	r L (õ s E	3L V	MRT	Q				
Spsbp1 ScTRB1 MmRanBP1 HsRanBP2D1 HsRanBP2D1 HsRanBP2D2 HsRanBP2D2 HsRanBP2D2 HsRanBP2D3 XIRanBP1 StspvarD2 Btppcti AtranBP1 StspvarD1 CeP592D1	77887777777871777 88799998990868999	MATTSSTHTTTTST			T K K K T T K K K Q Q K K K Q Q K	NNVAAASSNKKANNLLHKK		DR DR DR DR DR DR DR DR DR DR ER ER	I SW SW AW SF AW		TV: ACTINATIONALIAN		VS IA FA S- S- AD XA- S- S- XA- S- S- S- S- S- S- S- S- S- S- S- S- S-		Q C =		THLLQQQQH LQQLH	FAI FAI LAI LAI LAI LAI LAI LAI LAI LAI LAI L	SQ RFFFFFFFFFF RRRRRRRFFFFFFFFFFFFFFFFFF	NSSINTTTL - NTTSL	SW SE AE PPE PPE SC C C C C C C C C C C C C C C C C C C	VE NAANAA NAAEAA LAAVA VA NAA NAA VA	R GI N L K Q Q K L L E E E D D S - Q C C T S E E E D D S - Q C C T S E		L R NFF KFF KFF KFF F K KFF		MA	STD	DG	r L (Q S <u>R</u>	3L V	MRT	Q				
S p s b p 1 S c x B 1 Mm R a n B P 1 H s R a n B P 2 D 1 H m R a n B P 2 D 1 H m R a n B P 2 D 2 H m R a n B P 2 D 2 H m R a n B P 2 D 2 H m R a n B P 2 D 3 Mm R a n B P 1 B t s p v a r D 2 B t s p v a r D 2 A t R a n B P 1 B t s p v a r D 2 A t R a n B P 1 B t s p v a r D 2 A t R a n B P 1 B t s p v a r D 2 A t R a n B P 1 B t s p v a r D 2 A t R a n B P 1 B t s p v a r D 2 A t R a n B P 1 B t s p v a r D 2 A t R a n B P 1 B t s p v a r D 2 A t R a n B P 1 B t s p v a r D 1 C a P 5 A 2 D 2 C a P 5 A 2 D	77800999998990868998 77777787177777777777777777777777777	MATTSSTTTTTTSTL			T K K K T T K K K Q Q K K K Q Q Q	N V V A A A A S S N N V A A A A S S N N K A N N N A A A S S N K K A N N N K K A N N N K K A N N		DR DR DR DR DR DR DR DR ER ER	I SW SW AW SFF AW AV VW A AL SC VW Y		TVI ACTINATIONAL	S N A A D H A H A H A D H A H A H A H A H A H A H A H A H A H A	VIA FA- FA- S- S- S- S- S- S- S- S- S- S- S- S- S-		Q C =	T APPEKKELLVV PRVVDIH	ETT ELLEQQ EQQ EQQ EQQ EQQ EQQ EQQ EQQ EQQ	FAI LAI LAI LAI LAI LAI LAI LAI LAI LAI L	RFFRFFRFF RFFRFF RFFRFF RFFRFF	AGLLKQKKKK - LKKAKAKAKAKAKAKAKAKAKAKAKAKAKAKAKAKAKAK	SW SEE AAEEEED AKEEDD AKEEDD	VE NAANAA NAAEEAA LAAA VA MAA VA NAA NAA NAA	R GI N LL N D K K Q A L L E E E D D S - Q D C C T S G C D K S G C		L R NFF KKFF KKFF KKFF KKFF KFF KFF KFF		MA	STD	DG	r L (Q S <u>F</u>		MRT	Q				
S y s b y 1 S y s b y 1 M m R a n B P 1 H s n a n B P 2 H s n a n B P 2 D 1 H s n a n B P 2 D 1 H s n a n B P 2 D 2 H s n a n B P 2 D 2 H s n a n B P 2 D 3 X m R a n B P 2 D 3 X m R a n B P 1 B t s p v a r D 2 B t p P c t i B t s p v a r D 1 C e F 5 9 A 2 D 1 C e P 5 9 A 2 D 2	7780099999871871879987177777777777777777	MATTSSTTTTTTSTLE	PPPPPTTKPPKKSPGK		TKKKTTKKKQQKKKQQQS	NVVAAAAS NNVAAAS LLNKKANNL HMKKP	GGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	DR DR DR DR DR DR DR E R DR E R NN NN E E R NN NN E E R	I SW SW AW SF SF AW AU SF SF AW AL SC VW AL SC VV Z		TVI TVI ACTINATIONATIONATIONATIONATIONATIONATIONAT	S X A A D A D	VL FA- S- XA- XA- XA- XA- SE SE	Q M GGEEGGGGE - EGGGGEQA	Q C =	TAPPKKPLL PARKPPKKLLV PAKKVV PAKKVV PAKKVV PAKKVV PAKKVV PAKKVV PAKKVV	TTTLLQQQQQ EEQQH EEQQUEEE EEQQUEEE EEQQUEEEE EEQQUEEEEEE VKAI	FAI FAI LAI LAI LAI LAI LAI LAI LAI LAI LAI L	RFF RFF RFF RFF RFF RFF RFF RFF RFF	AGLINTTTL - NTTSLND	SW SKEEPPEED AEEEQD AEEEQD EAKEEQDD	VE NAA NAA EA LAA LAA VAA NAA NAA VAA NAA VAA NAA NAA NAA N	R G N L L N D K K Q Q K L L E E D S - K C C C T S G G A	R G I		LND KKEEEEEEDE - EEEEEKD	MA	STD	DG	r L (Q S <u>F</u>		MRT	Q				
S p s b p 1 S c r R B 1 M m R a n B P 1 H s R a n B P 2 D 1 H m R a n B P 2 D 1 H m R a n B P 2 D 2 H m R a n B P 2 D 2 H m R a n B P 2 D 3 M m R a n B P 2 D 3 M m R a n B P 1 B t s p v a r D 2 A t R a n B P 1 B t s p v a r D 2 A t R a n B P 1 B t s p v a r D 2 A t R a n B P 1 B t s p v a r D 2 S P h b a 1 S S S S S S S S S S S S S S S S S S S	778009999988777778788877777777778717777777777	MATTSSTTTTTTSTLE	PPPMDDTTTDDLTTGDSS -		TKKKTTKKKQQKKKQQQS -	NVVNAANNAANNAANNAANNAANNAANNAANNAANNAAN	GGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	DR DR DR DR DR DR DR DR ER NN NN ER ER	I SWWAWSSF AWWANL SFF AWWANL SCWYAU VWYAU		EA TV:: 1 TAAC	S N A A D A A D A A D A A D A A D A D A D A	VS IA FA FA S- S- AD S- S- S- S- S- S- S- S- S- S- S- S- S-	Q E G E E E G G G E - E G G G E Q A -	Q C E	TAAPPAKKLV - PVKKLLKAAP	PFLLE ECQ CO ECQ ECQ ECQ ECQ ECQ ECQ ECQ ECQ	FAI FAI LAI LAI LAI LAI LAI LAI LAI LAI LAI L	RFF RFF RFF RFF RFF RFF RFF RFF RFF RFF	AGSNNTTTL - NTTSLND	S KAPPEPPEDAKEEQDAKEEQDA	VE NAA NNAA EEAA LLAAA VIAA NMAACA III	R G N LK QQALLEEDDS - QCCT SEA	R G I		LND KKEEEEEEDE - EEEEEDEVD	MA	STD	DG	r L (<u>0</u> S <u>F</u>		MRT	Q				
S p s b p 1 S c x B 1 M m R a n B P 1 H s R a n B P 2 H m R a n B P 2 D 1 H m R a n B P 2 D 2 H m R a n B P 2 D 2 H m R a n B P 2 D 2 H s R a n B P 2 D 3 X m R a n B P 1 B t s p v a r D 2 B t p P c t i B t s p v a r D 1 C e F 5 9 A 2 D 1 C e P 5 9 A 2 D 2 S p h b a 1 C c x B 2 C c x B 2	778009999989908668998 777777787177776	MATTSSTTTTTTTSTLE - V	PPPPPPTTKPPLTTGDSS - G		TKKKTTKKKQQKKKQQQS - Q	NNVAAANNN NNAAANNN NNAAANNN NNAAANNN NNAAANNN NNAAANNN NN	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	DR DR DR DR DR DR DR DR DR DR DR DR DR D	I SWWASS AWWASS AWWAN ALL VWWALL SVWYL 		EA TV: TAACO	S N A A D A A D A A D A A D A A D A D A D A	VS FA- FA- S- S- S- S- S- S- S- S- S- S- S- S- S-		Q C E	T A A P P P K P P A A K K V P A A K K V P P A A K K V P P V A A K K V P A A K K K P A A K K V P A A K K V P A A K K V P A A K K V P A A K K V P A A K K V P A A K K V P A A K K V P A A K K V P A A K K V P A A K K K P A A A K K K P A A A K K K P A A A K K K P A A A K K K P A A A K K K P A A A K K K P A A A A	TTTLLCQQQQQH EEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE	FAII FAII LAI LAI LAI LAI LAI LAI LAI LAI LAI	RFF RFF RFF RFF RFF RFF RFF RFF RFF RFF	AGLNTTTL - NTTSLND	SW SEE ABE PPE PPE ABE SKEE PPE ABE SKEE PPE ABE SKEE SKEE PPE SKEE SKEE SKEE SKEE SKEE	VE NAA NNAA EEAA LLAA NMAA LLAA NMAA NVAA TTA	R G N L L N D Q K L L N D Q K L L E E E D D - K C C T S E E E C S - K C C T S E E E C S - K C C T S E E E C S - K C C T S E E E E C S - K C C T S E E E E E C S - K C C T S E E E E E E E E E E E E E E E E E E E	R R R R R K K K K K K K K K K K K K K K			MA	STD	DG	r L (0 S E		MRT	Q				
S p s b p 1 S c r R B 1 M m R a n B P 1 H s R a n B P 2 D 1 M m R a n B P 2 D 1 H m R a n B P 2 D 2 H m R a n B P 2 D 2 H m R a n B P 2 D 3 M m R a n B P 2 D 3 M m R a n B P 1 B t s p v a r D 2 A t R a n B P 1 B t s p v a r D 2 S c P 5 9 A 2 D 2 S c r N P 2 S c r N P 2	778009999989908688998 9999989908688998 99	MATTSSTTTTTTTSTLE-VV	PPPPPTTKPPKKSPGK - KD		TKKKTTKKKQQKKKQQQS QE	NNVAAASSNKKANNSKKP	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	DR DR DR DR DR DR DR DR DR DR DR DR DR D	I SWWAANFSSFWWWAALCOWYUAALCOWYUAALCOWYU		A VIII	S X A D D Y A D D Y A D D Y A D D Y A D D Y Y A D D Y Y A D D Y Y A D Y Y Y A D Y Y Y A D Y Y Y A D Y Y Y Y	VS IA FA FA S- S- S- S- S- S- S- S- S- S- S- S- S-		Q C =		VF FFTLLEQQ GQQQEEQQ EEQU EEQU EEQU EEU VKI	FAILAI FAILAI LAI LAI LAI LAI LAI LAI LAI LAI LA	SQ RFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFFF	- AGSNNTTTTL -NTTSLND	SW SKEE APPE PPE AKEE QD AKEE QD AKEE QD A KEE QD	V E NNAAAA EEAAAA NMMNVIAA T	R G N LK QQKLL E E E C D - QD C K S G G	R			MA	STD	DG	r L (0 S E		MRT	Q				
S p s b p 1 S c x R B 1 Mm R a n B P 1 H s R a n B P 2 D 1 H m R a n B P 2 D 1 H m R a n B P 2 D 2 H m R a n B P 2 D 2 H s R a n B P 2 D 2 H s R a n B P 2 D 3 X m R a n B P 1 B t s p v a r D 2 B t p p c t i B t s p v a r D 1 B t s p v a r D 1 C e F 5 9 A 2 D 2 S p h b a 1 S c x R B 2 S c x R B 2 S c x R B 2 S c x R D 2 S	778009999989908689998 992	MAHNSSHHHHHHHTSTLE-VVW	PPPPPTTKPPPKKSPGK - KDA		TKKKTTKKKQQKKKQQQS QED	NVVAAASSNKKANNSKKP	GGGGGGGGGGGGA GGGGGGGGGA GGGGGGGGA GGGGGG	DR DR DR DR DR DR DR NN E E R NN NN E E R NN NN E E R NN NN	I SWWASFFAWWASSFWWAALSVWYL		EA V (1) TAATI 11 TAATI 11 TAATI 11 TAATI 12 TAA	S N A D D A D D A D D A D D A D D A D D A D D A D D A D D A D D A D A D D A D	VIA FAA- S- S- AD AA- SE SE	Q M GGEEGGGGEIEGGGGEQAIII	Q C E	TEKKPPLLLVV-PVVDIHP		FAI FAI LAI LAI LAI LAI LAI LAI LAI LAI LAI L	RFF RFF RFF RFF RFF RFF RFF RFF RFF RFF	- AGSNNTTTTL - NTTSLND	SW SKEE PPE PPE PPE AKEE DD AKEE DD DA	V NNNAAAA LLVV - NAAACAA - AAACAAA 	R GI N LK QQ LL LE E C D - K C C T S G G				MA	STD	DG	r L (0 S E		MRT	Q				

Figure 4.—sbp1p is a structural homolog of the evolutionarily conserved RanBP family of proteins. (A) Gene structure of *S. pombe sbp1*. DNA sequence of *sbp1* and conceptual translation of its protein product. Nucleotides are numbered from the A of the initiating methionine codon as +1. Nucleotides corresponding to the two introns are in small italics. Amino acids are numbered in italics. The domain with highest similarity to other RanBPs is underlined. (B) Alignment of sbp1p to other proteins containing RanBDs. Multiple alignments were performed with proteins containing significant similarity to mouse Ran BP1. Dark shading indicates 60% or greater amino acid identity, and light shading indicates 60% or greater amino acid similarity. GenBank accession numbers are as follows: Spsbp1, (*S. pombe sbp1*) D86381; MmRanBP1, (*Mus musculus* RanBP1) X56045; HsRanBP1, (*Homo sapiens* RanBP1) X83617; ScYRB1, (*S. cerevisiae YRB1*) Z33503; HsRanBP2, (*H. sapiens* RanBP2) D42063; XlRanBP1, (*Xenopus laevis* RanBP1) Y09128; MmRanBP2, (*M. musculus* RanBP2) X87337; Btspvar2, (*Bos taurus* spliced variant with Ran-BD and cyclophilin domain, domain 1) L41691; AtRanBP1, (*A. thaliana* RanBP1) U62742; CeF59A2-1, (*Caenorhabditis elegans* clone F59A2, domain 2) Z34801; CeF59A2-2, (*C. elegans* clone F59A2, domain 2) Z34801; Sphba1, (*S. pombe hba1*) U38783; ScYRB2, (*S. cerevisiae YRB2*) Z38060; ScNUP2, (*S. cerevisiae* NUP2) X69964; HsHHCP045, (*H. sapiens*-expressed sequence tag HHCP045) M79174.

651





Figure 5.—sbp1p is a functional homolog of the evolutionarily conserved RanBP family of proteins. (A) sbp1p binds specifically to Ran-GTP. *E. coli*-produced GST-sbp1p (lane 1) or GST (lane 2) was separated electrophoretically and either transferred to filters (a and b) or stained with Coomassie brilliant blue (c). The filters were incubated with either 35 S-labeled Ran-GTP γ S (a) or 35 S-labeled Ran-GDP β S (b). (B) sbp1p binds to spi1p. A crude extract prepared from wild-type fission yeast (lane 1), or an equal amount of extract incubated with beads bound with either *E. coli*-produced GST-sbp1p fusion protein (lane 2) or GST (lane 3) was electrophoresed, transferred to filters, and immunoblotted with anti-spi1p antiserum. (C) sbp1p coactivates the rna1p-stimulated GTPase activity of Ran. Ran-GPT was incubated with rna1p and either GST (open circles) or GST-sbp1p (closed circles) in the indicated amounts, and the GTPase activity was monitored by determining the percent of remaining GTP bound to Ran.

brane, and the presence of spi1p was determined by immunoblotting using the previously described antispi1p antiserum. GST-sbp1p, but not GST alone, bound spi1p (Figure 5B).

sbp1p was also tested for its ability to stimulate the low endogenous GTPase activity of spi1p. Using a previously described *in vitro* assay (Bischoff *et al.* 1995), varying amounts of GST-sbp1p or GST were incubated with $[\gamma^{32}P]$ GTP and the GAP rna1p. GST-sbp1p, but not GST alone, was able to costimulate the GAP-catalyzed GTPase activity of spi1p by three- to fivefold, depending on the protein concentration (Figure 5C).

sbp1 is essential for viability in fission yeast: A diploid strain in which one copy of the *sbp1* (*med1*) open reading frame was replaced with the ura4 gene was constructed (see materials and methods), and tetrad analysis was carried out to determine if sbp1 was essential for viability. No tetrad contained more than two viable spores, and the viable spores were all ura⁻, indicating that these cells contained the wild-type copy of *sbp1*. To confirm that *sbp1* and no other essential gene was disrupted in this strain, the sbp1- Δ 1 diploid was transformed with the *LEU2*-based plasmid pREP41X-*sbp1*, in which the *sbp1* cDNA is under control of the medium strength *nmt1* promoter because expression from the full-strength promoter in pREP3X is toxic. Transformants that were leu⁺ were obtained, and the diploid was sporulated. All of the ura⁺ spores that were capable of germination and colony formation were also found to be leu⁺, indicating that spores in which the endogenous *sbp1* gene had been disrupted could grow only in the presence of a plasmid-borne copy of *sbp1*. The rescue was shown to be dependent on the transcription of *sbp1* because ura⁺ leu⁺ colonies were present when the nmt1 promoter was derepressed, which led to sbp1 transcription, but not when transcription was repressed by the presence of thiamine.

The phenotype of sbp1- Δ 1 cells was examined by growing these cells in liquid minimal media in the absence of thiamine to derepress transcription of the plasmid-borne copy of *sbp1* and then inhibiting promoter activity by the addition of thiamine to the media. Cells were examined periodically after promoter repression to monitor the development of the terminal phenotype. The percentage of terminally arrested cells, determined by the presence of a medial septum, increased steadily between 5 and 29 hr (Figure 6A). After 24 hr, the DNA and nuclear envelope morphology was examined by staining the living cells with Hoechst 33342 and DiOC_6 (Figure 6B). The cells look strikingly similar to cells harboring a temperature-sensitive mutation in *pim1* (Demeter *et al.* 1995; Sazer and Nurse 1994), harboring a null allele of *rna1* (Matynia *et al.* 1996), and to cells overexpressing either *rna1* (Matynia et al. 1996) or sbp1 (Figure 1B): they are binucleated, have condensed chromosomes, a fragmented nuclear envelope, and a medial septum.

DISCUSSION

Based on the complex phenotype of fission yeast cells in which the balance between the GTP- and GDPbound forms of the spi1p protein is expected to be perturbed by decreased GEF activity (Sazer and Nurse 1994) or increased GAP activity (Matynia *et al.* 1996), we have designed and carried out a screen for cDNAs that cause similar phenotypic consequences when overexpressed. Using a fission yeast cDNA library, the transcription of which is controlled by the thiamine regulatable *nmt1* gene promoter (Maundrell 1990), we have identified three categories of cDNAs.

One category, represented by a single cDNA, causes cells to arrest with multiple septa when overexpressed (M.K. Subramanian, D. McCollum, K.C.Y. Wong, X.



Figure 6.—*sbp1* is an essential gene whose deletion results in the accumulation of cells with a medial septum, condensed chromosomes, and an abnormal nuclear envelope. (A) The inviability of $sbp1-\Delta 1$ haploid cells is rescued by expression from pREP41X-*sbp1* but addition of thiamine (at t = 0) to repress transcription results in a progressive increase of the percentage of septated cells. (B) After 24 hr, live cells were double stained with Hoechst 33342 to visualize the DNA and DiOC₆ to visualize the nuclear envelope.

He, S. Sazer, L. Chang and K. Gould, unpublished results). Cells overexpressing the second class of cDNAs, which includes *mad2* and other components of the spindle checkpoint system, have some but not all characteristics of cells in which the spi1p system is perturbed (He et al. 1997). The third category of cDNAs includes seven clones that identify three different genes, med1, med2, and med3, the overproduction of which causes wild-type cells to exhibit all the phenotypes that were established as criteria for this screen, meaning that they phenocopy both a loss of pim1p GEF activity (Demeter et al. 1995; Sazer and Nurse 1994) and an increase in rna1p GAP activity (Matynia et al. 1996). Overexpression of med1, med2, or med3 results in the accumulation of binucleated cells with hypercondensed chromosomes, abnormal nuclear envelopes, and a medial septum. The terminal overproduction phenotypes of *med1* and *med2* are indistinguishable. Although overexpression phenotypes must be interpreted with caution, the biological relevance of the *sbp1* overexpression phenotype is supported by the observation that it is identical to the deletion phenotype. In addition to the pim1^{ts}-like phenotypes it shares with med1 and med2, med3 also accumulates DiOC₆staining material, presumably lipid or membranes, in the cytoplasm. Further characterization will be required to determine the nature of this cytoplasmic material. The genetic interaction of *med1*, *med2*, and *med3* with the spi1p GTPase system was established by demonstrating that overproduction of each of these three cDNAs is synthetically lethal with the *pim1-d1*^{ts} mutation, although further biochemical analysis is required to confirm a direct relationship between *pim1* and *med 2* or *med3*.

med1, med2, and *med3* are previously uncharacterized fission yeast genes that encode proteins with structural homologs in both mammals and budding yeast. Fission yeast med2p and its homologs have no informative domains, and their function is unknown. Its overexpression phenotype and genetic interaction with *pim1*, however, suggest that *med2* is likely to encode a regulator or target of the GTPase system. *med3* encodes a protein that shares sequence similarity with enzymes known to be involved in lipid metabolism, and its relationship to the spi1p GTPase system is currently under study. It is tempting to speculate that since the pim1-d1^{ts} mutant undergoes nuclear envelope fragmentation, the perturbation in intracellular lipid metabolism caused by *med3* overexpression exacerbates this membrane defect.

In this manuscript, we have focused on the characterization of one of these cDNAs, *med1*, which has the most toxic effect when expressed in pim1-d1^{ts} mutant cells. Because of its sequence similarity with a previously characterized family of proteins named RanBP (Figure 4B), *med1* was renamed *sbp1*. sbp1p is 51.4% identical to the human RanBP and 55.4% identical to the budding yeast homolog Yrb1p. These three proteins share significant sequence similarity in a region of the protein previously termed the RanBD (Beddow *et al.* 1995) or RanBP1 motif (Hartmann and Gorlich 1995), with a large group of proteins from several eukaryotic organisms, some but not all of which have been shown to bind specifically to the GTPase in its GTP-bound state (Figure 4B).

The functional similarity between the fission yeast, budding yeast, and mammalian BP1 proteins was established by demonstrating that, like RanBP1 (Bischoff *et al.* 1995) and Yrb1p (Schlenstedt *et al.* 1995), sbp1p specifically binds to the GTPase in its GTP-bound form and acts as a costimulator of the spi1p-GTPase activity in the presence of the spi1p-GAP protein rna1p. We propose that overproduction of sbp1p, the costimulator of the spi1p-GTPase, causes a perturbation in the ratio of Ran-GTP to Ran-GDP similar to that caused by inactivation of the GEF or overproduction of the GAP. In all three of these conditions, cells die with identical terminal phenotypes.

While this work was in progress, an Arabidopsis thaliana cDNA encoding a structural homolog of sbp1p was cloned on the basis of the abnormal phenotype it produced when overexpressed in wild-type fission yeast cells (Xia et al. 1996). It has also been found that the Saccharomyces cerevisiae and mammalian homologs of sbp1p, Yrb1p, and RanBP1, respectively, are also toxic when overproduced in cells carrying temperature-sensitive mutations in their respective Ran-GEFs (Hayashi et al. 1995), and that YRB1 overexpression increases the frequency of chromosome instability in wild-type cells (Ouspenski et al. 1995). These results are consistent with our finding that overexpression of sbp1p causes lethality when overexpressed in fission yeast. This overexpression, however, causes a specific cell cycle arrest after mitosis that is phenotypically identical to that seen when the balance between the GTP- and GDP-bound pools of spi1p is perturbed by other means (Demeter et al. 1995; Matynia et al. 1996; Sazer and Nurse 1994).

There are multiple RanBPs and RanBD-containing proteins in several organisms. For example, mammalian cells have Ran-GTP-binding proteins, RanBP1 and RanBP2, with similar biochemical properties (Beddow et al. 1995; Bischoff et al. 1995) but different intracellular localizations: RanBP1 is primarily cytoplasmic (Lounsbury et al. 1994; Schlenstedt et al. 1995), whereas RanBP2 is localized to the cytoplasmic filaments of the nuclear pore complex (Wu et al. 1995; Yokoyama et al. 1995). This suggests the possibility that each may independently mediate a different function of the GTPase system. We have shown that overproduction or depletion of sbp1p in fission yeast phenocopies perturbation of the GTPase system through either the pim1p GEF or the rna1p GAP (Demeter et al. 1995; Matynia et al. 1996; Sazer and Nurse 1994). Our characterization of sbp1p function in vivo indicates that it affects all of the essential functions of the spi1p GTPase system. This suggests that sbp1p regulates or influences the nucleotide-bound state of the spi1 GTPase and/or that it mediates the essential functions of the GTPase system.

Other RanBD-containing fission yeast proteins may mediate a subset of spi1p GTPase functions. The gene that encodes hba1p, the only other known RanBD-containing fission yeast protein, was cloned by virtue of its ability to confer resistance to Brefeldin A when overexpressed in wild-type cells (Turi *et al.* 1996). hba1p is the first RanBD-containing protein shown to be nuclear localized, setting it apart from RanBP1, RanBP2, and Yrb1p, all of which are cytoplasmic. hba1p is 25% identical and 64% similar to S. cerevisiae Yrb2, which was recently identified as a RanBD-containing protein based on its DNA sequence (Taura et al. 1997) and by virtue of its interaction with Ran in a two-hybrid screen (Noguchi et al. 1997). The phenotypes of null mutants of *hba1* and *yrb2* differ from those of other known components of the fission yeast or budding yeast GTPase systems, indicating that these proteins may influence some but not all of their downstream targets.

In our initial characterization, we were unable to demonstrate a significant physical interaction between hba1p and spi1p using either two-hybrid or spi1p-GTP overlay assays (Turi *et al.* 1996). Using more sensitive assays, however, Yrb2p has been shown to have a weak interaction with the spi1p homolog, Gsp1p (Noguchi *et al.* 1997; T. Taura and P. Silver, personal communication), suggesting that the same may be found for hba1p as well. dis3p, a previously cloned and characterized fission yeast protein implicated in mitotic progression, also binds to spi1p and Ran, but lacks the consensus RanBD. The specificity of binding by dis3p also differs from the previously characterized RanBD proteins in that it binds to the nucleotide-free form of the GTPase (Noguchi *et al.* 1996).

In summary, from a cDNA overproduction screen designed to identify regulators and/or downstream targets of the spi1 GTPase system, we have isolated three genes *med1, med2*, and *med3*, which cause a cell cycle arrest after mitosis identical to that resulting from an imbalance between spi1-GTP and spi1-GDP. *med1*, renamed *sbp1* because of its homology to the mammalian Ran-binding protein RanBP1, encodes a protein that binds specifically to the GTP-bound form of the GTPase and costimulates the rna1p catalyzed GTPase activity of spi1p. It remains to be determined if med2p and med3p are regulators or effectors of the spi1p GTPase system, or if they independently influence the mitosis-to-interphase transition.

We thank Ngoctuyen Ong for excellent technical assistance; Wendy Schrober in the Baylor Flow Cytometry Core Facility for FACS analysis; Nadja Pohl and Katarina Hentschel at the Resource Center/Primary Database of the German Human Genome Project, Max Planck Institute for Molecular Genetics, for mapping the *med1* gene; and Tetsuya Taura and Pam Silver (Harvard Medical School and Dana Farber Cancer Institute) for communication of results before publication. This work was funded by grants from the National Institutes of Health to S.S. (GM49119) and the Human Frontier Science Program to S.S., T.N., and H. Ponstingl (RG-0423/95M).

LITERATURE CITED

- Altschul, S. F., W. Gish, W. Miller, E. W. Myers and D. J. Lipman, 1990 Basic local alignment search tool. J. Mol. Biol. 2: 403–410.
- Beddow, A. L., S. A. Richards, N. R. Orem and I. G. Macara, 1995 The Ran/TC4 GTPase-binding domain: identification by expression cloning and characterization of a conserved sequence motif. Proc. Natl. Acad. Sci. USA 92: 3328–3332.
- Bischoff, F. R., and H. Ponstingl, 1991a Catalysis of guanine nucleotide exchange on Ran by the mitotic regulator RCC1. Nature 354: 80–82.
- Bischoff, F. R., and H. Ponstingl, 1991b Mitotic regulator protein RCC1 is complexed with a nuclear ras-related polypeptide. Proc. Natl. Acad. Sci. USA 88: 10830–10834.
- Bischoff, F. R., C. Klebe, J. Kretschmer, A. Wittinghofer and H. Ponstingl, 1994 Ran-GAP1 induces GTPase activity of nuclear Ras-related Ran. Proc. Natl. Acad. Sci. USA 91: 2587–2591.
- Bischoff, F. R., H. Krebber, E. Smirnova, W. Dong and H. Ponstingl, 1995 Co-activation of RanGTPase and inhibition of GTP dissociation by Ran-GTP binding protein RanBP1. EMBO J. 14: 705–715.
- Carey, K. L., S. A. Richards, K. M. Lounsbury and I. G. Macara, 1996 Evidence using a green fluorescent protein-glucocorticoid receptor chimera that the RAN/TC4 GTPase mediates an essential function independent of nuclear protein import. J. Cell Biol. 133: 985–996.
- Chi, N. C., E. J. H. Adam, G. D. Visser and S. A. Adam, 1996 RanBP1 stabilizes the interaction of Ran with p97 in nuclear protein import. J. Cell Biol. 135: 559–569.
- Coutavas, E., M. Ren, J. D. Oppenheim, P. D'Eustachio and M. G. Rush, 1993 Characterization of proteins that interact with the cell-cycle regulatory protein Ran/TC4. Nature 366: 585–587.
- Dasso, M., T. Seki, Y. Azuma, T. Ohba and T. Nishimoto, 1994 A mutant form of the Ran/TC4 protein disrupts nuclear function in *Xenopus laevis* egg extracts by inhibiting the RCC1 protein, a regulator of chromosome condensation. EMBO J. 13: 5732– 5744.
- Demeter, J., M. Morphew and S. Sazer, 1995 A mutation in the RCC1-related protein pim1 results in nuclear envelope fragmentation in fission yeast. Proc. Natl. Acad. Sci. USA 92: 1436–1440.
- Dingwall, C., S. Kandels-Lewis and B. Seraphin, 1995 A family of Ran binding proteins that includes nucleoporins. Proc. Natl. Acad. Sci. USA 92: 7525–7529.
- Forsburg, S. L., 1993 Comparison of Schizosaccharomyces pombe expression systems. Nucleic Acids Res. 21: 2955–2966.
- Hartmann, E., and D. Gorlich, 1995 A Ran-binding motif in nuclear pore proteins. Trends Cell Biol. 5: 192–193.
- Hayashi, N., N. Yokoyama, T. Seki, Y. Azuma, T. Ohba et al., 1995 RanBP1, a Ras-like G protein binding to Ran/TC4, inhibits RCC1 via Ran/TC4. Mol. Gen. Genet. 247: 661–669.
- He, X., T. E. Patterson and S. Sazer, 1997 The Schizosaccharomyces pombe spindle checkpoint protein mad2p blocks anaphase and genetically interacts with the anaphase-promoting complex. Proc. Natl. Acad. Sci. USA **94:** 7965–7970.
- Hoheisel, J. D., E. Maier, R. Mott, L. McCarthy, A. V. Grigoriev et al., 1993 High resolution cosmid and P1 maps spanning the 14 Mb genome of the fission yeast S. pombe. Cell 73: 109–120.
- Kadowaki, T., D. Goldfarb, L. M. Spitz, A. M. Tartakoff and M. Ohno, 1993 Regulation of RNA processing and transport by a nuclear guanine nucleotide release protein and members of the Ras superfamily. EMBO J. 12: 2929–2937.
- Kornbluth, S., M. Dasso and J. Newport, 1994 Evidence for a dual role for TC4 protein in regulating nuclear structure and cell cycle progression. J. Cell Biol. 125: 705–719.
- Lehrach, H., R. Drmanac, J. Hoheisel, Z. Larin, G. Lennon et al., 1990 Hybridization fingerprinting in genome mapping and sequencing, pp. 39–81 in *Genome Analysis, Volume 1: Genetic and Physical Mapping*, edited by K. E. Davies and S. M. Tilghman. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Leupold, U., 1970 Genetic methods for Schizosaccharomyces pombe. Methods Cell Physiol. 4: 169–177.

- X. He *et al.*
- Lounsbury, K. M., A. L. Beddow and I. G. Macara, 1994 A family of proteins that stabilize the Ran/TC4 GTPase in its GTP-bound conformation. J. Biol. Chem. 269: 11285–11290.
- Lounsbury, K. M., S. A. Richards, R. R. Perlungher and I. G. Macara, 1996 Ran binding domains promote the interaction of Ran with $p97/\beta$ -karyopherin, linking the docking and translocation steps of nuclear import. J. Biol. Chem. **271**: 2357–2360.
- Mahajan, R., C. Delphin, T. Guan, L. Gerace and F. Melchior, 1996 A small ubiquitin-related polypeptide involved in targeting Ran-GAP1 to nuclear pore complex protein RanBP2. Cell 88: 97–107.
- Matsumoto, T., and D. Beach, 1991 Premature initiation of mitosis in yeast lacking RCC1 or an interacting GTPase. Cell 66: 347– 360.
- Matunis, M. J., E. Coutavas and G. Blobel, 1996 A novel ubiquitin-like modification modulates the partitioning of the Ran-GTPase-activating protein Ran-GAP1 between cytosol and the nuclear pore complex. J. Cell Biol. **135**: 1457–1470.
- Matynia, A., K. Dimitrov, U. Mueller, X. He and S. Sazer, 1996 Perturbations in the spi1 GTPase cycle of *Schizosaccharomyces pombe* through its GAP and GEF components result in similar phenotypic consequences. Mol. Cell. Biol. **16**: 6352–6362.
- Maundrell, K., 1990 *nmt1* of fission yeast. J. Biol. Chem. 265: 10857-10864.
- Moreno, S., A. Kl ar and P. Nurse, 1991 Molecular genetic analysis of fission yeast *Schizosaccharomyces pombe*. Methods Enzymol. 194: 795–823.
- Noguchi, E., N. Hayashi, Y. Azuma, T. Seki, M. Nakamura *et al.*, 1996 Dis3, implicated in mitotic control, directly binds to Ran and enhances the GEF activity of RCC1. EMBO J. **15**: 5595–5605.
- Noguchi, E., N. Hayashi, N. Nakashima and T. Nishimoto, 1997 Yrb2p, a Nup2p-related yeast protein, has a functional overlap with Rna1p, a yeast Ran-GTPase-activating protein. Mol. Cell. Biol. 17: 2235–2246.
- Ouspenski, I. I., U. W. Mueller, A. Matynia, S. Sazer, S. J. Elledge et al., 1995 Ran-binding protein-1 is an essential component of the Ran/RCC1 molecular switch system in budding yeast. J. Biol. Chem. 270: 1975–1978.
- Sazer, S., 1996 The search for the primary function of the Ran-GTPase continues. Trends Cell Biol. 6: 81–85.
- Sazer, S., and P. Nurse, 1994 A fission yeast RCC1-related protein is required for the mitosis to interphase transition. EMBO J. 13: 606–615.
- Sazer, S., and S. Sherwood, 1990 Mitochondrial growth and DNA synthesis occur in the absence of nuclear DNA replication in fis-

sion yeast. J. Cell Sci. 97: 509-516.

- Schmidt, S., M. Sohrmann, K. Hofmann, A. Woollard and V. Simanis, 1997 The spg1p GTPase is an essential, dosage-dependent inducer of septum formation in *Schizosaccharomyces pombe*. Genes Dev. 11: 1519–1534.
- Schlenstedt, G., D. H. Wong, D. M. Koepp and P. A. Silver, 1995 Mutants in a yeast Ran binding protein are defective in nuclear transport. EMBO J. 14: 5367–5378.
- Shirayama, M., Y. Matsui and A. Toh-e, 1994 The yeast TEM1 gene, which encodes a GTP-binding protein, is involved in termination of M phase. Mol. Cell. Biol. 14: 7476–7482.
- Tani, T., R. J. Derby, Y. Hiraoka and D. L. Spector, 1995 Nucleolar accumulation of poly(A)⁺ RNA in heat shocked yeast cells: implication of nucleolar involvement in mRNA transport. Mol. Biol. Cell 6: 1515–1534.
- Taura, R., G. Schlenstedt and P. A. Silver, 1997 Yrb2p is a nuclear protein that interacts with Prp20p, a yeast Rcc1 homologue. J. Biol. Chem. 272: 31877–31884.
- Thompson, J. D., D. G. Higgins and T. J. Gibson, 1994 CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific Gap penalties and weight matrix choice. Nucleic Acids Res. 22: 4673– 4680.
- Turi, T., U. Mueller, S. Sazer and J. Rose, 1996 Characterization of a nuclear protein conferring Brefeldin A resistance in *Schizosaccharomyces pombe*. J. Biol. Chem. 271: 9166–9171.
- Worley, K. C., B. A. Wiese and R. S. Smith, 1995 BEAUTY: an enhanced BLAST-based search tool that integrates multiple biological information resources into sequence similarity search results. Genome Res. 5: 173–184.
- Wu, J., M. J. Matunis, D. Kraemer, G. Blobel and E. Coutavas, 1995 Nup358, a cytoplasmically exposed nucleoporin with peptide repeats, Ran-GTP binding sites, zinc fingers, a cyclophilin A homologous domain, and a leucine-rich region. J. Biol. Chem. 270: 14209–14213.
- Xia, G., S. Ramachandran, Y. Hong, Y.-S. Chan, V. Simanis *et al.*, 1996 Identification of plant cytoskeletal, cell cycle-related and polarity-related proteins using *Schizosaccharomyces pombe*. Plant J. 10: 761–769.
- Yokoyama, N., N. Hayashi, T. Seki, N. Panté, T. Ohba *et al.*, 1995 A giant nucleopore protein that binds Ran/TC4. Nature 376: 184–188.

Communicating editor: F. Winston