Journal of Cell Science 112, 1979-1988 (1999)
Printed in Great Britain © The Company of Biologists Limited 1999
JCS0300

Sto1p, a fission yeast protein similar to tubulin folding Cofactor E, plays an essential role in mitotic microtubule assembly

Ekaterina L. Grishchuk[1] and J. Richard McIntosh[2,*]

[1]Institute of Gene Biology, Moscow, Russia 117334
[2]Dept of MCD Biology, University of Colorado, Boulder, CO 80309-0347, USA
*Author for correspondence (e-mail: richard.mcintosh@colorado.edu)

Accepted 3 April; published on WWW 26 May 1999

SUMMARY

The proper functioning of microtubules depends crucially on the availability of polymerizable α/β tubulin dimers. Their production occurs concomitant with the folding of the tubulin polypeptides and is accomplished in part by proteins known as Cofactors A through E. In the fission yeast, *Schizosaccharomyces pombe*, this tubulin folding pathway is essential. We have taken advantage of the excellent cytology available in *S. pombe* to examine the phenotypic consequences of a deletion of *sto1*$^+$, a gene that encodes a protein similar to Cofactor E, which is required for the folding of α-tubulin. The interphase microtubule cytoskeleton in *sto1*-Δ cells is severely disrupted, and as cells enter mitosis their spindles fail to form. After a transient arrest with condensed chromosomes, the cells exit mitosis and resume DNA synthesis, whereupon they septate abnormally and die. Overexpression of Spo1p is toxic to cells carrying a cold-sensitive allele of the α- but not the β-tubulin gene, consistent with the suggestion that this protein plays a role like that of Cofactor E. Unlike its presumptive partner Cofactor D (Alp1p), however, Sto1p does not localize to microtubules but is found throughout the cell. Overexpression of Sto1p has no toxic effects in wild-type cells, suggesting that it is unable to disrupt α/β tubulin dimers in vivo.

Key words: Chaperone, Fission yeast, Leucine-rich repeat, Microtubule, Tubulin folding.

INTRODUCTION

Microtubules are an essential component of eukaryotic cells because they perform several vital functions. During interphase they contribute to the global organization of cytoplasm and to both the generation and maintenance of cell polarity. Microtubules become indispensable during mitosis when they provide the structural framework for accurate chromosome segregation. Different proteins control the behavior of microtubules at different times during a cell's life cycle, allowing these polymers to form either the relatively stable cytoplasmic network of interphase or the dynamic fibers of the mitotic spindle. Exactly how such factors regulate microtubule assembly and function is not yet known, and the relevant mechanisms are among the most interesting problems of cell physiology.

Microtubules are formed via the polymerization of dimers that assemble from α- and β-tubulins. Recent research has revealed a surprisingly complex biochemical pathway that is responsible for the correct folding of these polypeptides into polymerization competent forms. The initial folding, presumably cotranslational, of the nascent protein chains is carried out in an ATP-dependent manner by a multisubunit cytoplasmic chaperonin (for references see Melki et al., 1996; Vainberg et al., 1998). This interaction is facilitated by another cytoplasmic multisubunit complex, prefoldin (Alvarez et al., 1998; Geissler et al., 1998; Vainberg et al., 1998). The activities of these two complexes appear to be sufficient for the folding of actin, and perhaps γ-tubulin, but folding of α- and β-tubulins requires additional factors. Upon release from the cytoplasmic chaperonin, β-tubulin folding intermediates bind to Cofactor A protein, which acts as a buffer for β-tubulin (Archer et al., 1995, 1998; Melki et al., 1996; Tian et al., 1996). Cofactor B is thought to play an analogous role for α-tubulin (Tian et al., 1997). In addition to these two cofactors three other proteins, Cofactors C, D and E, are required for the formation of assembly-competent tubulin in vitro (Tian et al., 1996, 1997). The in vitro folding pathway mediated by these proteins appears to be symmetric for α- and β-tubulins. After the initial binding of α-tubulin by Cofactor B and β-tubulin by Cofactor A, the folding intermediates are passed onto Cofactors E and D, respectively. The pathways converge via the formation of a complex containing α- and β-tubulins and Cofactors E and D. Then, Cofactor C and GTP-hydrolysis promote the release of a native α/β dimer.

The importance of cofactor proteins for mammalian microtubule assembly is reflected in the results of a thorough analysis of their budding yeast homologs. The genes for all but one, Cofactor C, are found in the *S. cerevisiae* genome. Genetic evidence suggests that their protein products function in the

same pathway, which is important for microtubule stability and accurate chromosome segregation (Archer et al., 1995; Geiser et al., 1997; Hoyt et al., 1990, 1997; Stearns et al., 1990; Tian et al., 1997). This pathway is not, however, essential for cell viability, which is a puzzling contradiction of the requirement seen for these factors for the correct folding of tubulin in vitro.

The situation appears to be different in fission yeast, *Schizosaccharomyces pombe*, where mutations in cofactor proteins B and D lead to abnormal cell shape and a loss of cell polarity; deletion of Cofactor D (Alp1p) is lethal (Hirata et al., 1998; Radcliffe et al., 1998). In the temperature-sensitive mutant *alp1-1315* microtubules appear destabilized; their normal appearance is also perturbed by overexpression of *alp1*$^+$. Moreover, Alp1p is found along the interphase and spindle microtubules, and it can bind to taxol-stabilized microtubules in vitro (Hirata et al., 1998).

We have isolated a fission yeast gene, *sto1*$^+$, which encodes a protein similar to the human tubulin folding Cofactor E. Here we report results of the study aimed to answer two main questions concerning the role(s) of this protein in vivo: what are the phenotypic consequences of *sto1*$^+$ deletion or overexpression, and does Sto1p colocalize with microtubules? Our results show that unlike Cofactor E protein in *S. cerevisiae* (Pac2p), Sto1p has an essential function in the formation of normal cytoplasmic microtubules and the assembly of mitotic spindles. Consistent with its proposed role in the α-tubulin folding pathway, overexpression of Sto1p is harmful for cells carrying a mutation in the essential α-tubulin gene, *nda2*$^+$. However, unlike its presumptive partner, Alp1p, Sto1p does not localize to microtubules, and its overexpression has no toxic effects in wild-type cells.

MATERIALS AND METHODS

S. pombe methods

The yeast strains used in this study are described in Table 1. All media and growth conditions were as described by Moreno et al. (1991), unless otherwise stated. Edinburgh minimal medium (EMM) and malt extract (ME) were purchased from Bio101, Inc. (Vista, CA); yeast extract and agar were purchased from Difco (Detroit, MI). Yeast extract medium with supplements (YES) was used unless otherwise stated. To repress expression from the *nmt1* promoters, thiamine was added to 5 μg/ml. Phloxine B (Sigma, St Louis) was used at 5 mg/l to assess cell health; sick or dead cells cannot pump out this dye and thus become stained. A 10 mg/ml stock solution of thiabendazole (TBZ; Sigma, St Louis, USA) was prepared in dimethyl sulfoxide (DMSO).

Molecular techniques

Standard molecular techniques were used as described by Sambrook et al. (1989). For PCR we followed protocols from Innis et al. (1990). Sequencing was carried out using dideoxy chain termination (U.S. Biochemicals). Sequence analysis and comparisons employed the Genetics Computer Group package (University of Wisconsin) and the Basic Local Alignment Search Tool (NCBI). Mapping of *sto1*$^+$ to Chromosome 1 was performed using an *S. pombe* cosmid filter kindly provided by Dr E. Maier (Hoheisel et al., 1993) and with the help of the Reference Library Database, Max-Planck-Institut, Germany.

Isolation of the *sto1*$^+$ gene

We cloned *sto1*$^+$ as a suppresser of *tsm one* (*tsm1-512)*, a conditional mutation previously isolated (Grishchuk et al., 1998). An *S. pombe* genomic library in the shuttle vector pUR19 was the gift of Dr A. M. Carr (Barbet et al., 1992). All *S. pombe* transformations were done using lithium acetate procedures, either as described by Moreno et al. (1991) or as modified by Elble (1992). *Tsm1-512* cells were transformed with either a library or isolated plasmids, allowed to grow at 25°C, which is permissive for this strain, under nutritional conditions selective for the presence of the vector, and then replica plated onto minimal selective medium and YES at 36°C, a restrictive temperature for *tsm1-512*. Colonies capable of growth were picked from the original 25°C plates and tested for repeated rescue. The rescuing clones were then streaked on YES plates at 25°C to allow loss of the plasmid. Isolates which grew at 36°C and cosegregated with the ura$^+$ marker (present in pUR19 vector) were selected for further study. Plasmid DNAs were recovered from these isolates using a standard procedure (Moreno et al., 1991) and retested for their ability to rescue the temperature sensitivity of *tsm1-512*.

Using this procedure we isolated 22 genomic plasmids, at least 18 of which were independent isolates. Restriction maps of their inserts and PCR with sequence-specific primers revealed that 20 of them were derived from the same genomic sequence, representing five overlapping fragments. The smallest of the inserts, p1G#12 (3.2 kb), was used for further analysis. The rescue by the two remaining plasmids was much weaker, and they were not studied further.

Sequencing of p1G#12 revealed two open reading frames (ORFs) from Chromosome 1 (GenBank accession number z69730); one of these, CDS10 (minimal complementing fragment 19711-22065 nucleotides), was sufficient for rescue of the temperature-sensitivity. Since *tsm1*$^+$ is located on Chromosome 2 (Grishchuk et al., 1998), the rescue of *tsm1-512* was conferred by an extragenic suppressor. This conclusion was confirmed by homologous integration of the p1G#12 plasmid and subsequent crosses between the resulting strain and *tsm1-512*.

Four presumptive introns were identified in *sto1*$^+$ on the basis of sequence analysis. To verify their presence the appropriate fragments were sought by PCR, using *sto1*$^+$-specific primers and a cDNA library as a template. PCR products were resolved on a low-melting agarose gel and sequenced. The resulting sequence is identical to Alp21p (P. A. Radcliffe and T. Toda, accession number Q10303).

Sto1$^+$ gene disruption

Deletion of the entire ORF for *sto1*$^+$ was achieved with two different constructs. The first was made by subcloning the downstream 1.2 kb *Cla*I-*Kpn*I fragment (nucleotides 21675-22870) and the 480 bp fragment of the upstream sequence of *sto1*$^+$ into pSPORT1 vector (Gibco-BRL, Grand Island, NY) containing the *ura4*$^+$ gene. The

Table 1. *S. pombe* strains used in this study

Strain	Genotype	Source
PN513	*leu1-32 ura4-D18 h*$^-$	P. Nurse
D2-6	*ade6-M210/ade6-M216 leu1-32/leu1-32 ura4-D18/ ura4-D18 h*$^+$*/h*$^-$	This study
D3642	*ade6-M210/ade6-M216 leu1-32/leu1-32 ura4-D18/ ura4*$^+$ *h*$^+$*/h*$^-$	This study
McI#207	*nda2-52 leu1-32 ura4-D18 h*$^-$	This study
McI#154	*nda3-311 leu1-32 ura4-D18 h*$^-$	This study
McI#177	*sto1D::ura4*$^+$*/sto1*$^+$ *ade6-M210/ ade6-M216 leu1-32/leu1-32 ura4-D18/ura4-D18 h*$^+$*/h*$^-$	This study

upstream 480 bp flanking region was obtained by PCR using p1G#12 as a template. Products of the PCR reaction were purified by electrophoresis and cut with *Xba*I in the PCR primer 5′ GGG TCT AGA ATC AAC GTG GGA AAG A 3′ and *Eco*RI (nucleotide 19457) before subcloning into pSPORT1. The *ura4*$^+$ gene, sandwiched between the DNA sequences that flanked *sto1*$^+$, was cut from the pSPORT1 vector with *Eco*RI and *Kpn*I restriction enzymes and used for transformations.

The second knockout construct was obtained by PCR using the *ura4*$^+$ gene as a template and the following primers: 5′ CTG TAT TTT AAA AGA AGC AAA ACT AGA GCA TCG ATT TTG GTC CTT CAA GTA ATA ACT TGG CTG TTC CAA CAC CAA TG 3′ and 5′ AAA GAA ACA AAA AAC TTA AAC TGC CCA AAT TTT TAT AGC AAT CCA TTT TCT TTC CCA CGT TGA TTA ATC TAC TCA GCA TT 3′. These primers contain 68 bp of the sequence upstream (‘oligopro’) and 65 bp of the sequence downstream (‘oligoterm’) of the *sto1*$^+$ open reading frame, respectively. The 3′ ends of these primers include 17 bp sequences specific to the DNA flanking the *ura4*$^+$ gene.

Products of the PCR reaction were purified by gel electrophoresis and transformed into a freshly prepared diploid strain (strain #D2-6, Table 1). Stable ura$^+$ colonies were picked and tested for insertions by colony PCR. Briefly, a small sample of cells from each ura$^+$ colony was boiled for 10 minutes in 20 μl of water, clarified by centrifugation, and 5 μl of the supernatants were used in PCR. Typical conditions for the PCR reactions were: 200 μM dNTP, 1.5 mM $MgCl_2$, 1 μM primers each; the first cycle included 2.3 minutes at 94°C and subsequent 40 cycles were done at 94°C (40 seconds), 44°C (1.5 minutes) and 72°C (1.5 minutes). DNA products of the PCR reactions were resolved on an agarose gel, and the strains that produced the desired PCR bands (two strains for each of the knockout constructs) were used for tetrad analysis. Homologous integration for one representative integrant from each of the constructs was confirmed by Southern blotting.

Tagging and overexpression of *sto1*$^+$ and human Cofactor E (CFE)

The genomic copy of *sto1*$^+$ was amplified by PCR with *Pfu* polymerase (Stratagene, La Jolla. California) using p1G#12 as a template. The 5′ primer GGA ATT CCA TAT GCA TAT ATC CAC TGG AA was immediately upstream of the start codon; the 3′ primer was either TCC CCC CGG GCC ATG GAA TTA GTA TAT TTA A, which includes 170 bp downstream of the stop codon, or CGC GGA TCC CGA CGT TTC CAC TTT CCT T, which introduces a *Bam*HI restriction site before the stop codon. The first PCR product was subcloned into pREP42pk1, kindly provided by Dr H. Browning (University of Colorado) and into pREP1, pREP41 and pREP81 (Maundrell, 1993). The product from the second pair of primers was used to subclone the *sto1*$^+$ gene in frame with the S65T allele of the green fluorescent protein (GFP) in the pREP3GFP vector, kindly provided by Dr R. R. West (University of Colorado). All these constructs rescued the *sto1Δ* strain. Rescue was determined by transforming these and control plasmids with the same nutritional marker (*LEU2*) into the McI#177 strain, which was then sporulated in ME. Spores were germinated at 32°C (or as indicated) on EMM supplemented with adenine and thiamine (or without thiamine) to repress the promoter. The ura$^+$, leu$^+$ colonies were obtained for all constructs carrying the *sto1*$^+$ gene, but not for the control plasmids.

cDNA for human Cofactor E (GenBank accession number U61232) was kindly provided by Dr N. Cowan (New York University Medical Center). The cDNA was subcloned into pREP1 and tested for rescue of *sto1Δ* lethality, as outlined above. The N-terminal fusion with GFP was obtained using a pREP42-GFP vector, kindly provided by Dr S. Sazer (Baylor College of Medicine). The continuity of fusion ORFs for this and Sto1-GFP constructs was confirmed by sequencing.

Cell cultures

The phenotype of *sto1Δ* cells was analyzed by comparing a control diploid strain (D3642, Table 1) with one in which one of the two *sto1*$^+$ genes had been replaced by the *ura4*$^+$ gene (McI#177, Table 1). Diploid cells were grown to log phase at 25°C in EMM supplemented with leucine, harvested and sporulated in malt extract medium supplemented with leucine. Spores were isolated by repeated treatments with glusulase (NEN, Boston) until no vegetative cells could be found. Spores were germinated at 32°C by inoculation into EMM supplemented with leucine and adenine. At different times samples were taken to count cells and spores with a hemocytometer and to fix in ethanol for analysis by flow cytometry (FACS) and microscopy (see below). At 14, 17 and 24 hours after inoculation cells were also fixed for immunostaining of microtubules (see below).

The cells that grew from spores in medium lacking uracil could either be haploid *sto1Δ::ura4*$^+$ or occasionally arising diploid ura$^+$ cells. Examination by microscopy and analysis of the DNA content by FACS revealed that upon prolonged incubation (40-48 hours) the *sto1Δ* culture consisted almost entirely of healthy, growing diploid cells. The fraction of the ura$^+$ diploid cells at earlier times was estimated by plating different volumes of a diluted cell suspension on EMM agar plates, supplemented with adenine and leucine at 32°C, and by counting the numbers of colonies several days later. Efficiency of plating was estimated by comparing colony-forming units at 0 hours on EMM agar plates supplemented with adenine, leucine and uracil at 32°C with the number of spores, determined by counting with a hemocytometer. At 20 and 24 hours after inoculation, less than 8% and 13% of the cells, respectively, were diploid. Hence, the majority of the cells in this culture grown for 24 hours in the absence of uracil were *sto1*$^-$, while the diploid cells represented only a small fraction of the total.

Cytological techniques

Cellular localizations of DNA and septum material were visualized by staining with DAPI (4′,6′,diamidino-2-phenylindole dihydrochloride, Sigma, St Louis) and calcofluor white M2R (Sigma, St Louis), respectively. Cells were fixed either in 3.7% formaldehyde (Electron Microscopy Sciences, Ft. Washington) as described by Moreno et al. (1991), or in ice-cold 70% ethanol in preparation for flow cytometry analysis. Immunofluorescent staining of microtubules was carried out essentially as described (Alfa et al., 1993), using a mouse monoclonal antibody against α-tubulin (kindly provided by Dr M. Fuller, Stanford University) and Texas Red-labeled goat anti-mouse antibodies (Jackson Labs, PA). Stained cells, or cells carrying plasmids with GFP-fusion proteins, were viewed with a Zeiss epifluorescence microscope and recorded in digital form, using a Empix CCD camera and the MetaMorph imaging program (Universal Imaging, West Chester, PA). For flow cytometry cells were processed as described by Alfa et al. (1993); the final concentration of RNase was 1 mg/ml. Samples were analyzed using a FACScan (Becton Dickinson; San Jose, CA) and the Lysis II software package.

RESULTS

Sto1p is similar to Pac2p of *S. cerevisiae* and to human cofactor E for tubulin assembly

Sto1$^+$ (suppressor of tsm one) was identified through its ability to suppress the temperature-sensitivity of the mitotic mutant *tsm1-512*, which has abnormal cytoplasmic microtubules and is defective in the formation of metaphase spindles (Grishchuk et al., 1998). When present on a high copy plasmid, a genomic copy of *sto1*$^+$ improves the appearance of microtubules in *tsm1-512* cells. The predicted protein sequence responsible for this rescue contains 511 amino acids and has a molecular mass of 58 kDa. Sequence analysis shows that Sto1p is similar to Pac2p of *S. cerevisiae* (24% identity and 48% similarity over

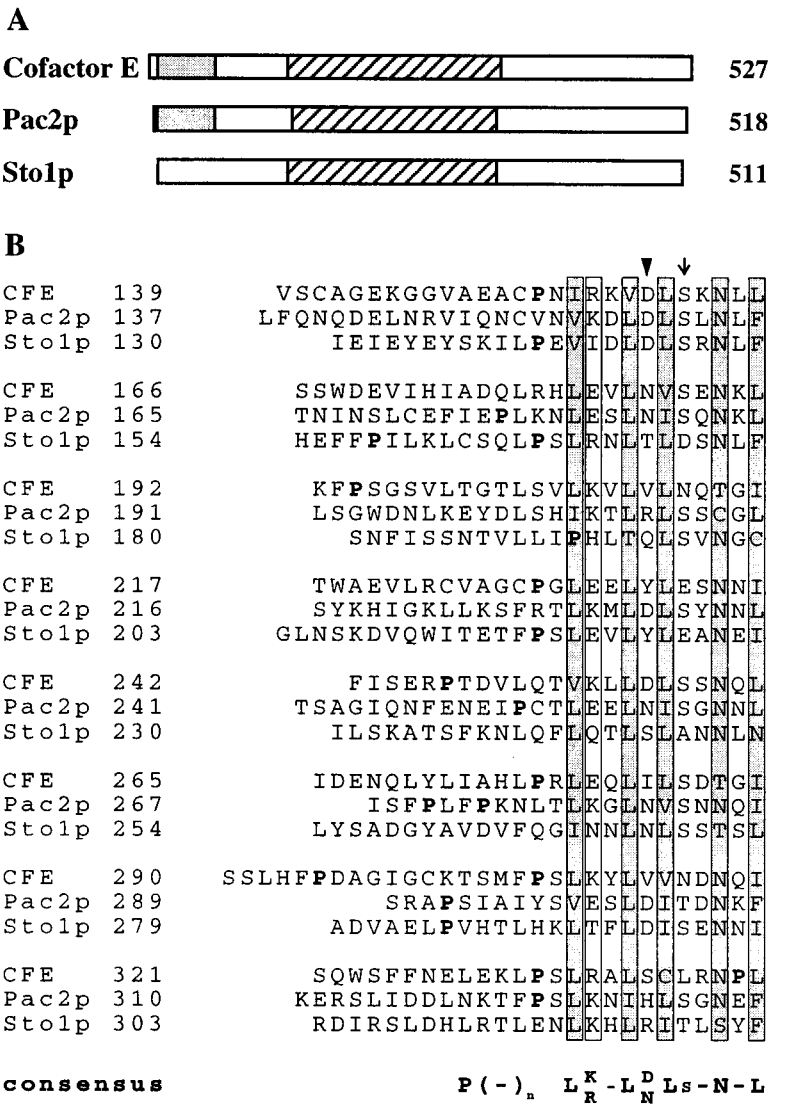

Fig. 1. Sto1p is similar to Pac2p (GenBank # U16814) and human Cofactor E (CFE, GenBank #U61232).
(A) Schematic representation of the domain composition in these proteins. Gray boxes indicate the presumptive microtubule-binding domains, while hatched boxes depict the leucine-rich repeats (LRR). Numbers correspond to amino acids. (B) Sequence alignment of segments containing LRRs. Positions rich in leucine and a conserved asparagine residue are marked with dark boxes. Light box indicates a position rich in charged amino acids. The position marked with an arrowhead is 50% occupied by asparagine or aspartic acid, while the position marked with an arrow is rich in serine. Prolines are shown in bold.

its entire length) and human cofactor E (25% identity and 46% similarity). Pac2p and Cofactor E are slightly more similar to each other (30% identity and 54% similarity) than either of them is to Sto1p. Alignments of these protein sequences with the GCG program 'Compare' revealed multiple regions of sequence similarity scattered throughout their lengths. When these regions of similarity were manually aligned, a derived consensus was found to match a loosely conserved leucine-rich repeat, LRR (Fig. 1). Such repeats are found in a number of proteins and are thought to be important for protein-protein interactions (Kobe and Deisenhofer, 1994; Ohkura and Yanagida, 1991).

As reported previously Pac2p and Cofactor E share similarity at their N-terminal ends with the microtubule-binding domain present in a number of different microtubule-associated proteins (Hoyt et al., 1997; Tian et al., 1996). Sto1p, however, appears to lack this domain.

Cofactor E (CFE) has been isolated as one of the proteins required for the folding of α- and β-tubulins in vitro (Tian et al., 1996, 1997), a function that is consistent with the phenotypes of *pac2* mutants in *S. cerevisiae* (Geiser et al., 1997; Hoyt et al., 1997; Vega et al., 1998). To analyze phenotypic consequences of depleting Sto1p in *S. pombe*, and to better understand its role in microtubule biogenesis, we examined the phenotype of a *sto1*$^+$ knockout strain.

The *sto1*$^+$ is essential for growth

The entire ORF of *sto1*$^+$ was deleted from a diploid ura$^-$ strain (D2-6, Table1), using a one step integration of the *ura4*$^+$ gene at that locus, as described in Materials and Methods. Homologous integrants were first identified by colony PCR and then confirmed by Southern blotting. A total of 85 tetrads from four independent disruptants were dissected on YES medium. Lethality and viability segregated 2:2, and upon replica plating to minimal medium with appropriate supplements, no viable ura$^+$ colonies were found, indicating that *sto1*$^+$ is an essential gene. Examination by microscopy revealed that the *ura4*$^+$ spores germinated and the resulting cells divided several times before dying. The average number of cells in the dead colonies varied from 6 to 10 and was not dependent on temperature (20-36°C).

To examine the phenotype of the *sto1Δ* cells more closely, the diploid strain in which one copy of *sto1*$^+$ had been replaced with *ura4*$^+$ (strain McI#177, Table 1) was sporulated and the

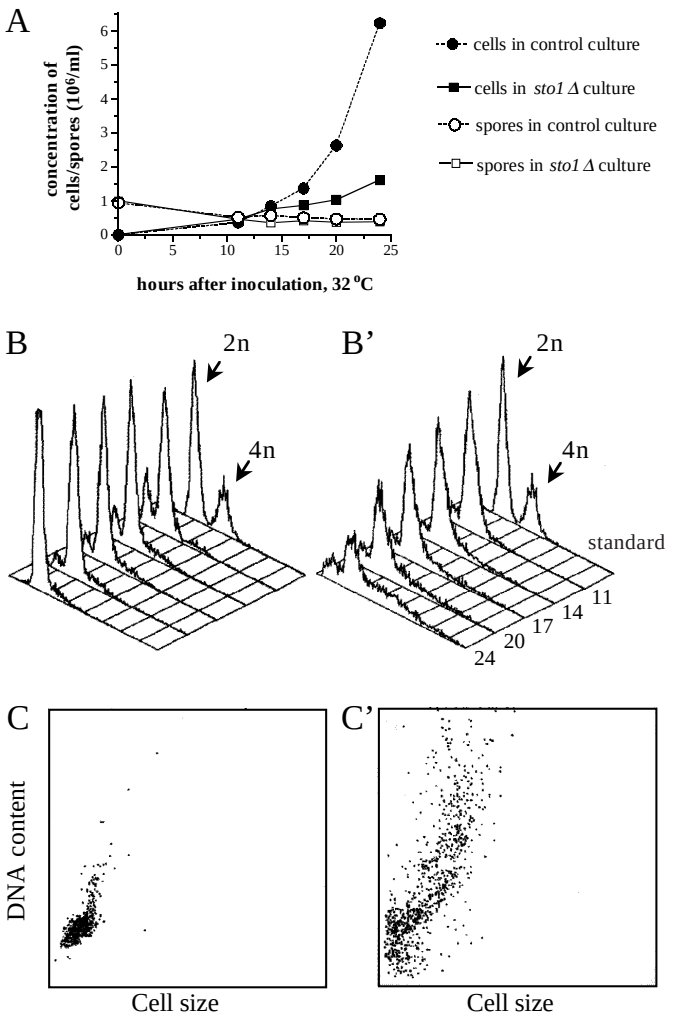

Fig. 2. The *sto1*$^+$ gene is essential for growth in fission yeast. S*to1Δ* and wild-type cultures were obtained by germinating spores from a heterozygous diploid strain in which one copy of *sto1*$^+$ was replaced with the *ura4*$^+$ gene, and from a wild-type *ura4*$^+$/*ura4-D18* diploid control strain. The spores were inoculated at 32°C in minimal medium without uracil. Results of a single experiment are shown, but similar results were obtained in duplicate experiments. (A) Number of cells and spores in the *sto1Δ* and control cultures as counted with a hemocytometer. (B and B′) DNA content of cells in control (left panel) and *sto1Δ* (right panel) cultures. Cells were fixed in ethanol at indicated times and processed for flow cytometry. Numbers show hours after inoculation. 5,000 cells were counted for each data set. The 'standard' was a starved wild-type diploid culture, which shows 2n and 4n peaks of DNA. (C and C′) Two dimensional plots of the DNA content vs cell size (measured by the amount of forward scattered light) 24 hours after inoculation of the wild-type (left panel) and *sto1Δ* spores (right panel).

resulting cells were compared to those obtained from sporulating a control *ura4*$^+$/*ura4-D18* diploid strain (D3642, Table 1). In minimal medium lacking uracil the *ura4*$^-$ spores do not germinate for at least 24 hours at 32°C. Germination of the *sto1Δ* spores, which are ura$^+$, occurred with kinetics similar to those of the control culture, but subsequent cell growth was significantly impaired (Fig. 2A). Analysis of the DNA content revealed the progressive accumulation of cells with abnormally large and heterogeneous amounts of DNA (Fig. 2B′). At the same time many of the cells continued to increase in size, which is indicative of a failure in mitotic process(es) (Fig. 2C′).

To better understand why *sto1*$^-$ cells failed to divide normally, we examined their morphology with DAPI to stain DNA and calcofluor to visualize newly synthesized septa. At 20 and 24 hours of incubation more than 54% and 60% of cells, respectively, looked distinctly abnormal (2,214 cells examined). Less than 3.2% of the 1,224 cells examined in comparable control cultures were abnormal at the same times. Four categories of mutant phenotypes could be distinguished: cells with highly condensed chromosomes and no septum (Fig. 3A, a, arrow), cells with a septum and condensed but unseparated chromosomes (Fig. 3A, b, arrow), cells with two chromatin masses on one side of the septum (Fig. 3A, a, arrowhead), and cells that were abnormally septated (Fig. 3A, b and c), which precluded visualization of their DAPI-staining material.

Fig. 3B describes the relative frequencies of these categories at 20 and 24 hours following the initiation of germination. Cells with condensed chromosomes were the most abundant at 20 hours. They were noticeably longer than exponentially growing cells in control cultures, which is most likely to have arisen from a mitotic arrest. Only a minor fraction of the abnormal cells (~6%) showed signs of nuclear division. Interestingly, abnormally septated cells predominated 4 hours later. Wild-type cultures showed <3% abnormal cells, all categories combined, at each time point examined. We concluded that after germination, the *sto1*$^-$ cells divided a few times, presumably because of Sto1p stored in the spores, and then arrested transiently with condensed chromosomes. The arrest was followed by septation, including massive over-septation, leading to cell death.

Sto1p is required for formation of normal cytoplasmic microtubules and for spindle assembly

To examine the organization of microtubules in the *sto1Δ* cells we used immunofluorescence with antibodies to tubulin (Fig. 4, a and a′). This technique allows the visualization of fission yeast microtubules during different mitotic stages and in interphase, when numerous cytoplasmic microtubules of various lengths can be seen (Hagan and Hyams, 1988). Significantly smaller fractions of cells showed microtubule staining in the *sto1Δ* cultures than in wild-type control cultures, in which ~50% of the cells stained with antitubulin (1,874 cells examined). At 14, 17 and 20 hours after inoculation 28%, 30% and 24% of the cells in *sto1Δ* cultures were stained (averages from 3 experiments in which 2,802 cells were examined). Each *sto1Δ* cell that did stain contained only 1-2 microtubules per cell (Fig. 4, b′,c′), while the wild-type cells that were stained showed up to 10 tubulin-containing fibers. Apparently the interphase microtubules in the mutant strain were abnormal.

Many of the *sto1Δ* cells contained condensed chromosomes but no mitotic spindles, suggesting that the spindle microtubules failed to polymerize. The cultures also contained some cells with numerous, well organized interphase microtubules (e.g. Fig. 4, d,d′), which served as an internal control for cell fixation and staining. The majority of these cells were bigger than wild-type haploid cells, and they contained large nuclei, suggesting that they arose from diploid sto1$^+$ spores present in the preparation (see Materials and Methods).

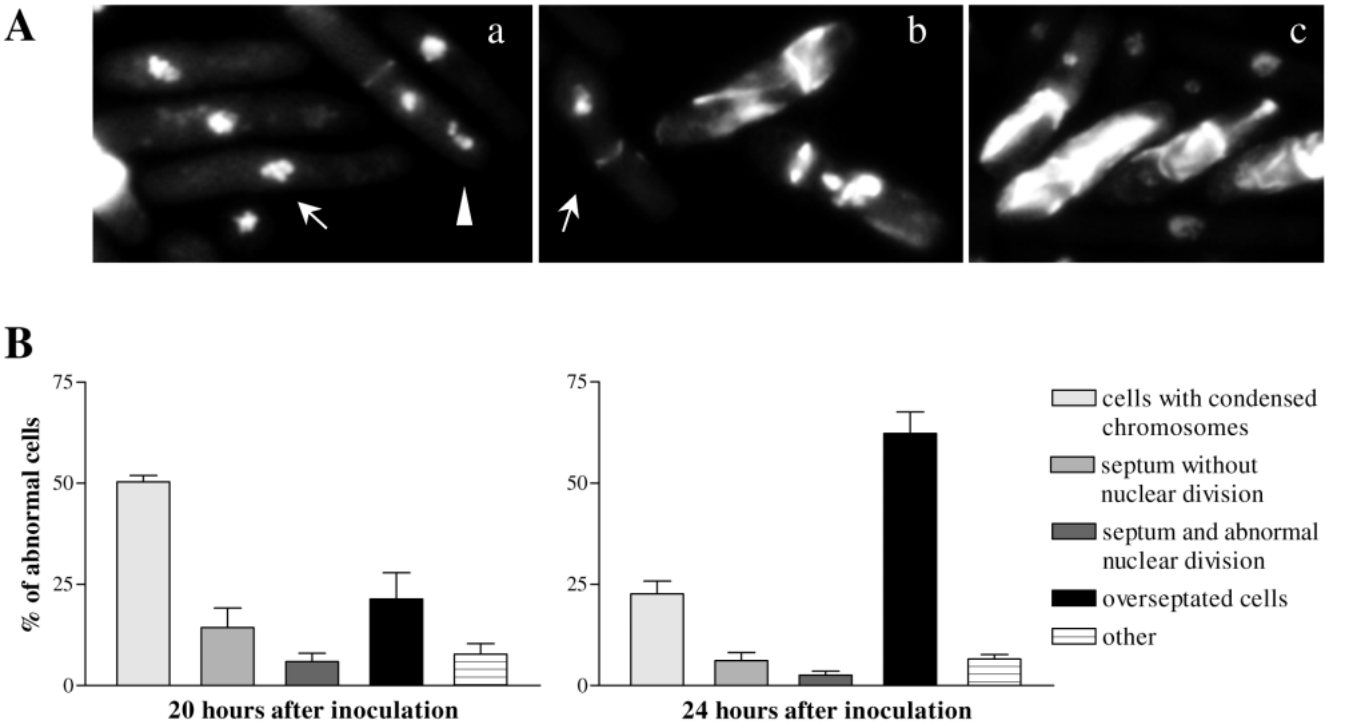

Fig. 3. *Sto1Δ* cells arrest in mitosis. (A) 20 hours (a) and 24 hours (b and c) after the initiation of germination, cells were fixed and stained with DAPI and calcofluor. The arrow in a points to three cells with condensed chromosomes. The arrowhead in a points to a cell that has undergone abnormal nuclear division, resulting in two nuclei on one side of the septum. The arrow in b points to a cell that has a septum without having gone through nuclear division. Brightly stained cells on b and c are examples of overseptated cells. (B) Distribution of phenotypes among cells with abnormal morphology obtained in three experiments. Cells that did not fall into first four categories (e.g. cells with smeared nuclear DNA) were grouped into the 'other' category. 54% of the 1,218 cells examined at 20 hours after inoculation (left graph), and 60 % of the 996 cells examined at 24 hours after inoculation (right graph) were abnormal, while the frequency of abnormality in cells germinating from wild-type spores was <3%.

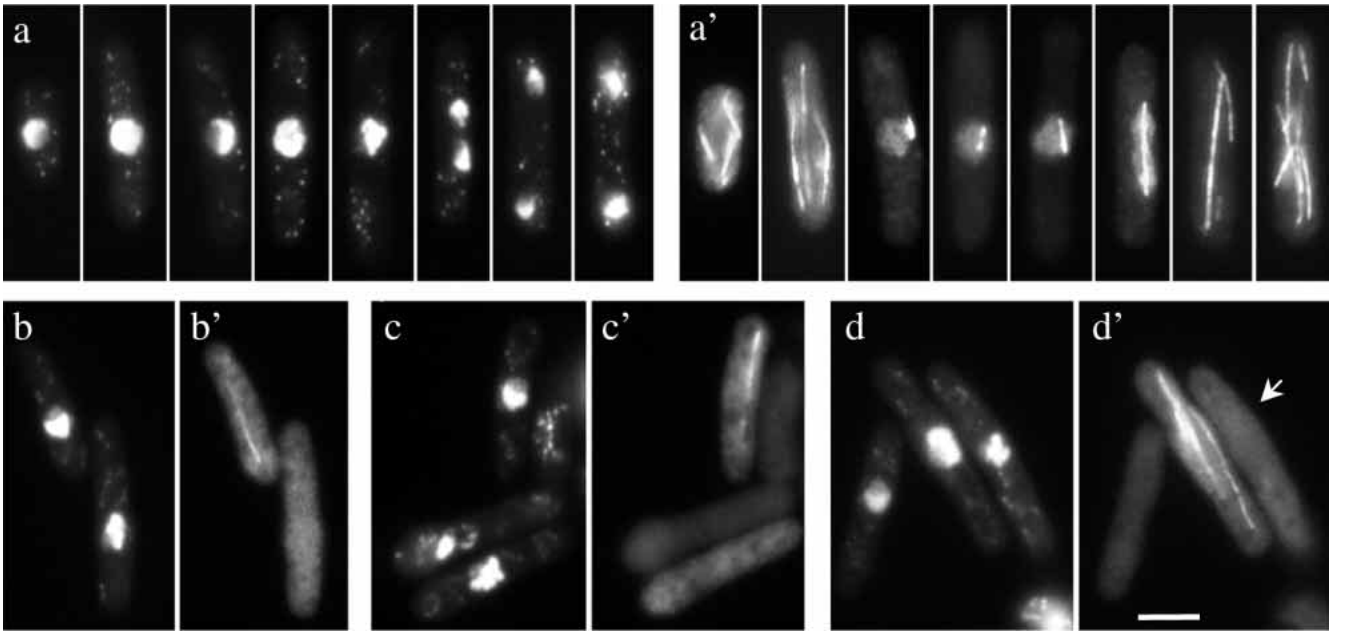

Fig. 4. Morphology of cytoplasmic and spindle microtubules in wild-type haploid cells and in cells from a *sto1Δ* culture. (a-d) DNA stained with DAPI, (a′-d′) tubulin staining. (a and a′) are wild-type cells at different stages of the cell cycle; all other images are of the cells in a *sto1Δ* culture. Most cells lack microtubules, though some interphase cells contain one or two (b-c′). Cells with condensed chromosomes (such as the cell marked with an arrow in d′) have no microtubule staining, but a few large cells with large nuclei (presumably sto1$^+$ diploid) appear normal. Bar, 5 μm.

We concluded that in the absence of Sto1p, normal cytoplasmic and spindle microtubules fail to form.

The sequence similarity between Sto1p and Cofactor E suggested that the latter gene might rescue the lethality of *sto1Δ* cells. We have tested this possibility using a human cDNA for Cofactor E (a generous gift from N. Cowan) expressed under the regulation of the *nmt1*$^+$ promoter in the pREP1 vector, which is marked with *LEU2*. This construct was transformed into the diploid strain in which one copy of *sto1*$^+$ was replaced with *ura4*$^+$ (strain McI#177, Table 1). The transformed cells were sporulated and the resulting spores were plated at 25°C-36°C on medium lacking uracil and leucine (data not shown). No colonies grew under conditions either partially repressive for the *nmt* promoter (in the presence of thiamine) or those that allow its full expression (without thiamine). This implies that human Cofactor E can not substitute for the essential function of the *sto1*$^+$ gene. We concluded that the differences between Sto1p and CFE are too great for one to serve as a functional replacement of the other.

Overproduction of Sto1p is harmful for cells carrying a mutation in the α-tubulin gene

Overproduction of one component of a multiprotein complex can often disrupt the assembly and/or functioning of the complex as a whole. Indeed, when Alp1p (Cofactor D) is overproduced in wild-type *S. pombe* cells, it leads to a loss of normal microtubules (Hirata et al., 1998). To examine whether elevated intracellular levels of Sto1p affected *S. pombe* microtubules, the *sto1*$^+$ gene was expressed under the regulation of wild-type and mutant copies of the *nmt1* promoter (Basi et al., 1993). S*to1*$^+$ expressed in the presence of thiamine from the weakest of these alleles, pREP81, was sufficient to fully rescue the growth of *sto1Δ* cells, as assessed by colony size and color on EMM plates containing phloxine, which stains the sick and dead cells. Gross overproduction of this gene under the control of the wild-type *nmt1*$^+$ promoter, derepressed by the absence of thiamine, did not significantly affect the growth of either *sto1Δ* or wild-type cells at 32°C. Moreover, the microtubules appeared normal in wild-type cells growing under conditions permitting maximal overexpression of the *sto1*$^+$ gene (data not shown).

Overexpression was also not toxic in *nda2-52* (McI#207, Table 1) and *nda3-311* cells (McI#154, Table1), which carry cold-sensitive alleles of α and β-tubulin, respectively, when grown at permissive conditions (32°C). At 25°C, however, which is semipermissive for both of these tubulin mutants, the growth of *nda2-52* cells was severely inhibited by overproduction of Sto1p, while *nda3-311* cells grew normally (Fig. 5). To examine whether overproduction of Sto1p perturbed microtubule dynamics in *nda2-52* cells, this strain was grown at 32°C in the presence of the microtubule destabilizing agent thiabendazole (TBZ). The overexpression of Sto1p from the wild-type *nmt* promoter increased the TBZ-sensitivity of *nda2-52* cells (Fig. 6), while TBZ-sensitivities of wild-type and *nda3-311* cells were not altered.

Sto1p does not localize to microtubules

Unlike Pac2p and human Cofactor E, the sequence of Sto1p lacks a presumptive microtubule binding domain. To ask whether Sto1p localizes to fission yeast microtubules we created a copy of *sto1*$^+$ tagged at its C terminus with the Green

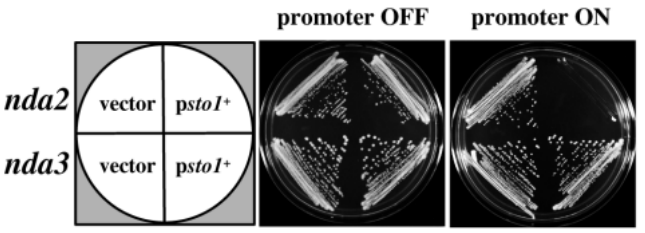

Fig. 5. Overproduction of Sto1p is toxic for *nda2-52* cells. Cells were transformed with vector (pREP1) or p*sto1*$^+$ (pREP1*sto1*$^{+)}$ and grown for 7 days at 25°C on EMM plates supplemented with uracil and thiamine (promoter off) or without thiamine (promoter on).

Fluorescent Protein (GFP; see Materials and Methods). This fusion protein is functional, since when expressed under the regulation of the *nmt1*$^+$ promoter in the pREP3X vector, it can rescue cells lacking the wild-type copy of *sto1*$^+$. When wild-type cells (strain McI#37, Table 1) transformed with this construct were grown on minimal medium with thiamine we observed faint fluorescence throughout the cell (Fig. 7). Upon removal of thiamine the general staining became brighter. Some of the Sto1-GFP fusion protein accumulated in brightly stained dots, which clumped in one or two unevenly shaped inclusions. The same was observed in wild-type cells, whose microtubules were disrupted by incubation with 100-400 μM TBZ and/or treatment at 0°C, as well as in *nda2-52* and *nda3-311* cells, when these strains were grown at permissive (32°C) or restrictive (20°C) temperatures. The microtubules were also not fluorescent in *sto1Δ* cells carrying the pREP3X-Sto1-GFP plasmid, or in *nuc2-663* cells, which arrest in metaphase at restrictive temperatures (data not shown). We concluded, therefore, that Sto1p does not localize to microtubules and is found throughout the cell.

This finding prompted us to examine whether human Cofactor E, a microtubule-associated protein in vitro (Tian et al., 1996), failed to substitute for Sto1p, because of its different cellular localization. This does not, however, appear to be the case. A GFP tagged copy of human CFE was expressed in wild-type *S. pombe* cells from the *nmt* promoter in the pREP42 vector. This fusion protein showed no localization to microtubules or any specific compartment of the fission yeast cell at any stage of the cell cycle (not shown). We therefore concluded that the presumptive microtubule-binding domain of human CFE was insufficient to target the GFP fusion of this protein to *S. pombe* microtubules.

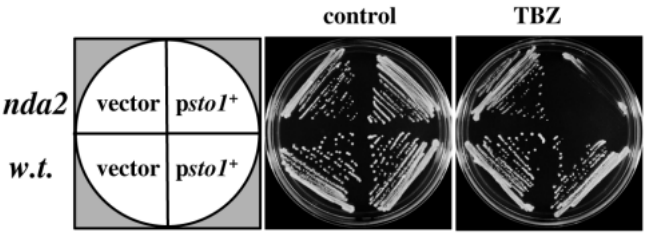

Fig. 6. Overproduction of Sto1p increases the sensitivity of *nda2-52* cells to TBZ. Wild-type (PN513, Table 1) and *nda2-52* cells were transformed with vector (pREP1) or p*sto1*$^+$ (pREP1*sto1*$^{+)}$ and grown for 3 days at 32°C on EMM plates supplemented with uracil and with either 0.8% DMSO (control) or 0.5 μg/ml TBZ with 0.8% DMSO (TBZ).

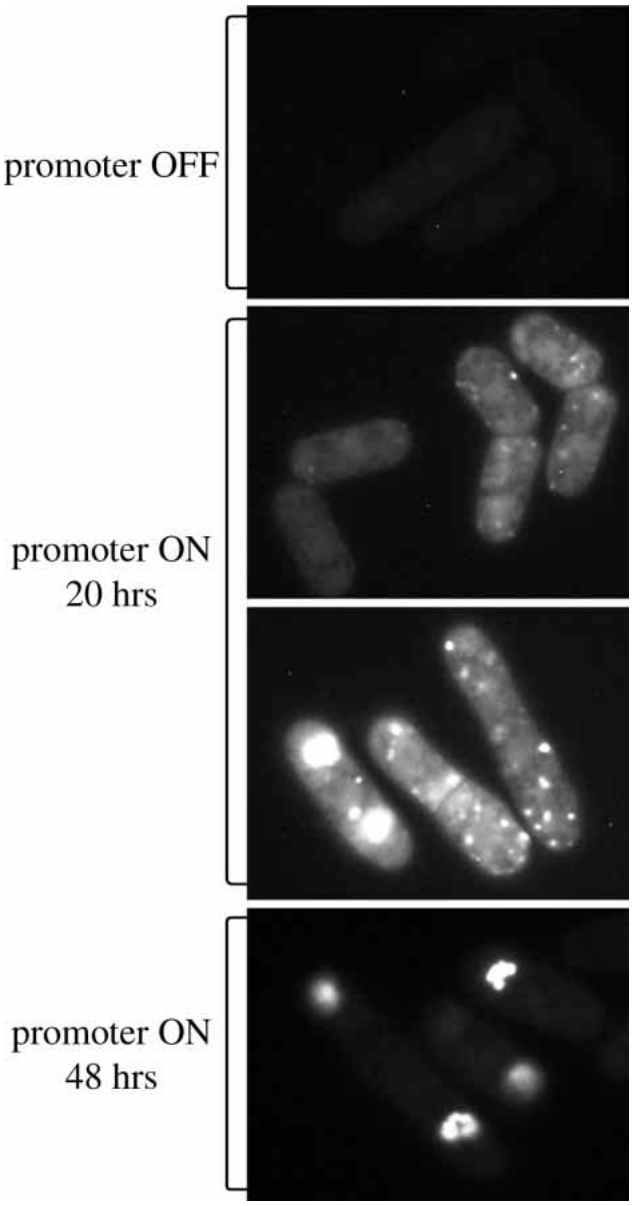

Fig. 7. Sto1p does not localize to microtubules. Photographs of wild-type haploid cells carrying plasmids expressing the Sto1-GFP fusion protein. Cells were grown at 25°C in EMM supplemented with uracil and thiamine (promoter off) or without thiamine for 20 and 24 hours (promoter on). To reflect the relative brightness of GFP fluorescence in these cells, the top three images were recorded by integrating identical number of frames. The different intensities of fluorescence in cells of the same panel most probably reflect different numbers of plasmids present. The image of cells grown without thiamine for 48 hours (promoter on, 48 hours) was recorded at a shorter exposure to allow better visualization of the brightly staining objects.

DISCUSSION

The availability of a pool of polymerizable α/β tubulin dimers is crucial for proper microtubule dynamics and function. The dimers are produced concomitant with folding of the tubulin polypeptides in a pathway carried out in part by the Cofactor proteins. In budding yeast, a failure in cofactor function often leads to cold-sensitive microtubules and to increased chromosome instability, but the cells grow as wild-type at permissive temperatures. In fission yeast the same pathway appears to be essential (Hirata et al., 1998; Radcliffe et al., 1998), and in this way it is more similar to the folding of α- and β-tubulins in mammals, as identified in the in vitro reactions using isolated Cofactors and denatured tubulins (Gao et al., 1994; Tian et al., 1996, 1997). This may reflect the fact that several features of the microtubule physiology in *S. pombe* are more similar to those in mammalian cells than are those of budding yeast (reviewed by Hagan, 1998). Unlike *S. cerevisiae*, *S. pombe* contains microtubules that serve to define and maintain its cell polarity (Beinhauer et al., 1997; Hirata et al., 1998; Mata and Nurse, 1997; Radcliffe et al., 1998). Like in higher eukaryotes, cytoplasmic microtubules depolymerize during mitosis, which is followed by a prompt formation of the intranuclear spindle. In contrast, budding yeast cytoplasmic microtubules persist throughout mitosis and the metaphase spindle forms earlier in the cell cycle (Byers and Goetsch, 1975; Hagan and Hyams, 1988).

We have isolated a fission yeast gene, *sto1*$^+$, which encodes a protein similar to human Cofactor E. Homology between these proteins is implied not only by their overall sequence similarity, but also by the presence of a newly identified central domain, which contains leucine rich repeats. As expected, *sto1*$^+$ is an essential gene. The lethality of *sto1Δ* strain could not, however, be rescued by ectopically expressed human Cofactor E. It has previously been shown that mammalian Cofactor A and several prefoldin subunits could substitute some functions of the respective *S. cerevisiae* genes (Archer et al., 1995; Geissler et al., 1998). Human CFE is the first protein in this pathway to be functionally tested in fission yeast. Further work is required to evaluate whether the tubulin folding pathway of *S. pombe* is different from that in humans or whether Sto1p is involved in some additional aspect of microtubule control and function.

Several results of our study suggest that Sto1p plays a role in microtubule biogenesis in *S. pombe* homologous to that of human Cofactor E. Examination of the phenotype of cells lacking the *sto1*$^+$ gene revealed that Sto1p is required for the formation of normal cytoplasmic microtubules and for spindle assembly. In *sto1Δ* cells the interphase microtubules are rarely seen, and a punctuate staining, such as reported for *alp1-315* mutant cells, was not observed (Hirata et al., 1998). In *sto1Δ* cells spindles fail to form and after a transient arrest with condensed chromosomes, cells die, apparently from massive abnormal septation. An important role of Sto1p in regulating microtubule dynamics is also implied by its ability, when moderately overexpressed, to rescue the temperature-sensitivity of *tsm1-512* mutant, which is defective in the formation of metaphase spindle and interacts genetically with α- and β-tubulin mutants (data not shown, Grishchuk et al., 1998). Our efforts to clone the *tsm1*$^+$ gene have not yet been successful, but it has been mapped genetically to a ~25 kb region on Chromosome 2. The open reading frames in this region, however, show no sequence similarity to any of the proteins implicated in the tubulin folding pathways.

Consistent with the proposed role of Sto1p in the α-tubulin folding pathway, overexpression of Sto1p is toxic for cells carrying a mutation in α-, but not β-tubulin. This inhibition of

growth could be attributed to the destabilization of microtubules in *nda2-52* cells, because overproduction of Sto1p increased the sensitivity of this strain to the microtubule destabilizing agent, TBZ. A similar phenotype has previously been reported for overproduction of Pac2p in budding yeast (Vega et al., 1998). It should be noted, however, that the phenotypes of the *nda2-52* and *nda3-311* mutants are different, e.g. with respect to their sensitivity to microtubule depolymerizing drugs (Toda et al., 1983). While the observed toxicity of Sto1p overproduction in the *nda2-52* mutant background is consistent with its presumptive role as an α-tubulin binding protein, further analysis of different tubulin mutants is desirable. To directly examine physical interaction between Sto1p and α-tubulin, we immunoprecipitated epitope tagged Sto1p expressed in *sto1Δ* background. No Nda2p could be detected under these conditions (E. L. Grishchuk, L. Aveline and J. R. McIntosh, unpublished result), presumably because the interaction is weak (Tian et al., 1997), and in *S. cerevisiae* its detection requires overproduction of both proteins (Vega et al., 1998).

We had a strong expectation that Sto1p in vivo would be found along microtubules, mainly because human Cofactor E behaves in vitro as a microtubule-associated protein (Tian et al., 1996). Both human CFE and Pac2p contain a short region of similarity to the microtubule binding domain of CLIP-170, which is also found in several other microtubule associated proteins (Hoyt et al., 1997; Pierre et al., 1992; Tian et al., 1996). Furthermore, microtubule association was reported for Alp1p (Cofactor D), a presumptive partner for Sto1p (Hirata et al., 1998). However, we did not observe localization of the Sto1-GFP fusion protein to microtubules or any other specific cellular compartment at any stage of the cell cycle. The same result was obtained by indirect immunofluorescence with another, pk1-epitope tagged (Southern et al., 1991), functional copy of Sto1p (data not shown). We concluded that Sto1p does not localize to microtubules in vivo or its microtubule association is too transient or weak to be detected by these methods.

The α- and β-tubulin pathways in vitro appear to be symmetrical (Tian et al., 1997). This is seen in part by the ability of both Cofactors E and D, when present in excess, to disrupt α/β tubulin dimers by binding to their respective tubulin polypeptides. This mechanism is presumably responsible for the toxicity of overproduction of Alp1p in *S. pombe* (but not of the Cofactor D homolog in budding yeast), which leads to microtubule destabilization and can be rescued by 'adding back' β-tubulin (Hirata et al., 1998; Hoyt et al., 1990). Overproduction of Sto1p, however, has no toxic effects in wild-type cells and their microtubules appear normal. Similar results were reported for overexpression of Pac2p in budding yeast (Vega et al., 1998). We concluded that Sto1p is unable to disrupt the α/β tubulin dimers in vivo. We interpret this result as a demonstration of the asymmetry of the α- and β-tubulin metabolisms in vivo. Indeed, it is well known that yeast cells are exquisitely sensitive to the overproduction of β-tubulin, while overexpression of α-tubulins is not very toxic (Adachi et al., 1986; Hiraoka et al., 1984; Weinstein and Solomon, 1990). Further work is needed to reveal how the kinetic characteristics of tubulin's interaction with the Cofactor proteins and other parameters, such as their microtubule association, contribute to the regulation of the overall microtubule integrity and dynamics.

This work was supported in part by GM-33787 from the National Institute of Health and PRP-84 from the American Cancer Society to J.R.M. We are grateful to Drs A. M. Carr, N. Cowan, M. Fuller, H. Browning, S. Sazer and R. R. West for providing plasmids, antibodies and other reagents, and to members of the McIntosh lab for helpful discussions, critical reading of the manuscript and technical assistance. We also thank Dr G. V. Savchenko for assisting with testing the ability of various *sto1*$^+$ constructs to complement the null-allele.

REFERENCES

Adachi, Y., Toda, T., Niwa, O. and Yanagida, M. (1986). Differential expressions of essential and nonessential α-tubulin genes in *Schizosaccharomyces pombe*. *Mol. Cell Biol.* **6**, 2168-2178.

Alfa, C., Fantes, P., Hyams, J., McLeod, M. and Warbrick, E. (1993). *Experiments with Fission Yeast*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Alvarez, P., Smith, A., Fleming, J. and Solomon, F. (1998). Modulation of tubulin polypeptide ratios by the yeast protein Pac10p. *Genetics* **149**, 857-864.

Archer, J. E., Vega, L. R. and Solomon, F. (1995). Rbl2p, a yeast protein that binds to β-tubulin and participates in microtubule function *in vivo*. *Cell* **82**, 425-434.

Archer, J. E., Magendantz, M., Vega, L. R. and Solomon, F. (1998). Formation and function of the Rbl2p-β-tubulin complex. *Mol. Cell Biol*. **18**, 1757-1762.

Barbet, N., Muriel, W. J. and Carr, A. M. (1992). Versatile shuttle vectors and genomic libraries for use with *Schizosaccharomyces pombe*. *Gene* **114**, 59-66.

Basi, G., Schmid, E. and Maundrell, K. (1993). TATA box mutations in the *Schizosaccharomyces pombe nmt1* promoter affect transcription efficiency but not the transcription start point or thiamine repressibility. *Gene* **123**, 131-136.

Beinhauer, J. D., Hagan, I. M., Hegemann, J. H and Fleig, U. (1997). Mal3, the fission yeast homologue of the human APC-interacting protein EB-1 is required for microtubule integrity and the maintenance of cell form. *J. Cell Biol*. **139**, 717-728.

Byers, B. and Goetsch, L. (1975). Behavior of spindles and spindle plaques in the cell cycle and conjugation of *Saccharomyces cerevisiae*. *J. Bacteriol*. **124**, 511-523.

Elble, R. (1992). A simple and efficient procedure for transformation of yeasts. *Biotechniques* **13**, 18-20.

Gao, Y., Melki, R., Walden, P. D., Lewis, S. A., Ampe, C., Rommelaere, H., Vandekerckhove, J. and Cowan, N. J. (1994). A novel cochaperonin that modulates the ATPase activity of cytoplasmic chaperonin. *J. Cell Biol*. **125**, 989-996.

Geiser, J. R., Schott, E. J., Kingsbury, T. J., Cole, N. B., Totis, L. J., Bhattacharyya, G., He, L. and Hoyt, M. A. (1997). *Saccharomyces cerevisiae* genes required in the absence of the *CIN8*-encoded spindle motor act in functionally diverse mitotic pathways. *Mol. Biol. Cell.* **8**, 1035-1050.

Geissler, S., Siegers, K. and Schiebel, E. (1998). A novel protein complex promoting formation of functional α- and γ-tubulin. *EMBO. J*. **17**, 952-966.

Grishchuk, E. L., Howe, J. L. and McIntosh, J. R. (1998). A screen for genes involved in the anaphase proteolytic pathway identifies *tsm1*$^+$, a novel *Schizosaccharomyces pombe* gene important for microtubule integrity. *Genetics* **149**, 1251-1264.

Hagan, I. M. (1998). The fission yeast microtubule cytoskeleton. *J. Cell Sci*. **111,** 1603-1612.

Hagan, I. M. and Hyams, J. S. (1988). The use of cell division cycle mutants to investigate the control of microtubule distribution in the fission yeast *Schizosaccharomyces pombe*. *J. Cell Sci.* **89**, 343-357.

Hiraoka, Y., Toda, T. and Yanagida, M. (1984). The *NDA3* gene of fission yeast encodes β-tubulin: a cold-sensitive *nda3* mutation reversibly blocks spindle formation and chromosome movement in mitosis. *Cell* **39**, 349-358.

Hirata, D., Masuda, H., Eddison, M. and Toda, T. (1998). Essential role of tubulin-folding cofactor D in microtubule assembly and its association with microtubules in fission yeast. *EMBO. J*. **17**, 658-666.

Hoheisel, J. D., Maier, E., Mott, R., McCarthy, L., Grigoriev, A. V., Schalkwyk, L. C., Nizetic, D., Francis, F. and Lehrach, H. (1993). High resolution cosmid and P1 maps spanning the 14 Mb genome of the fission yeasts *S. pombe*. *Cell* **73**, 109-120.

Hoyt, M. A., Stearns, T. and Botstein, D. (1990). Chromosome instability

mutants of *Saccharomyces cerevisiae* that are defective in microtubule-mediated processes. *Mol. Cell. Biol*. **10**, 223-234.

Hoyt, M. A., Macke, J. P., Roberts, B. T. and Geiser, J. R. (1997). *Saccharomyces cerevisiae PAC2* functions with *CIN1, 2* and *4* in a pathway leading to normal microtubule stability. *Genetics* **146**, 849-857.

Innis, M. A., Gelfand, D. H., Sninsky, J. J. and White, T. J. (1990). *PCR protocols: A guide to Methods and Applications*. Academic Press Inc., NY.

Kobe, B. and Deisenhofer, J. (1994). The leucine-rich repeat: a versatile binding motif. *Trends Biochem. Sci*. **19**, 415-421.

Mata, J. and Nurse, P. (1997). *tea1* and the microtubular cytoskeleton are important for generating global spatial order within the fission yeast cell. *Cell* **89**, 939-949.

Maundrell, K. (1993). Thiamine-repressible expression vectors pREP and pRIP for fission yeast. *Gene* **123**, 127-130.

Melki, R., Rommelaere, H., Leguy, R., Vandekerckhove, J. and Ampe, C. (1996). Cofactor A is a molecular chaperone required for β-tubulin folding: functional and structural characterization. *Biochemistry* **35**, 10422-10435.

Moreno, S., Klar, A. and Nurse, P. (1991). Molecular genetic analysis of fission yeast *Schizosaccharomyces pombe*. In *Methods in Enzymology* (ed. C. Guthrie and G. Fink), pp. 795-823. Academia Press Inc., NY.

Ohkura, H. and Yanagida, M. (1991). *S. pombe* gene *sds22*$^{+}$ essential for a midmitotic transition encodes a leucine-rich repeat protein that positively modulates protein phosphatase-1. *Cell* **64**, 149-157.

Pierre, P., Scheel, J., Rickard, J. E. and Kreis, T. E. (1992). CLIP-170 links endocytic vesicles to microtubules. *Cell* **70**, 887-900.

Radcliffe, P., Hirata, D., Childs, D., Vardy, L. and Toda, T. (1998). Identification of novel temperature-sensitive lethal alleles in essential β-tubulin and nonessential α2-tubulin genes as fission yeast polarity mutants. *Mol. Biol. Cell*. **9**, 1757-1771.

Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*, edn 2. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Southern, J. A., Young, D. F., Heaney, F., Baumgartner, W. and Randall, R. E. (1991). Identification of an epitope on the P & V proteins of simian virus 5 that distinguishes between two isolates with different biological characteristics. *J. Gen. Virol*. **72**, 1551-1557.

Stearns, T., Hoyt, M. A. and Botstein, D. (1990). Yeast mutants sensitive to antimicrotubule drugs define three genes that affect microtubule function. *Genetics* **124**, 251-262.

Tian, G., Huang, Y., Rommelaere, H., Vandekerckhove, J., Ampe, C. and Cowan, N. J. (1996). Pathway leading to correctly folded β-tubulin. *Cell* **86**, 287-296.

Tian, G., Lewis, S. A., Feierbach, B., Stearns, T., Rommelaere, H., Ampe, C. and Cowan, N. J. (1997). Tubulin subunits exist in an activated conformational state generated and maintained by protein cofactors. *J. Cell Biol*. **138**, 821-832.

Toda, T., Umesono, K., Hirata, A. and Yanagida, M. (1983). Cold-sensitive nuclear division arrest mutants of the fission yeast *Schizosaccharomyces pombe. J. Mol. Biol.* **168**, 251-270.

Vainberg, I. E., Lewis, S. A., Rommelaere, H., Ampe, C., Vandekerckhove, J., Klein, H. L. and Cowan, N. J. (1998). Prefoldin, a chaperone that delivers unfolded proteins to cytosolic chaperonin. *Cell* **93**, 863-873.

Vega, L. R., Fleming J. and Solomon, F. (1998). An α-tubulin mutant destabilizes the heterodimer: phenotypic consequences and interactions with tubulin-binding proteins. *Mol. Biol. Cell*. **9**, 2349-2360.

Weinstein, B. and Solomon, F. (1990). Phenotypic consequences of tubulin overproduction in *Saccharomyces cerevisiae:* differences between α-tubulin and β-tubulin. *Mol. Cell Biol*. **10**, 5295-5304.