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RESEARCH ARTICLE

The evolutionary conserved *BER1* gene is involved in microtubule stability in yeast

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Abstract In yeast, microtubules are dynamic filaments necessary for spindle and nucleus positioning, as well as for proper chromosome segregation. We identify a function for the yeast gene *BER1* (Benomyl REsistant 1) in microtubule stability. *BER1* belongs to an evolutionary conserved gene family whose founding member Sensitivity to Red light Reduced is involved in red-light perception and circadian rhythms in Arabidopsis. Here, we present data showing that the *ber1*Δ mutant is affected in microtubule stability, particularly in presence of microtubule-depolymerising drugs. The pattern of synthetic lethal interactions obtained with the *ber1*Δ mutant suggests that Ber1 may function in N-terminal protein acetylation. Our work thus suggests that microtubule stability might be regulated through this post-translational modification on yet-to-be determined proteins.

Keywords Microtubule · Kinetochore · Spindle checkpoint · Yeast · Protein N-acetylation · SRR1

Abbreviations

APC	Anaphase-promoting complex
FACS	Fluorescence activated cell sorting
WT	Wild type

Introduction

Sensitivity to Red light Reduced (*SRR1*) is an *Arabidopsis* gene involved in red light response, where it acts in the phytochrome B pathway. It is also required for normal circadian rhythms (Staiger et al. 2003). Remarkably, this gene is conserved in eukaryotes, where it is found as single copy gene without any recognizable functional domain. However, *SRR1* homologues might not necessarily function in circadian rhythms or light perception, since this gene is also found in organisms lacking such processes (e.g. yeast). In order to understand the molecular function of SRR1-related proteins, we studied the *Saccharomyces cerevisiae* homologue of SRR1, the product of the open reading frame *YLR412W*. The deletion of this gene did not lead to any obvious phenotype as previously reported by systematic analysis of gene knock-out (see *Saccharomyces* Genome Database at http://www.yeastgenome.org). However, our results indicate that the yeast SRR1-homologue is required for proper microtubule function, and we renamed the corresponding gene *BER1* for benomyl resistant 1 (see below).

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Microtubules are polar and dynamic filaments composed of alpha and beta-tubulin heterodimers. The polymerization of the tubulin dimers takes place mainly at the plus end of microtubules, which is much more dynamic than the minus end (Howard and Hyman 2003; Winsor and Schiebel 1997). Microtubule plus ends bind different proteins, such as kinetochore-associated proteins, as well as regulators of microtubule dynamics, whereas minus ends are generally bound to a microtubule-organizing centre (MTOC) (Howard and Hyman 2003). *S. cerevisiae* produces two types of microtubules, which are both nucleated at spindle pole bodies (SPBs). SPBs are the MTOCs of yeast and are imbedded in the nuclear envelope. Cytoplasmic microtubules are nucleated on the cytoplasmic side of SPBs, and function in nuclear migration and spindle positioning within the cell. Microtubules emanating from the nuclear side of the SPBs form the mitotic spindle and ensure proper chromosome segregation at anaphase (Winsor and Schiebel 1997).

Within the spindle, microtubule-kinetochore attachment is tightly monitored during mitosis by the spindle checkpoint (Yu 2002). This checkpoint functions to arrest the cell at the metaphase-anaphase transition as long as a single kinetochore is not bipolarly attached to opposite spindle poles. This survey mechanism ensures the faithful segregation of sister chromatids to daughter cells. In case of misattachment, metaphase arrest involves a number of proteins such as Mad1-Mad3, Bub1, Bub3 and inhibition of the anaphase-promoting complex (APC), an E3 ubiquitin-ligase. Upon correct attachment of all kinetochores, the APC is activated and triggers anaphase through targeting of securin/Pds1 to the proteasome (Yu 2002). Securin is the inhibitor of separase, a protease involved in the resolution of sister-chromatid cohesion (Yu 2002).

Protein acetylation is a widely spread process classified in two distinct pathways: directed acetylation of side chains and N-terminal acetylation of nascent proteins. This latter pathway affects many different proteins including proteasome components (Polevoda and Sherman 2003). Here, we report the functional characterization of the *BER1* gene. Using a genetic approach, based on synthetic lethal screens, we show that cells lacking Ber1 require *BUB1*, *BUB3* and *NKP2* for survival. Furthermore, we show that *ber1*Δ cells are specifically resistant to microtubule-destabilizing drugs such as benomyl and nocodazole. Analyses of our synthetic-lethality data suggest that Ber1 is involved in N-acetylation and/or proteasome biogenesis. Although tubulin acetylation was proposed to modulate microtubule dynamics in higher eukaryotes (Westermann and Weber 2003), this modification has not (yet) been shown in yeast. Thus, our data indicate that Ber1 is involved in proper kinetochore function and perhaps other microtubule-related processes, and suggest that it might act through N-acetylation of one or several non-identified proteins.

Materials and methods

Media, yeast strains and plasmids

Nocodazole and benomyl were diluted in DMSO and were added at 10 μg/ml unless specified.

Strains are derivatives of W303 or S288C. WT is BY4741 or BY4742. The *ber1*Δ mutant was created by deleting the complete ORF of the *YLR412W* locus in WT strains with the NatMX4 cassette (Goldstein and McCusker 1999). All strains were grown in rich medium (YPD) at room temperature, unless indicated otherwise. The *nnf1-17* strain (Euskirchen 2002) was a kind gift from Ghia Euskirchen, *ipl1-321* (Chan and Botstein 1993) and *cse4-1* (Stoler et al. 1995) were a kindly provided by Peter Sorger. Strain for the chromosome loss assay was a kind gift from Andrew Murray. WT and *ber1*Δ mutant were transformed with either empty plasmid or plasmids expressing *BUB1* or *bub1-5* under *GAL* promoter described (Farr and Hoyt 1998).

Synthetic genetic array

The synthetic lethal screens were performed as described (Tong et al. 2001). Two independent screens were performed using two different strains as bait: for the first screen, the strain from the knock-out collection in which *YLR412W* (=*BER1*) was deleted with the *KanMX* marker was transformed with a linearized plasmid bearing the *NatMX* cassette to replace the *KanMX* marker with the *NatMX* marker by homologous recombination (Goldstein and McCusker 1999). The resulting yeast strain was crossed with Y3084 and a progeny was selected to get the following genotype: *mfa1*Δ*::MFa1pr-LEU2 can1*Δ*::MFA1pr-HIS3 his3*Δ *leu2*Δ *ura3*Δ *met15*Δ *lys2*Δ *ber1::NatMX4*. The second screen was performed using Y2922 in which the *BER1* open reading frame was replaced by the *NatMX4* cassette, giving the following genotype: *mfa1*Δ*::MFA1pr-HIS3 can1*Δ *his3*Δ *LEU2 ura3*Δ *MET15 lys2*Δ *ber1::NatMX4*.

Chromosome loss and FACS analyses

The chromosome loss assay was performed as described (Hieter et al. 1985). For FACS analyses, overnight pre-cultures were diluted to a final concentration of about 5×10^6 cells/ml and grown in YPD at 30°C. At 10^7 cells/ml, cultures were either blocked with alpha-factor or with 15 μg/ml nocodazole. Then cultures were washed and released in YPD. Aliquots were taken at indicated times, spun and resuspended in ethanol 70%. Samples were then spun and resuspended in 250 μl Tris–HCl pH 7.4 + 200 μg/ml RNase A and incubated for 2 h at 37°C. After centrifugation,

samples were resuspended in 250 µl of 50 mM sodium citrate pH 7.0 + 10 µg/ml propidium iodide and analysed by flow cytometry.

Microscopy

Cells were mounted in complete minimal medium shortly before viewing. To visualize GFP-labelled microtubules, full-frame pictures of fields of cells were taken at nine focal planes (0.2 µm step size) using an Olympus BX50 fluorescence microscope, a piezo motor, and the TILLVision software (TILLphotonics, Martinsried, Germany) essentially as described (Kusch et al. 2002). The pictures were projected on one single plane using the maximum intensity projection method. Picture analyses were performed using ImageJ.

Results

BER1 shows synthetic lethality with kinetochore and spindle checkpoint genes

SRR1-related proteins encoded by single copy genes can be identified in most eukaryotes for which genome information is available (Staiger et al. 2003). All the proteins presented on our alignment (Fig. 1) contain a centrally located SRR1 domain (defined by Pfam) present in numerous proteins. However, in this set of proteins, including in backer's yeast Ber1, the homology extends over the entire protein (Fig. 1). This analysis did unfortunately not suggest any potential biochemical activity for this protein family. We thus decided to follow a functional genomic approach in yeast in order to generate working hypotheses for the molecular function of SRR1-related proteins.

To gain insight into the function of Ber1, we first performed two independent genome-wide synthetic genetic screens using *ber1*Δ in two different genetic backgrounds as bait. The synthetic interactions that were identified in both screens are shown in Table 1. Analysis of these interactions showed that genes involved in kinetochore function are clearly overrepresented in this data set. Accordingly, Nkp2 is a protein of unknown function that localizes to the kinetochore and to the spindle pole body and Bub1/3 localize to the kinetochore during mitosis and function in the spindle checkpoint (Gillett et al. 2004). These latter two proteins have been discovered due to their high sensitivity to nocodazole (Hoyt et al. 1991), a microtubule depolymerising drug that affects microtubules in a manner similar to

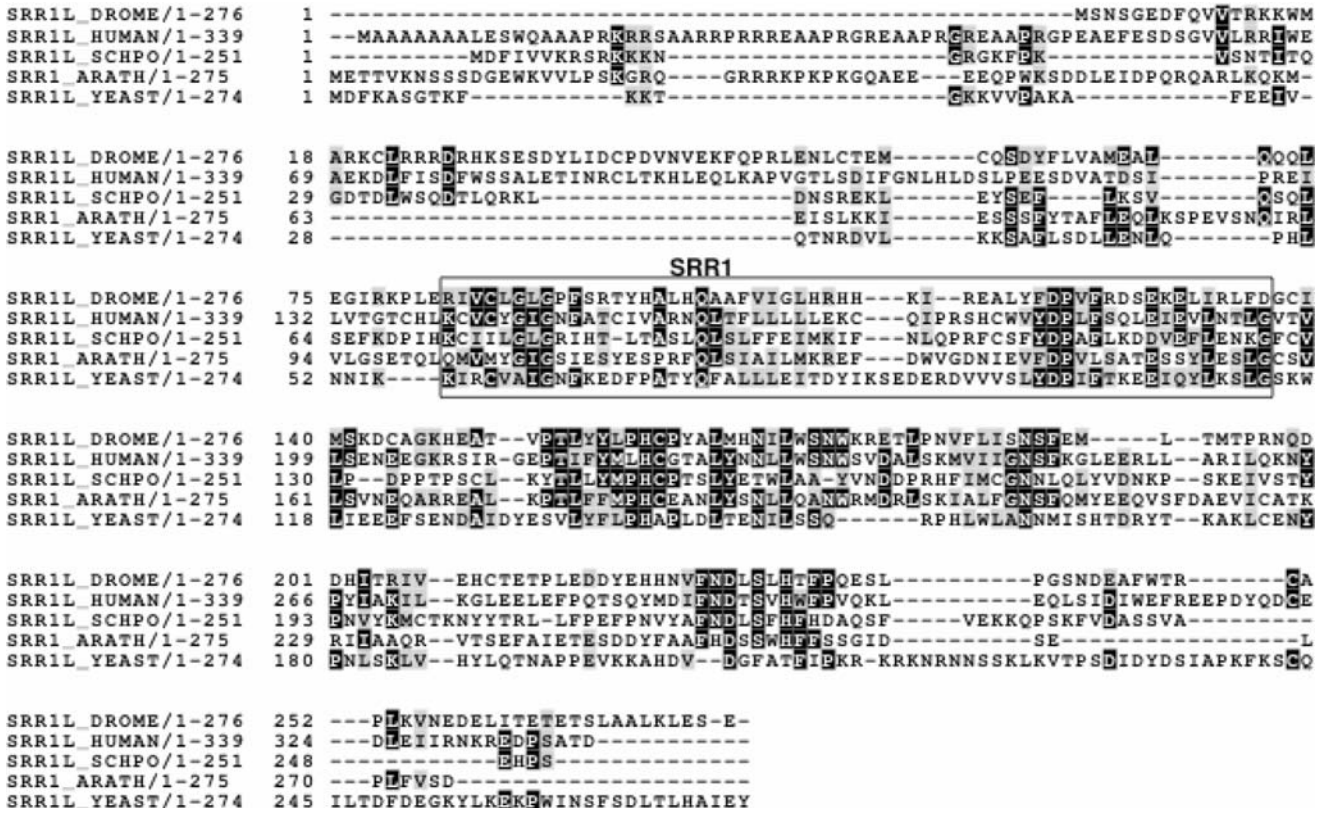

Fig. 1 Multiple sequence alignment of the SRR protein family. The *Arabidopsis* SRR1 homologs have been retrieved from UniProtKB/Swiss-Prot database (Bairoch et al. 2005) using BLAST with standard parameters (Altschul et al. 1997), and re-aligned using T-Coffee (Notredame et al. 2000). The SRR1 domain, as defined in Pfam 22.0 (Bateman et al. 2004), is shown in the *black box*

benomyl. These results suggested a role for Ber1 in the spindle checkpoint, in the kinetochore, or in a more general microtubule-related process.

Further analyses of the synthetic lethal data also suggested a role for Ber1 in protein N-acetylation or proteasome biosynthesis (Fig. 2). Indeed, when the published (Csank et al. 2002) synthetic genetic interactions for each gene found in the synthetic lethal screens (see Table 1) were compared, three disruptions were found to show a very similar genetic interaction pattern as *ber1*Δ: namely *rpn4*Δ, *ard1*Δ and *nat1*Δ. Rpn4 is a transcription factor that stimulates the expression of several proteasome subunits (Xie and Varshavsky 2001), whereas Ard1 and Nat1 are the main subunits of the N-terminal acetyltransferase (Polevoda and Sherman 2003). This enzyme has been shown to be involved in many processes including protein stability, protein function or protein–protein interactions (Polevoda and Sherman 2003).

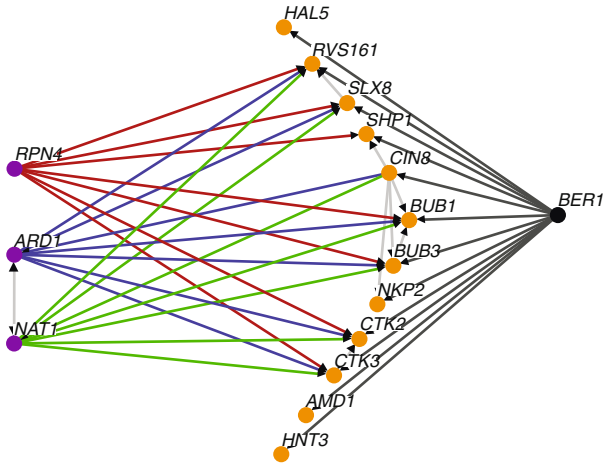

Fig. 2 Analysis of synthetic lethal screen results performed with *ber1*Δ as bait. *Dark grey* lines represent *ber1*Δ synthetic lethal/sick interactions. *Orange dots* represent the common results of two independent screens. *Violet dots* represent genes that exhibit synthetic lethal/sick interactions with genes found in the *ber1*Δ screens. The scheme was made using Osprey

Confirmation of the involvement of Ber1 in kinetochore function

In order to get further insights into the putative involvement of *Ber1* in kinetochore function, the *ber1*Δ mutant was crossed with temperature-sensitive mutants of the following essential components of the kinetochore: the aurora kinase Ipl1 (Chan and Botstein 1993), which regulates microtubule-kinetochore attachment (Buvelot et al. 2003); Nnf1, a component of the central MIND kinetochore complex that contributes to linking the inner and outer kinetochore layers (De Wulf et al. 2003); Ndc10 (Goh and Kilmartin 1993), which is a key component of the inner kinetochore that is necessary for kinetochore assembly (Espelin et al. 2003); and Cse4 (Stoler et al. 1995), a centromere-specific histone variant involved in kinetochore assembly (Meluh et al. 1998). Altogether, these proteins are quite representative of the different components of the kinetochore. All double mutants grew at permissive temperature and were all unable to grow at the restrictive temperature (Fig. 3). Notably, at the semi-restrictive temperature, *ipl1-321 ber1*Δ and *cse4-1 ber1*Δ double mutants were slightly

Table 1 Common synthetic lethal screen results of two independent screens made with *ber1*Δ

ORF	Gene name	Biological process
YJL165C	HAL5	Cation homeostasis
YML035C	AMD1, AMD3	Purine nucleotide metabolism
YOR026W	BUB3	Mitotic spindle checkpoint
YOR258W	HNT3	Biological process unknown
YEL061C	CIN8	Mitotic anaphase B, mitotic sister chromatid segregation, mitotic spindle organization and biogenesis in nucleus
YER068C-A		Biological process unknown
YGR188C	BUB1	Mitotic spindle checkpoint
YJL006C	CTK2	Protein amino acid phosphorylation, regulation of transcription from RNA polymerase II promoter
YDR444W		Biological process unknown
YER116C	SLX8	Protein sumoylation, response to DNA damage stimulus, telomere maintenance
YML112W	CTK3	Protein amino acid phosphorylation, regulation of transcription from RNA polymerase II promoter
YLR315W	NKP2	Biological process unknown
YBL058W	SHP1	Glycogen metabolism, proteasomal ubiquitin-dependent protein catabolism, sporulation, telomere maintenance
YCR009C	RVS161	Bipolar bud site selection, endocytosis, response to osmotic stress

Results listed here were found in two independent screens. *BER1* and genetically linked genes were found as expected but were removed from this list

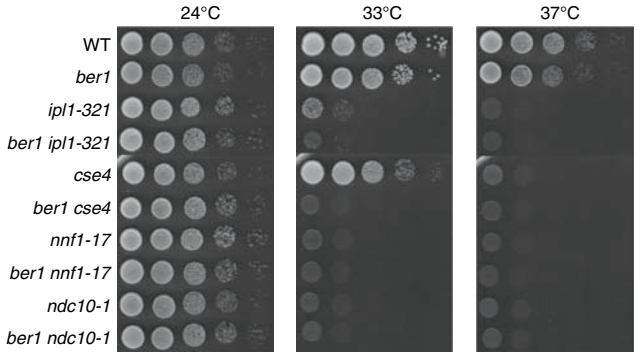

Fig. 3 *BER1* shows genetic interactions with kinetochore mutants *cse4* and *ipl1-321*. Ten-fold dilution series of indicated strains were *spotted* on YPD plates and grown at indicated temperature for 36 h

and strongly, respectively, defective for growth when compared to parental strains. Interestingly, it has been shown that Cse4 is phosphorylated by Ipl1 and that both proteins act in the same pathway (Buvelot et al. 2003). These results indicate a putative role of Ber1 in kinetochore function. However, this could be via an indirect effect, e.g. through a Ber1-dependent alteration of the predicted Cse4 N-acetylation (Fig. 2).

Another hypothesis is that Ber1 is acting directly on the spindle checkpoint, since spindle checkpoint components are localized at the kinetochore at least during mitosis (Gillett et al. 2004). In this case, Ber1 should localize to the kinetochore. Despite efforts made to localize Ber1-GFP or Ber1-Venus, we could not detect any signal for the corresponding constructs (notably, when the tags were inserted at the C-terminus of *BER1* in the genome). However, a diffuse fluorescent signal was detected in the cytoplasm and in the nucleus when Ber1-GFP was expressed under the control of an inducible *GAL1* promoter on a plasmid (data not shown). This is in agreement with observations made during large-scale analyses of protein localization (Huh et al. 2003). A similar subcellular localisation was reported for SRR1-GFP in Arabidopsis (Staiger et al. 2003). However, no enrichment of the signal was seen on microtubules or at kinetochores.

The cell-cycle and the spindle checkpoint are normal in the *ber1*Δ mutant

In order to test the hypothesis that Ber1 plays a role in the spindle checkpoint, and thus in a mechanism monitoring the chromosome-spindle attachment, we assessed the chromosome loss rate in the *ber1*Δ mutant (Hieter et al. 1985). The rate of chromosome loss was slightly higher in the *ber1*Δ mutant under all tested conditions (Fig. 4a). This rate was however notably lower than the one in spindle checkpoint mutants such as *mad* and *bub* (Warren et al. 2002).

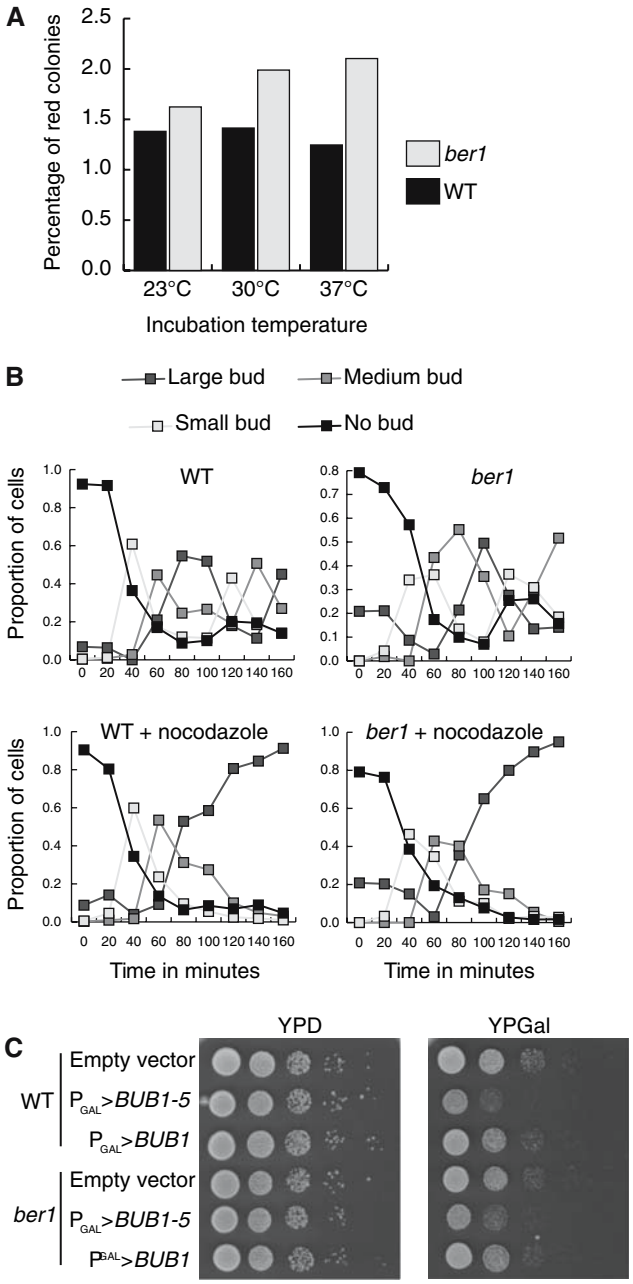

Fig. 4 Spindle checkpoint is normal in *ber1*Δ mutant. **a** *Ade2*-ochre mutant strains carrying an additional chromosome bearing ochre suppressor were plated and incubated at indicated temperatures. The percentage of *red* colonies was recorded. More than 2,400 colonies were analysed for each trial. **b** Cells of indicated cultures were blocked in alpha-factor and released in rich medium with or without 10 μg/ml nocodazole. *Aliquots* were taken at indicated times, resuspended in PBS buffer and cells were classified according to the bud size. **c** WT and *ber1*Δ cells were transformed with plasmids expressing *BUB1* of *bub1-5* under *GAL1* promoter. After pre-culture in selective medium supplemented with sucrose, cells were spotted on YPGal and incubated for 2 days at 24°C

The *ber1*Δ mutant was then analysed for its ability to activate and silence the spindle checkpoint. We first assessed cell-cycle progression by synchronizing cells with

alpha-factor and releasing them in rich medium. Microscopic examination revealed normal cell-cycle progression in the *ber1*Δ mutant (Fig. 4b). These results were confirmed by FACS analysis (data not shown) and are consistent with the normal growth rate of the *ber1*Δ mutants (Fig. 5c). Mitotic checkpoint activation was then directly tested by synchronizing cells with alpha-factor and releasing them in the presence of nocodazole. Microscopic examination showed that in the presence of nocodazole, *ber1*Δ mutants behaved as the WT, indicating that checkpoint activation was normal in the absence of Ber1 (Fig. 4b).

Mitotic checkpoint exit was tested by arresting cells in the presence of nocodazole (thus activating the checkpoint) and then releasing cells into rich medium. The *ber1*Δ mutation did not affect the timing needed to release cells as observed by FACS analysis (Supplementary data 1). In addition, the *ber1*Δ mutation did not rescue the effect of over-expressing the *bub1-5* mutant (Fig. 4c), which has been shown to cause slower growth because of constitutive activation of the spindle checkpoint (Farr and Hoyt 1998). Taken together, these results suggest a role for Ber1 in kinetochore function, but not in the spindle checkpoint, which appeared to be normal in the mutant. One possibility is that Ber1 could directly affect the microtubules.

Loss of Ber1 increases resistance to microtubule-depolymerising drugs

To test this hypothesis, the *ber1*Δ mutant was grown on medium containing either nocodazole or benomyl. The growth of the mutant was greater than the WT in presence of these microtubule-depolymerising drugs (Fig. 5a, b). This phenotype was reproduced in independent knock-out mutants and in knock-out mutants obtained in different WT backgrounds. The phenotype was observed in the two mating types as well as in a liquid assay (Fig. 5c). The yeast orthologue of *SRR1* was therefore called *BER1*, for benomyl resistant. For unexplained reasons, the overexpression of the *BER1* gene was only able to partially rescue the *ber1*Δ mutant. The *ber1*Δ mutant was crossed with several tubulin mutants in order to confirm genetically what was observed with drugs (Fig. 5d). The *tub2-423 ber1*Δ double mutant exhibited very slow growth at 37°C, validating the requirement of Ber1 for normal microtubule-mediated processes in case of chemical or genetic alteration of microtubules. Cell-wall integrity and resistance to other drugs affecting the cell-cycle were tested and shown to be identical in WT and *ber1*Δ cells, suggesting that drug import into *ber1*Δ cells was normal (Fig. Supplementary data 2). Furthermore, these results suggest that the effect of the *BER1* deletion on microtubule function is specific.

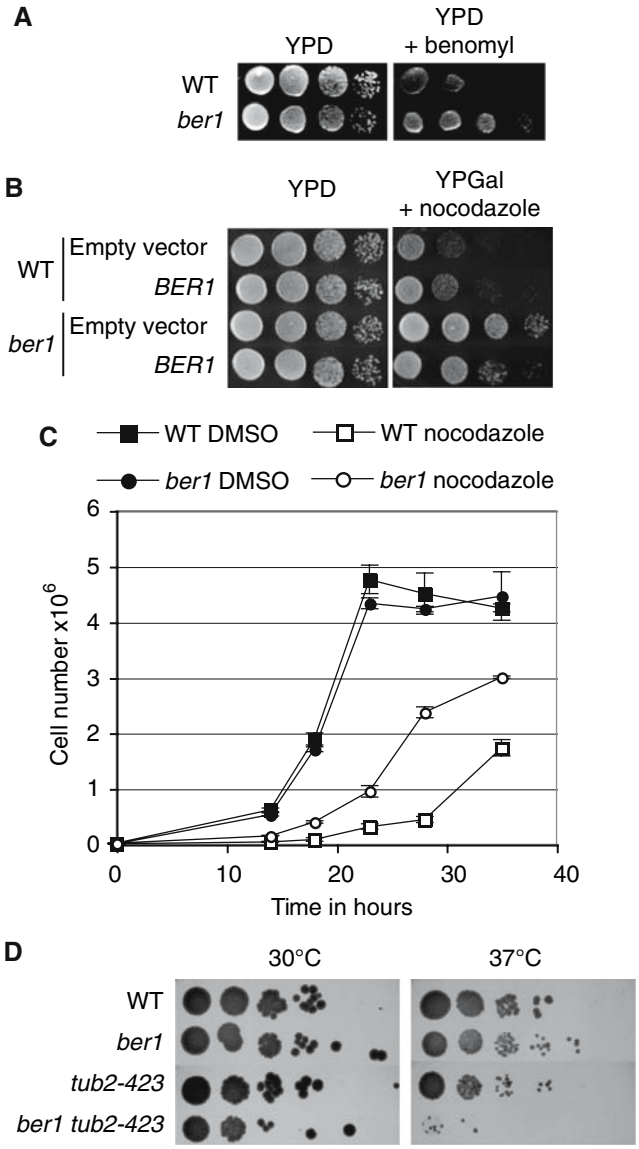

Fig. 5 The *ber1*Δ mutant is resistant to benomyl and nocodazole and is synthetic lethal with *tub2-423*. **a** Ten-fold serial dilutions of indicated strains were spotted on YPD or YPD plus 10 μg/ml benomyl plates. Plates were incubated at 23°C between 3 and 4 days. **b** Ten-fold serial dilutions of indicated strains carrying empty vector or *BER1* under the control of *GAL1* promoter were *spotted* on YPAD (glucose) or YPGal (galactose) plus nocodazole plates. Plates were incubated at 23°C between 3 and 4 days. **c** Growth curve of WT and *ber1*Δ mutants in liquid YPAD at 23°C supplemented with 15 μg/ml nocodazole or the drug vehicle (DMSO) alone. Cell numbers were recorded using a cell counter. **d** Ten-fold serial dilutions of indicated strains were incubated at indicated temperatures for 3 days

Microtubules are resistant to nocodazole in *ber1*Δ mutant cells

The role of Ber1 with respect to microtubules was further studied by crossing the *ber1*Δ mutant to a strain bearing a GFP-Tub1 reporter, which allows the visualization of

microtubules in vivo (Straight et al. 1997). In rich medium, the average length and number of cytoplasmic microtubules per cell were normal in the *ber1*Δ mutant (Supplementary data 3). However, in the presence of low concentrations of nocodazole, the microtubules were less efficiently depolymerised in the *ber1*Δ mutant (Fig. 6). In the WT, after treatment for 20 min with 3 μg/ml nocodazole, the cytoplasmic microtubules started to disappear and they were greatly

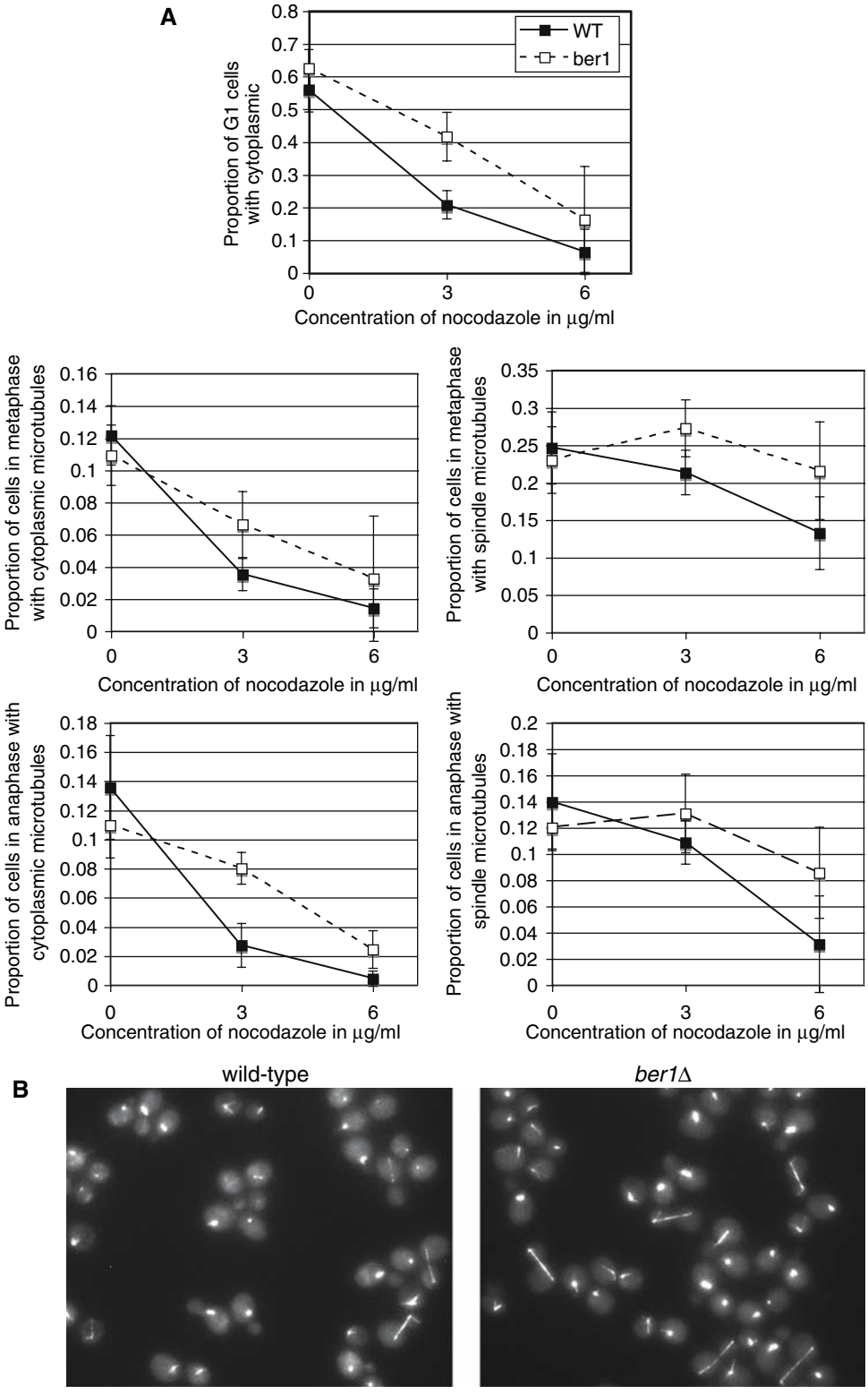

Fig. 6 Microtubules are resistant to nocodazole in the *ber1*Δ mutant. WT and *ber1*Δ mutant strains expressing Tub1-GFP were observed. **a** The number of cells exhibiting indicated microtubules were counted after incubation of an exponentially growing culture with indicated concentration of nocodazole for 20 min. At least 200 cells were counted for each sample and the results expressed as proportion of the whole population (with 1 = 100%). Graphs are results of at least three independent experiments. *Filled square*: WT. *Open square*: *ber1*Δ mutant. **b** Representative pictures of WT and *ber1*Δ cells after incubation in 3 μg/ml nocodazole for 20 min

affected at a concentration of 6 μg/ml in our assay conditions. The spindle microtubules were more resistant and only disappeared at 6 μg/ml. In the *ber1*Δ mutant, incubation in 3 or 6 μg/ml of nocodazole led to reduced depolymerization, with about twice as many *ber1*Δ cells retaining cytoplasmic and spindle microtubules as compared to the WT (Fig. 6). One explanation for this resistance could be that the tubulin levels are higher in the *ber1*Δ mutant than in the WT. However immuno-blotting analyses ruled out this possibility (data not shown), suggesting that microtubules are more resistant to depolymerization in the absence of Ber1.

Discussion

In this study, we characterize the function of *BER1*, the yeast orthologue of the Arabidopsis *SRR1* gene. A synthetic lethal screen approach suggested that Ber1 is implicated in a number of cellular functions, and most prominently in kinetochore function. The spindle checkpoint was shown to be normal in the *ber1*Δ mutant (Fig. 4), but microtubules were more stable in this mutant when cells were treated with microtubule-depolymerising drugs (Fig. 6). This phenotype is not common, since most of the mutants of factors affecting microtubules such as Bim1, Bik1 and Stu2 are more sensitive to such drugs (Al-Bassam et al. 2006; Berlin et al. 1990; Schwartz et al. 1997; Winsor and Schiebel 1997).

Synthetic lethal screen analyses revealed that Rpn4, Ard1, Nat1 and Ber1 share a wide set of genetic interactions (Fig. 2). Our data therefore suggest that Ber1 may function in protein N-terminus acetylation and/or proteasome biogenesis. Since proteasome subunits are heavily N-acetylated (Csank et al. 2002), the role of Ber1 in proteasome assembly might be a secondary consequence of its role in protein modification. Thus, we suggest that Ber1 might be an accessory factor for the N-terminal acetyltransferase. We would however like to point out that with the currently available data this is only a working hypothesis.

According to the N-terminus acetylation model, Ber1 might affect tubulin function via N-acetylation of regulators such as Bim1, Bik1 and/or Stu2 (Wolyniak et al. 2006). Those proteins, are predicted to be acetylated at their N-termini (Csank et al. 2002), and this might interfere with their stability or function and hence, with microtubule dynamics. Our genetic analysis of Ber1 function at the kinetochore supports this model. Indeed, the fact that the *ber1*Δ mutant is synthetic lethal with the temperature-sensitive *cse4-1* mutant, which is defective in the inner-kinetochore complex, and synthetic sick with *ipl1-321*, first suggested that the kinetochore is affected in absence of Ber1 (Fig. 3). These kinetochore defects must be quite specific, since no synthetic lethality was observed with other mutants of the kinetochore such as *nnf1-17* and *ndc10-1* suggesting that the overall integrity of the kinetochore is maintained in the *ber1*Δ mutant. In addition, since Nnf1 is part of the MIND complex, which makes a link between microtubules and centromeric DNA, it is likely that the microtubule-kinetochore interactions are normal. Interestingly, among the temperature-sensitive alleles of essential genes that were tested for synthetic lethality by manual crosses, Cse4 is the only one that is predicted to be acetylated at the N-terminus. If this modification is essential for the stability or function of the Cse4-1 mutant protein and if Ber1 plays a role in the N-terminus acetylation, this could explain the *cse4 ber1* double mutant phenotype. However, the fact that none of *NAT1*, *ARD4* and *BER1* genes are essential indicates that the suggested N-acetylation of Cse4 is not an absolute requirement for the function of the wild-type protein.

In conclusion, our analysis of the pattern of synthetic lethal interaction obtained with *ber1*Δ mutants suggests that Ber1 may be involved in protein N-acetylation. Future experiments will address this hypothesis biochemically and hopefully identify the targets of Ber1p activity. Such experiments may allow us to shed light on the biochemical function of other members of the SRR1 family, in particular Arabidopsis SRR1 for which genetic studies have shown that it is involved in light perception and circadian rhythms (Staiger et al. 2003). Proteasome subunits are heavily N-acetylated (Csank et al. 2002) and the proteasome is very important for light perception and circadian rhythms in Arabidopsis (Hellmann and Estelle 2002; Mas 2005). It will thus be important to test whether Arabidopsis SRR1 may also have such a protein modification role.

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