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A New Function of Skp1 in the Mitotic Exit of Budding Yeast *Saccharomyces cerevisiae*

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We previously reported that Skp1, a component of the Skp1-Cullin-F-box protein (SCF) complex essential for the timely degradation of cell cycle proteins by ubiquitination, physically interacts with Bfa1, which is a key negative regulator of the mitotic exit network (MEN) in response to diverse checkpoint-activating stresses in budding yeast. In this study, we initially investigated whether the interaction of Skp1 and Bfa1 is involved in the regulation of the Bfa1 protein level during the cell cycle, especially by mediating its degradation. However, the profile of the Bfa1 protein did not change during the cell cycle in *skp1-11*, which is a *SKP1* mutant allele in which the function of Skp1 as a part of SCF is completely impaired, thus indicating that Skp1 does not affect the degradation of Bfa1. On the other hand, we found that the *skp1-12* mutant allele, previously reported to block G2-M transition, showed defects in mitotic exit and cytokinesis. The *skp1-12* mutant allele also revealed a specific genetic interaction with Δ*bfa1*. Bfa1 interacted with Skp1 via its 184 C-terminal residues (Bfa1-D8) that are responsible for its function in mitotic exit. In addition, the interaction between Bfa1 and the Skp1-12 mutant protein was stronger than that of Bfa1 and the wild type Skp1. We suggest a novel function of Skp1 in mitotic exit and cytokinesis, independent of its function as a part of the SCF complex. The interaction of Skp1 and Bfa1 may contribute to the function of Skp1 in the mitotic exit.

Keywords: Skp1, Bfa1, *skp1-12*, mitotic exit, *S. cerevisiae*

SKP1 encodes a conserved kinetochore protein that is essential for both the G1-S and G2-M transitions of the cell cycle in the budding yeast *Saccharomyces cerevisiae* (Connelly and Hieter, 1996). Skp1 also forms a component of the SCF (Skp1-cullin-F-box protein) complex, which functions as an E3 ubiquitin ligase for the ubiquitin-mediated proteolysis of cell cycle regulators, including Sic1, at the G1/S transition of the cell cycle (Feldman *et al.*, 1997). The SCF complex is composed of the subunits Skp1, Rbx1, Cdc53, and one of a large number of F-box proteins (Zheng *et al.*, 2002). The substrate specificity of the SCF complex is determined by the interchangeable F-box protein subunit, which recruits a specific set of substrates for ubiquitination to the core complex of Skp1, Rbx1, Cdc53, and the E2 enzyme Cdc34 (Seol *et al.*, 1999). The temperature sensitive mutant alleles of *SKP1*, *skp1-11* and *skp1-3*, produce non-functional Skp1 as a component of the SCF complex and they are unable to accomplish the G1/S transition at the restrictive temperature. Apart from its function as a component of the SCF complex, Skp1 associates with Sgt1 and Hsp90 to modulate the assembly and turnover of the centromere binding factor 3 (CBF3) complex for proper kinetochore function (Lingelbach and Kaplan, 2004). Skp1 also interacts with Bub1 to activate the spindle checkpoint in response to defects in kinetochore tension (Kitagawa *et al.*, 2003). The *skp1-4* and *skp1-AA* mutant alleles show deficiencies of *SKP1* function at the kinetochore. In addition, the *skp1-12* mutant was reported to display a severe defect in the progression of mitosis and to accumulate large, budded, elongated cells with G2 DNA content, suggesting that *SKP1* is required for the G2/M transition (Bai *et al.*, 1996). Skp1 is also associated with the components of the RAVE (regulator of the (H^+)-ATPase of the vacuolar and endosomal membrane) complex, which enhances the activity of V-ATPase for endocytosis, intracellular lysosomal targeting, and protein processing (Seol *et al.*, 2001). Taken together, these reports strongly suggest

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that Skp1 is a multifunctional protein involved in many critical cellular pathways, apart from its function in the ubiquitin-mediated proteolysis of SCF.

The activation of the mitotic exit network (MEN) leads to the inactivation of cyclin-dependent kinase (CDK) and cytokinesis at the end of mitosis in the budding yeast *S. cerevisiae*. The key regulator of MEN is Tem1, which is positively controlled by Lte1 and negatively controlled by Bfa1 and Bub2 (Bardin *et al.*, 2000). Tem1 is positively regulated at the end of mitosis and ultimately triggers Cdc14 phosphatase to degrade the mitotic cyclins and to activate the mitotic CDK inhibitor, Sic1, thus ending mitosis (Pereira *et al.*, 2000). However, Bfa1 inactivates Tem1 to arrest the cell cycle during late anaphase in the presence of spindle defects or DNA damage. Bfa1 is, in turn, negatively regulated by polo kinase Cdc5 through direct phosphorylations (Wang *et al.*, 2000). The amount of Bfa1 and its phosphorylation oscillate throughout the cell cycle; the Bfa1 protein level is relatively low at G1 and is gradually accumulated and phosphorylated (Hu *et al.*, 2001).

We previously reported on the direct interaction between Skp1 and Bfa1 (Lee *et al.*, 2001). In this study, we investigated the biological function(s) of the interaction between Bfa1 and Skp1 during the cell cycle.

Materials and Methods

Yeast culture

S. cerevisiae cells were grown in YPAD media (1% yeast extract, 2% bactopepton, 100 mg/ml adenine and 2% glucose) or in a synthetic complete (SC) drop-out medium prepared with minimal medium (YNB) and the necessary supplements.

Yeast strains, gene disruption, and tagging

The *S. cerevisiae* strains used in this study are listed in Table 1. The genes on the specific chromosomal loci were deleted or tagged by homologous recombination with the cassettes described by Longtine *et al.* (Longtine *et al.*, 1998). Genomic *BFA1* was replaced with *KAN* by transforming *KAN* DNA fragments amplified from pFA6a-KanMX6 with the KS226 and

Table 1. Strains used in this study

Strain	Genotype	Source
w303a	MATa *ura3-1 trp1-1 ade2-3 leu2-3,112 his3-11,15*	Lab stock
YSK193	MATa *can1-100 ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-1 skp1-11* (y552)	Elledge, S.
YSK194	MATa *can1-100 ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-1 skp1-12* (y554)	Elledge, S.
YSK334	w303a *Δbfa1::HIS3*	Lab stock
YSK475	the same as YSK193 except *Δbfa1::KAN*	Lab stock
YSK476	the same as YSK194 except *Δbfa1::KAN*	Lab stock
YSK1370	W303a *BFA1-TAP*	Lab stock
YSK1470	w303a *Δbfa1::HIS3 BFA1-TAP/pRS304 Sic1-3HA-KAN*	This study
YSK1414	*skp1-11 Δbfa1::KAN BFA1-TAP/pRS304 Sic1-3HA-his5*$^+$	This study
YSK1942	*skp1-12 Δbfa1::KAN BFA1-TAP/pRS304 Sic1-3HA- his5*$^+$	This study

HA; hemagglutinin.

Table 2. Oligonucleotides used in this study

KS207	5'TGAATGTACTCAAGATAACGGTAAAGAAACAGTTATAAGAAGGCTAAAGGCTCGTTCAGAATGACACGTATAG 3'
KS209	5'CTTATATACCGCCTCCATCCTCGGTAGGTACTTCTGACACTGGCACGGTAAGCTTGGTGAGCGCTAGGAGTCAC 4'
KS226	5'TGAATGTACTCAAGATAACGGTAAAGAAACAGTTATAAGAAGGCTAAAGGGGAATTCGAGCTCGTTTAAAC 3'
KS284	5'CTTATATACCGCCTCCATCCTCGGTAGGTACTTCTGACACTGGCACGGTACGGATCCCCGGGTTAATTAA 3'
KS327	5' AACGGTACCCGGAGCAAGAGATAGTCTGAG 3
KS363	5'**CCTATATGTATGAAATCAGGAACATGGTAATCAATTCGACAAAAGAT***TCCATGGAAAAGAGAAG* 3' bold; part of genomic *BFA1* sequence, italic; TAP sequence from pBS1479
KS364	5'**GTACTCAAGATAACGGTAAAGAAACAGTTATAAGAAGGCTAAAGGG***TACGACTCACTATAGGG* 3' bold; part of genomic *BFA1* sequence, italic; TAP sequence from pBS1479
KS373	5' CGC**ACTAGT**TCAGGTTGACTTCCCCGCGGA 3'
KS466	5'GAGAAGATTCAAGCCAAAGGCATTGTTTCAATCTAGGGATCAAGAGCATCGGATCCCCGGGTTAATTAA 3'
KS467	5'GTAAAATGTTGATGTTAATGCCCCTTTGCAAATAAATGTAGAATAAGTAAGAATTCGAGCTCGTTTAAAC 3'

KS284 oligonucleotides into YSK193 and YSK194 in order to construct strains YSK475 and YSK476, respectively. *HIS3* DNA fragments amplified from pRS303 with oligonucleotides KS207 and KS209 were used to replace genomic *BFA1* with *HIS3* in strains YSK334, YSK1470, YSK1414, and YSK1942. The strain YSK1370, in which the chromosomal *BFA1* is tagged with *TAP* (protein A-TEV protease site-calmodulin binding peptide), was constructed by transforming PCR amplified TAP (tandem affinity purification) fragments from pBS1479 with KS363 and KS364. *BFA1-TAP* amplified by from YSK1370 with KS327 and KS373 were transformed to construct YSK1470, YSK1414, and YSK1942. The chromosomal *SIC1* was tagged with *3HA* by transforming PCR-amplified *3HA* using pFA6a-3HA- KanMX6 for YSK1470 and pFA6a-3HA-His3MX6 for YSK1414 and YSK1942 as templates.

Protein analysis

Yeast strains were grown to 0.5 at OD_{600} in YPAD. The cells were arrested with 0.2 M hydroxyurea (Sigma) for 4-5 h and released by washing with 2 volumes of dH_2O. The preparations of the yeast cell extract and its immunoblots were performed according to the standard procedures (Lim *et al.*, 2004). PAP (peroxidase anti-peroxidase, Sigma) and anti-HA (Roche) were used in the immunoblots.

Yeast two-hybrid assay

SKP1, *skp1-11* and *skp-12* were subcloned into pJG4-5. The full-length and deletion mutants of *BFA1* were fused to the DNA-binding domain in pGilda. The yeast strain EGY48 was co-transformed with these constructs and the reporter plasmid pSH18-34. A yeast two-hybrid assay was performed as described by Kim *et al.* (Kim *et al.*, 2004). The β-galactosidase activity was assessed for protein-protein interactions

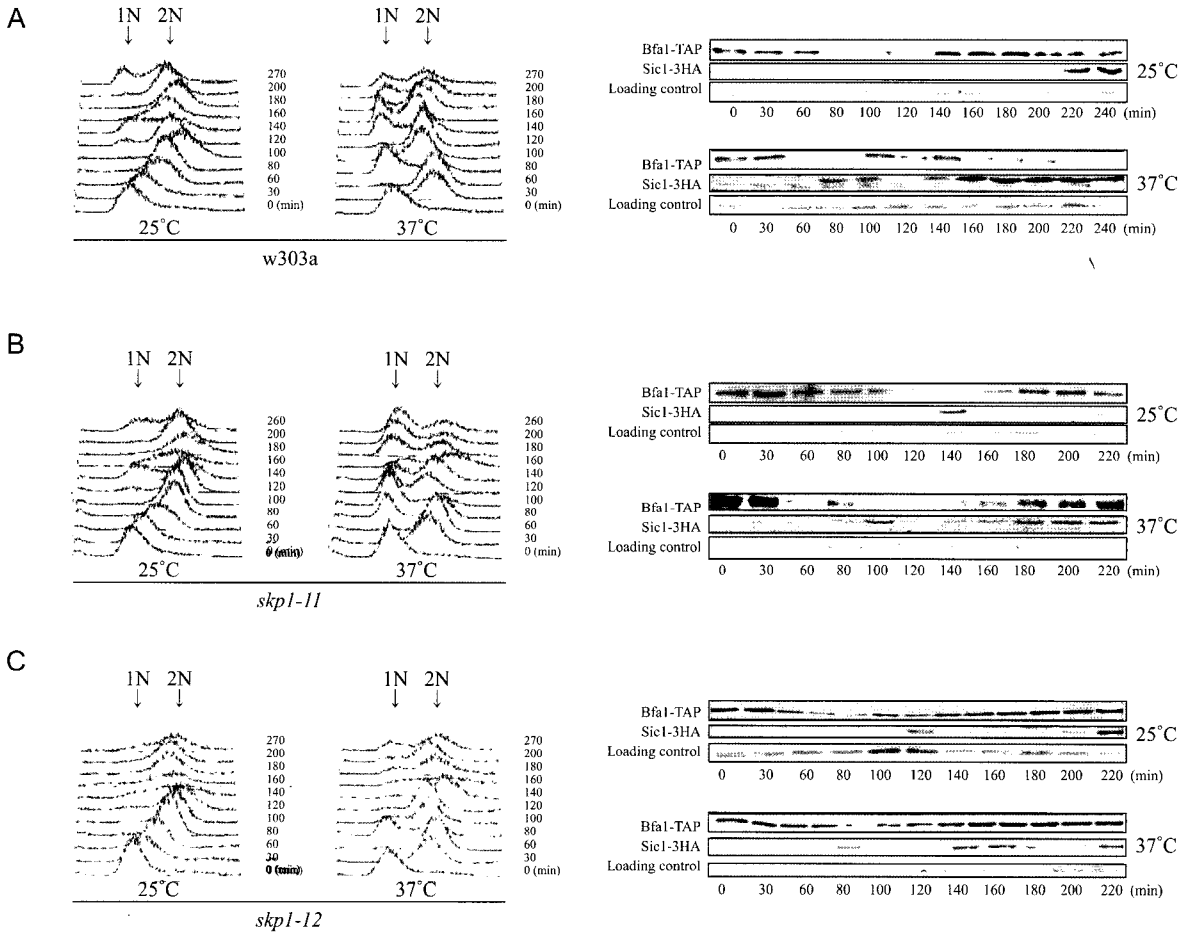


Fig 1. Bfa1 protein profiles during the cell cycle in *skp1* mutants. (A) Wild type (YSK1470), (B) *skp1-11* (YSK1414), and (C) *skp1-12* (YSK1942) cells in which chromosomal *BFA1* and *SIC1* had been respectively tagged with TAP and *3HA* were cultured and arrested with 0.2 M hydroxyurea (HU) for 4 hrs at 25°C. They were then released in YPAD both at the permissive (25°C) and the restrictive (37°C) temperatures and incubated for up to 4 hr. Aliquots of cells were taken at each indicated time point after the release, and their DNA content (FACS) and the protein levels of Bfa1 and Sic1 (western blots using TAP and HA antibodies) were analyzed.

as described by Jwa and Song (Jwa and Song, 1998).

Microscope techniques

The yeast cells were analyzed by fluorescence microscopy after being fixed with 70% EtOH and washed three times with 1X PBS. The DNA was visualized using 1 mg/ml DAPI (4',6'-diamidino-2-phenylindole). Differential interference contrast (DIC) and fluorescence microscopy were performed with a 100×/1.4 oil immersion objective using a PerkinElmer Ultraview spinning disk confocal attached to a Nikon TE2000 inverted microscope.

Flow cytometry

Flow cytometry analyses were carried out using a Becton Dickinson fluorescence-activated cell analyzer as described by Leem (Leem, 1999).

Results and Discussion

Malfunction of Skp1 as an SCF does not change the profile of Bfa1 during the cell cycle

Numerous cell cycle regulatory proteins that exhibit oscillating protein levels during the cell cycle are controlled by ubiquitin-mediated proteolysis. We initially investigated whether the Bfa1 protein is regulated by Skp1 through SCF-mediated degradation based on the physical interaction of Bfa1 with Skp1 in the SCF complex (Lee *et al.*, 2001), and with the changes in the protein level of Bfa1 during the cell cycle. We compared the Bfa1 profiles during the cell cycle in the wild type *SKP1* with those in the temperature-sensitive *skp1-11* and *skp1-12* mutants in order to investigate the Skp1 regulation of Bfa1. Sic1, the mitotic CDK inhibitor that accumulates during mitosis and is degraded by SCF for G1/S transition, was used as a marker for cell cycle progression and SCF function. Therefore, we constructed strains in which the chromosomal *BFA1* was tagged with TAP (protein A-TEV protease site-calmodulin binding peptide) and the *SIC1* was tagged with 3HA in *skp1-11* (YSK1414), *skp1-12* (YSK1942), and the wild type (YSK1470). Each of these strains that grew to mid-log at the permissive temperature was synchronized with hydroxyurea treatment (HU) and released to YPDA. The protein levels of endogenous Bfa1 and Sic1 were then examined during the cell cycle at both the permissive and the restrictive temperatures of *skp1* mutations. Since the cells were arrested with HU, lanes of 0 min indicate S phase proteins.

As previously reported, the levels of Bfa1 fluctuated during the cell cycle in the wild type and in *skp1-11* at the permissive temperature. Bfa1 levels were high during mitosis and decreased at G1 (Fig. 1A and B). Sic1p, used as a marker for SCF function, also demonstrated its usual pattern in both wild type and *skp1-11* at the permissive temperature. Sic1p initially appears during mitosis, shows the highest accumulation at G1 when the level of Bfa1 is the lowest, then disappears during G1/S (Fig. 1A and B). The cell cycle proceeded much faster at 37°C (the restrictive temperature) than at 25°C (the permissive temperature), resulting in similar protein patterns at two different temperatures but with different time courses.

The Bfa1 in the wild type showed the accumulated feature at S phase due to the arrest with HU, and decreased at 120 min of 25°C and 60 min of 37°C (Fig. 1A). The Bfa1 level rose again at 140 min of 25°C and at 100 min of 37°C after Sic1 degradation, indicating that the cells underwent the S phase of the following cell cycle (Fig. 1A). This protein profile of Bfa1 was consistent with the DNA content by FACS (fluorescence acfivated cell sorter) analysis (Fig. 1A).

The cell cycle of *skp1-11* is blocked during the G1 phase at the restrictive temperature of 37°C because this mutant allele of *SKP1* leads to a malfunction in the SCF complex. The quantity of Bfa1 was the highest in the first lane because of the S phase arrest by HU. A similar blot was obtained as in the wild type cells at 25°C. The levels of Bfa1 decreased at around 140 min when Sic1 accumulation was high, indicating the G1 phase of the next cycle (Fig. 1B). While Sic1 remained elevated as time elapsed at 37°C due to the lack of SCF function in *skp1-11*, Bfa1 continued to fluctuate (Fig. 1B). These results demonstrated that the protein level of Bfa1 fluctuates, yet it does not seem to be affected by a defect of Skp1 function as a component of SCF. Because the lack of Skp1 function as a part of SCF in *skp1-11* did not change the protein profile of Bfa1, we wanted to confirm this result by examining the effect of ubiquitin-mediated proteolysis on Bfa1. Thus, we studied the profile of Bfa1 in *rpn9-1* cells that have defects in proteasome function due to the mutation of *RPN9*, a component of the proteasome complex (Takeuchi *et al.*, 1999). However, we could not detect any change in the Bfa1 profile in these cells (data not shown). These observations are consistent with our previous report that Bfa1 mutation on several key residues in its putative F-box motif, which may be essential to interact with Skp1 to be degraded by SCF, does not affect its interaction with Skp1 and its function in mitotic exit and cytokinesis (Lee *et al.*, 2001). In short, the interaction of Bfa1 and Skp1 does not control the profile of Bfa1 during the cell cycle.

Bfa1 profile as well as the DNA content of skp1-12 suggests that a skp1-12 mutant can not exit from mitosis

We also examined the Bfa1 profile during the cell cycle in *skp1-12*. The *skp1-12* allele was previously

reported to be able to function as a component of SCF but not to progress into mitosis (Bai *et al.*, 1996). We observed that Bfa1 levels decreased at 60 min of 25°C and 80 min of 37°C (Fig. 1C). However, the Bfa1 levels remained elevated after the first round of the cell cycle, both at 25°C and 37°C, and its accumulation was more obvious at 37°C (Fig. 1C), suggesting that the cells proceed to the next G1 phase by finishing the first cell cycle but they were unable to finish mitosis from the second round, especially at 37°C. Consistent with this observation, most cells showed 2N DNA content and Sic1 accumulation. Therefore, the *skp1-12* allele is likely to have defects in the process of terminating mitosis rather than in the initiation of mitosis.

C-terminal 184 residues of Bfa1 (Bfa1-D8) is sufficient to interact with Skp1

We then mapped the domain of Bfa1 to bind to Skp1 in an effort to better understand the biological function of the interaction between Bfa1 and Skp1. We examined the physical interaction and its intensity between Skp1 and various Bfa1 mutants with yeast two-hybrid assays using different Bfa1 deletion mutants constructed in our laboratory (Kim *et al.*, 2004, Fig. 2A). Our previous study demonstrated that most Bfa1 functions involved in the spindle orientation checkpoint and mitotic exit are concentrated in 184 amino acids in the C-terminus (Bfa1-D8) (Kim *et al.*, 2004). The capability of Bfa1 to interact with Skp1 was also evident in Bfa1-D8 or other deletion mutants that include D8 such as D1 (Fig. 2A). In addition to the observation that *skp1-12* has defects in mitotic exit, the physical association of Skp1 with Bfa1-D8 that is responsible for Bfa1 function in the mitotic exit, suggests that their interaction may be involved in the control of mitotic exit, as shown in Fig. 1C.

Bfa1 interacts with Skp1-12 more strongly than with the wild type Skp1

We then studied whether the Bfa1-D8 domain that mainly binds to the wild type Skp1 interacts with different affinities to the Skp1 mutant proteins, Skp1-11 and Skp1-12. When the intensity of each interaction

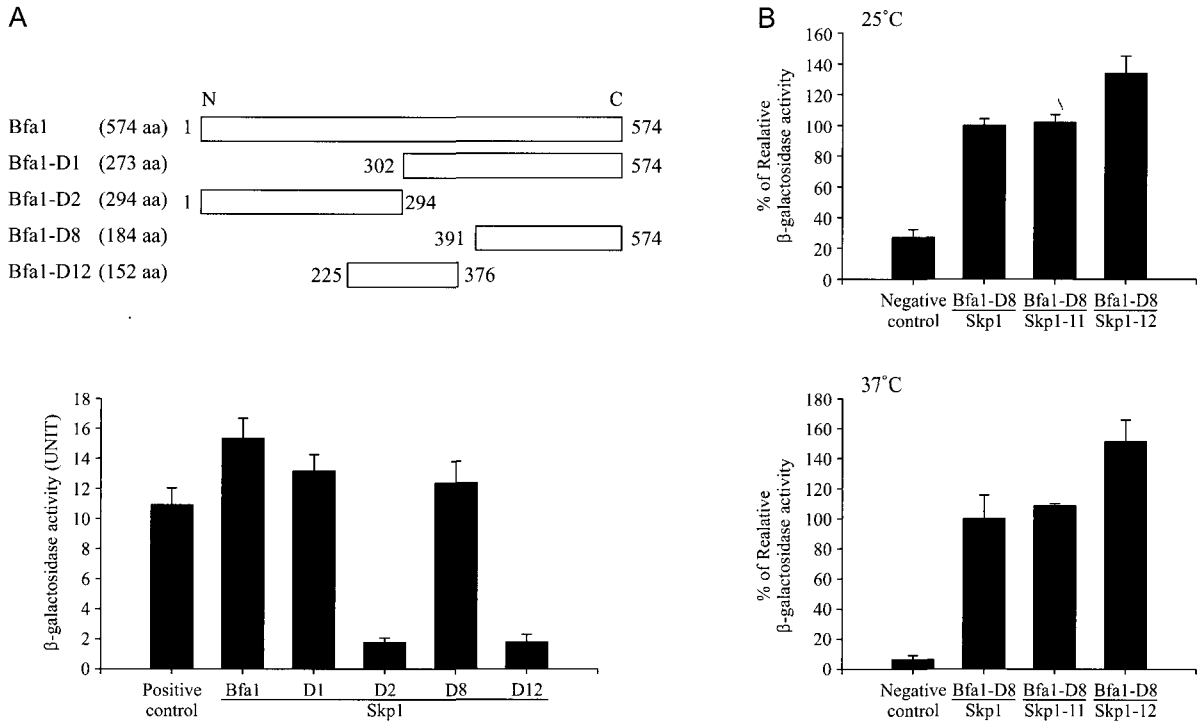
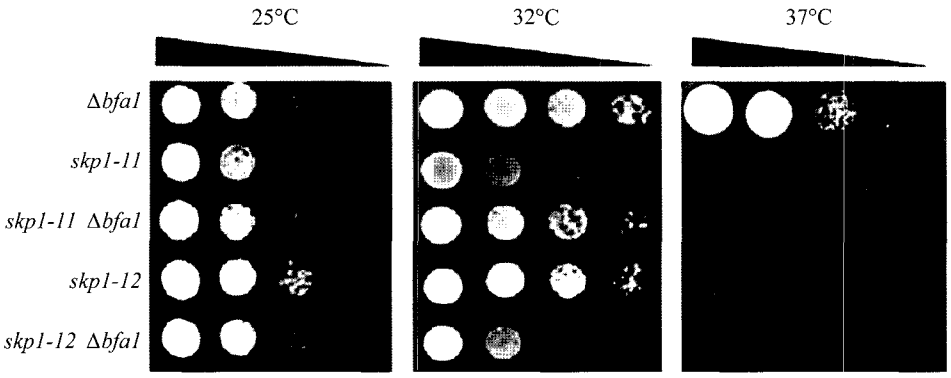


Fig. 2. Increased interaction between Skp1 and Bfa1 in the *skp1-12* mutant. (A) The interaction of Skp1 with several Bfa1 deletion mutants was measured by yeast two hybrid assays using pJG4-5/*SKP1* and each pGilda/*BFA1* deletion mutant shown. The intensity of the interaction was quantified by the β-galactosidase activity with a positive control (pJG4-5/*TEM1* and pGilda/*BFA1*). The β-galactosidase activity was assayed three times for each sample and the average was plotted with the standard deviations. (B) The interaction of Bfa1-D8 with Skp1 mutants was measured by yeast two-hybrid assays at both the permissive (25°C) and the restrictive (37°C) temperatures. *SKP1*, *skp1-11* and *skp-12* subcloned into pJG4-5 and pGilda/*BFA1-D8* were used. Their interactions were quantified by β-galactosidase assays. Bfa1-D2 (pGilda/*BFA1-D2*) and Skp1 (pJG4-5/*SKP1*) were used for negative control. The relative β-galactosidase activity of each interaction of Bfa1-D8 and Skp1 was represented as a percentage. Three independent experiments were performed and the average was plotted with the standard deviations.

A



B

hrs \ Strain / Phenotype(%)	Wild type (w303a)	*skp1-11* (YSK193)	*skp1-12* (YSK194)	*skp1-12 Δbfa1* (YSK334)	*Δbfa1* (YSK476)
0	2.7 ± 0.8	5.5 ± 0.5	6.3 ± 2.3	4.5 ± 1.3	1.1 ± 1.2
3	1.7 ± 0.3	3.3 ± 2.6	3.5 ± 2.6	4.3 ± 0.6	1.8 ± 1.0
6	2.7 ± 1.9	4.7 ± 0.3	4.7 ± 0.3	8.2 ± 1.6	0.5 ± 0.5
9	0.0 ± 0.0	2.8 ± 1.3	2.8 ± 1.3	2.8 ± 1.9	0.0 ± 0.0
0	17.1 ± 2.2	32.7 ± 4.3	32.7 ± 4.3	22.5 ± 4.5	9.9 ± 0.1
3	7.8 ± 0.3	18.7 ± 3.6	18.7 ± 3.6	26.3 ± 2.3	15.3 ± 1.1
6	9.5 ± 2.2	38.2 ± 3.5	38.2 ± 3.5	36.0 ± 1.8	19.2 ± 1.8
9	7.3 ± 0.8	39.7 ± 2.9	39.7 ± 2.9	54.2 ± 4.1	12.5 ± 2.6

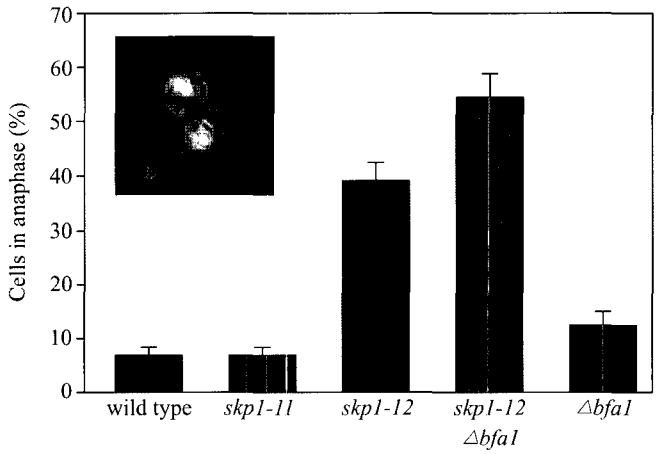


Fig. 3. Specific genetic interaction of *Δbfa1* with *skp1-12*. (A) Cells of *Δbfa1* (YSK334), *skp1-11* (YSK193), *skp1-11Δbfa1* (YSK475), *skp1-12* (YSK194), and *skp1-12Δbfa1* (YSK 476) were serially diluted by tenfold, spotted onto YPAD plates, and incubated respectively at 25°C, 32°C and 37°C for 3 days. (B) Wild type cells (W303), *Δbfa1* (YSK334), *skp1-11* (YSK193), *skp1-12* (YSK194), and *skp1-12Δbfa1* (YSK476) were cultured to mid-log at 25°C, shifted to 37°C, and incubated for up to 9 h. (Table) After being shifted to incubation at 37°C, the cells were taken at every 3 h, stained with DAPI, and their phenotypes were analyzed. A total of 200 cells from each strain were counted at each time point and sorted according to their phenotypes. Three independent experiments were performed and the average percentage of cells with the indicated phenotypes (nucleus in the bud neck or cells in late anaphase) was demonstrated with standard deviations. (Graph) The percentage of cells in late anaphase at 9 h was plotted from the table.

was measured by yeast two-hybrid β-galactosidase assays both at 25°C and 37°C, we observed that Bfa1-D8 has a stronger interaction with the Skp1-12 mutant protein than with the wild type Skp1 or Skp1-11 (Fig. 2B). Also, the interaction of Bfa1-D8 with Skp1-12 was further increased at 37°C and was about 50% stronger than the interaction with the wild type (Fig. 2B).

BFA1 shows the specific genetic interaction with skp1-12

We then examined the genetic interaction between *BFA1* and *skp1-12* in order to understand whether the increased physical interaction between Bfa1 and Skp1-12 has any meaning in their biological function. We constructed a Δ*bfa1skp1-12* double mutant (YSK476) and compared its lethality with *skp1-12* (YSK194) and Δ*bfa1* (YSK334). The double mutation of Δ*bfa1* and *skp1-12* showed synthetic lethality and led to cell death, even at a lower temperature (32°C) than the restrictive temperature 37°C (Fig. 3A). We also constructed a Δ*bfa1skp1-11* double mutant (YSK475) and examined its synthetic lethality to verify whether the genetic interaction between Δ*bfa1* and *skp1-12* is *skp1-12* allele-specific. However, we could not detect any difference in the viability between *skp1-11* (YSK193) and Δ*bfa1skp1-11* (Fig. 3A), which demonstrated that *BFA1* shows a specific genetic interaction with *skp1-12*.

skp1-12 cells have defect in mitotic exit and cytokinesis

Our results thus far strongly suggest that the *skp1-12* allele of *SKP1* has defects in mitotic exit and that the genetic and physical interaction between Bfa1 and Skp1-12 may be involved in the regulation of mitotic exit. We examined the phenotypes of *skp1-12* (YSK194) and Δ*bfa1skp1-12* (YSK476) after incubation at the restrictive temperature of 37°C for up to 9 h in order to verify the defects of *skp1-12* in mitotic exit. We also compared the phenotypes of the *skp1-12* and Δ*bfa1skp1-12* mutants with those of the wild type, Δ *bfa1* (YSK334), and *skp1-11* (YSK 193) cells after incubation at 37°C for up to 9 h. The number of cells in early mitosis (nucleus positioned in the bud neck with large sized bud) and in late anaphase (divided each nucleus into two daughter cells without cytokinesis) was then scored in each strain. Only in *skp1-12* and Δ*bfa1skp1-12*, were the cells in late anaphase highly increased and further augmented with prolonged incubation at 37°C (Fig. 3B). In contrast, the number of cells in early mitosis did not vary among these strains (Fig. 3B). These results strongly support that *skp1-12* has defects in mitotic exit, which differs from the original report that it showed the defect in G2/M transition.

Interaction of Skp1 and Bfa1 may contribute to Skp1 function in mitotic exit

This study was based on our previous result that revealed the physical interaction between Bfa1 and Skp1 (Lee *et al.*, 2001). Since the well-known function of *SKP1* lies on the component of E3 ubiquitin ligase, the SCF complex, we initially tried to find the biological meaning of this interaction in the ubiquitin-mediated proteolysis of Bfa1 during the cell cycle. However, in the present study we showed that the Bfa1 protein profile did not change in the *skp1-11* mutant allele that loses Skp1 function as the SCF complex for G1/S transition. These results indicate that Bfa1 protein levels are independent of SCF-mediated proteolysis, although its protein level fluctuates in a cell cycle-dependent manner.

On the other hand, we recently found that the *skp1-12* allele of *SKP1* has defects in the exit from mitosis. We also observed the specific genetic interaction of *skp1-12* with Δ*bfa1* and the increased physical interaction between Bfa1 and Skp1-12. These observations altogether demonstrate the novel function of Skp1 in mitotic exit and suggest that the interaction of Skp1 and Bfa1 may contribute to the function of Skp1 in mitotic exit. The novel role of Skp1 in mitotic exit described in this paper is consistent with the recent report that CBF3, which is controlled by Skp1, regulates septin dynamics and cytokinesis at the end of mitosis, independent of its kinetochore function in chromosome segregation (Gillis *et al.*, 2005). Further studies investigating the mechanism underlying Skp1 interaction with Bfa1 and the coordination of the mitotic exit to cytokinesis and septin dynamics are necessary to understand the function of Skp1 in the mitotic exit.

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