The Journal of Microbiology, December 2001, p.286-292
Vol. 39, No. 4
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Characterization of a Putative F-box Motif in Ibd1p/Bfa1p, a Spindle Checkpoint Regulator of Budding Yeast *Saccharomyces cerevisiae*

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(Received August 20, 2001 / Accepted November 20, 2001)

During mitosis, the proper segregation of duplicated chromosomes is coordinated by a spindle checkpoint. The bifurcated spindle checkpoint blocks cell cycle progression at metaphase by monitoring unattached kinetochores and inhibits mitotic exit in response to the misorientation of the mitotic spindle. Ibd1p/Bfa1p is a spindle checkpoint regulator of budding yeast in the Bub2p checkpoint pathway for mitotic exit and its disruption abolishes mitotic arrest when proper organization of the mitotic spindle is inhibited. Ibd1p/Bfa1p localizes to the spindle pole body, a microtubule-organizing center in yeast, and its overexpression arrests the cell cycle in 80% of cells with an enlarged bud at mitosis and in 20% of cells with multiple buds. In this study, we found that the C-terminus of Ibd1p/Bfa1p physically interacts with Skp1p, a key component of SCF (Skp1/cullin/F-box) complex for ubiquitin-mediated proteolysis of cell cycle regulators as well as an evolutionally conserved kinetochore protein for cell cycle progression. A putative F-box motif was found in the C-terminus of Ibd1p/Bfa1p and its function was investigated by making mutants of conserved residues in the motif. These Ibd1p/Bfa1p mutants of a putative F-box interacted with Skp1p *in vitro* by two-hybrid assays as wild type Ibd1p/Bfa1p. Also, these Ibd1p/Bfa1p mutants displayed the overexpression phenotypes of wild type Ibd1p, when overexpressed under inducible promoters. These results suggest that a putative F-box motif of Ibd1p/Bfa1p is not essential for the interaction with Skp1p and its function in mitotic exit and cytokinesis.

Key words: mitosis, spindle checkpoint, IBD1/BFA1, SKP1, F-box motif, SCF (Skp1/cullin/F-box protein) complex, *S. cerevisiae*

Cells ensure the fidelity of each division through checkpoints that control cell cycle progressions by monitoring the successful completion of preceding processes. During mitosis, the spindle checkpoint monitors the assembly and the orientation of the mitotic spindle for equal segregation of replicated chromosomes (37). By sensing defects of the microtubule cytoskeleton, the spindle checkpoint arrests cells at metaphase and prevents the exit from mitosis through the regulation of Cdk activity (37). Genetic studies in budding yeast have identified several components of the spindle checkpoint by isolating mutants that could no longer sense spindle depolymerization and died rapidly in the presence of microtubule-depolymerizing drugs such as nocodazole or benomyl (19, 27). These components include *MAD1, MAD2, MAD3, BUB1, BUB2,* and *BUB3.* It has been suggested that the spindle checkpoint bifurcates into two separate signaling pathways, the *MAD/BUB* spindle assembly checkpoint for metaphase arrest and the *BUB2*-dependent pathway to block mitotic exit and cytokinesis (1, 26).

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Bub1p, Bub3p, Mad1p, Mad2p, and Mad3p form a conserved spindle assembly checkpoint that monitors the attachment of bipolar microtubules to the kinetochores of sister chromatids, and delays the metaphase-to-anaphase transition in response to spindle assembly defects by inhibiting Cdc20p, a key component of the anaphase-promoting complex (APC) (10, 20). Homologues of yeast Mad1p, Mad2p, Bub1p, and Bub3p have also been identified and localized to unattached kinetochores in higher eukaryotes including mammalian cells (6, 7, 29, 35, 36), suggesting that the mechanisms of the spindle assembly checkpoint are conserved in eukaryotes (39).

Bub2p is present in the spindle pole body and forms a separate branch of the spindle checkpoint pathway to control mitotic exit and timing of cytokinesis (12, 13, 26). In fission yeast, Cdc16p, a Bub2p homologue, interacts with Byr4p to coordinate the onset of cytokinesis (15, 21). *IBD1/BFA1* was identified as a putative homologue of *byr4* in budding yeast and has been reported to function in the *BUB2*-dependent spindle checkpoint for mitotic exit and cytokinesis (1, 25, 26). Recently, the Bub2p checkpoint including Bub2p, Ibd1p/Bfa1p, and Tem1p has been

shown to monitor the anaphase spindle orientation and to inhibit mitotic exit and cytokinesis when nuclear migration into the bud is delayed (3, 4, 32).

SKP1 encodes a conserved kinetochore protein that is essential for both G1-S and G2-M transitions (8). Also, Skp1p forms a component of the SCF complex that functions as an E3 ubiquitin ligase for ubiquitin-mediated proteolysis of cell cycle regulators including Sic1p at the G1-S transition of the cell cycle (11). SCF complex is composed of the subunits Skp1p, Rbx1p, Cdc53, and any one of a large number of different F-box proteins (2, 11, 22, 28, 30, 33). The substrate specificity of 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 Skp1p, Rbx1p, Cdc53 and the E2 enzyme Cdc34 (23, 31, 34, 38).

In this study, we found that the fully functional C-terminus of Ibd1p/Bfa1p physically interacts with Skp1p, a key component of the SCF (Skp1/cullin/F-box) complex as well as an evolutionally conserved kinetochore protein for cell cycle progression. Interaction of Ibd1p/Bfa1p with Skp1p led us to investigate the presence of a putative F-box in Ibd1p/Bfa1p. A putative F-box motif was found in the C-terminus of Ibd1p/Bfa1p and its function was investigated by making mutants of conserved residues in the motif by site-directed mutagenesis.

Materials and Methods

Strains and culture conditions

All *S. cerevisiae* strains used in this study are listed in Table 1. Yeast cells were grown in YPD medium (1% yeast extract, 2% bactopeptone and 2% glucose) or in a synthetic complete (SC) drop-out media prepared with minimal medium (YNB) and necessary supplements. To induce expressions from *GAL* promoter, cells grown to mid log phase in 2% glucose were transferred to SC drop-out media with raffinose for 4 h. Then, 2% galactose was added to this culture and cells were incubated in 2% raffinose/galactose for 8-14 h at 29°C.

Yeast two-hybrid assay

Yeast two-hybrid screen was performed by following Gyuris *et al.* (17). The *IBD1-D1* that encodes the 279 C-terminal amino acids of Ibd1p was subcloned into pLex202+PL (pLexA202+PL/*IBD1-D1*) and was used as bait for the screen. For an interactor hunt, EGY48 with pLexA202+PL/*IBD1-D1* was transformed with the cDNA library in pJG4-5 where the expression of each cloned cDNA was under the control of *GAL1* inducible promoter. A total of 50,000 colonies were screened. Positive interaction was verified by leucine assay in EGY48 where chromosomal *LEU2* was replaced by LexAop6-*LEU2*. True positives only grew on SC-leu drop-out media in the presence of galactose/raffinose but did not with glucose, since the cDNA is only expressed with galactose/raffinose.

Table 1. The *S.cerevisiae* strains used in this study

Strains	Genotypes
EGY48	MATa LEU::LexAop6-LEU2 ura3 his3 trp1 GAL^+
W303	MATa ura3-1 trp1-1 ade2-1 leu2-3112 his3-11.15
YSK8	*MATa IBD1::His3 ura3-1 trp1-1 ade2-1 leu2-3112 his3-11.15*

Co-precipitation and immunoblotting

To detect the co-precipitation of Ibd1p and Skp1p, EGY48 cells transformed with *IBD1*-GST and *SKP1*-HA were grown to 5×10^6 cells/ml and proteins were extracted in H-buffer (25 mM Tris-HCl pH 7.4, 15 mM EGTA pH 7.5, 15 mM $MgCl_2$, 0.1% Triton X-100, 10% glycerol, 1 mM NaN_3, 0.6 mM odium vanadate pH 7.0, 1X protease cocktail in DMSO (Boehringer Mannheim Co.), 1 mM DTT, 5 mg/ml PMSF) by beadbeating. Cell lysate was incubated with GST-bead (Sigma) for 2 hr in 4°C and the GST-bead was washed twice with H-buffer. SDS sample buffer (6X) was added to the bead, the bead was boiled for 5min, and the proteins were resolved on a 10% SDS-PAGE. The resolved proteins were transferred onto nitrocellulose membranes and incubated with either monoclonal mouse anti-GST antibody or polyclonal mouse anti-HA antibody (Upstate Lab.). Bound antibodies were detected with anti-mouse IgG-HRP (Jackson Immunochemicals) and enhanced chemiluminescence (ECL) reagents.

Construction of mutants in a putative F-box of Ibd1p

Mutagenic primers were phosphorylated at the 5' ends by T4 polynucleotide kinase at 37°C for 60 min and were stopped by heating at 65°C for 10 min. Using pLexA202 +PL/*IBD1-D1*, pMW20/*IBD1*, pMW20/*IBD1-D1,* and pMW20/*IBD1-D8* as respective templates, each mutated Ibd1p strand in a putative F-box was constructed by PCR with phosphorylated mutagenic primers and *Pfu* DNA polymerase (Stratagene). Then, the non-mutated DNA strands of the template were digested with *DpnI* at 37°C for 1 h and transformed into *DH5α*. Mutagenic primers used are listed in Table 2. The 441-444 mutation was labeled as M441, the 468-469 mutation as M468, and both 441-444 and 468-469 mutations as DM (double mutant). M441 mutant was constructed with 5' oligonucleotide KS173 and 3' oligonucleotide KS174, M468 mutant with 5' oligonucleotide KS175 and 3' oligonucleotide KS176. The DM mutant was made using M441 as a template for PCR with 5' oligonucleotide KS175 and 3' oligonucleotide KS176. Mutants constructed in each vector were verified by DNA sequence analysis.

Microscopic techniques

S. cerevisiae cells harvested by centrifugation were fixed

Table 2. Oligonucleotides used in this study

Oligonucleotides	Sequences	Usage
KS173	5'attcctgagataaacgatggaggagatggcaaaagtatgaaaaaacgtagc3'	5' of M441 & DM
KS174	5'gctacgttttttcatacttttgccatctcctccatcgtttatctcaggaat3'	3' of M441 & DM
KS175	5'gccaagttaatacgccatttgacgctaatgacaatgatggcgtttatc3'	5' of M468 & DM
KS176	5'gataaacgccatcattgtcattagcgtcaaatggcgtattaacttggc3'	3' of M468 & DM

for 1 min by adding 1 ml 70% ethanol, washed three times and resuspended in PBS. Fixed cells were stained with 1 μg/ml 4',6-diamidino-2 phenylindole (DAPI) (Sigma), and observed with Fluorescence Leica DMR microscopy using an 100 × objective. Photographs were taken using a Leica DC200 and were scanned by a Leica DC viewer.

Results and Discussion

Interaction of the C-terminus of Ibdlp/Bfalp with Skplp

The C-terminus of Ibd1p/Bfa1p for 279 amino acids was enough to induce overexpression phenotypes of Ibd1p/Bfa1p as well as the localization and functions of Ibd1p/Bfa1p, and was named Ibd1-D1p (25). *IBD1-D1* was subcloned into pLexA202 + PL to be used as bait for a yeast two-hybrid screen (pLexA202 + PL/*IBD1-D1*). For the interactor hunt, EGY48 with pLexA202 + PL/*IBD1-D1* was transformed with the cDNA library in pJG4-5 where the expression of each cloned cDNA was under the control of *GAL1* inducible promotor. A total of 50,000 colonies were screened, and five colonies for real positive interactions and three false positives were identified. Two colonies among five real positives contained partial cDNA of *SKP1* to encode an interacting protein. The full ORF of *SKP1* was subcloned into pJG4-5 and two-hybrid assay was performed with *IBD1-D1* to verify the interaction between Skp1p and the C-terminus of Ibd1p/Bfa1p (Fig. 1A). True positive interaction was verified by leucine assay in EGY48 where chromosomal *LEU2* is replaced by LexAop6-*LEU2*. Skp1p showed a positive interaction only with Ibd1-D1p on galactose/raffinose but not on glucose, indicating the real positive interaction (Fig. 1A). The direct interaction of Skp1p and Ibd1-D1p was further confirmed by co-immunoprecipitation. GST-tagged Ibd1-D1p and HA-tagged Skp1p was co-expressed and purified with anti-GST antibody. The HA-tagged Skp1p was co-purified with GST-tagged Ibd1-D1p and detected with anti-HA antibody as shown in Fig. 1B.

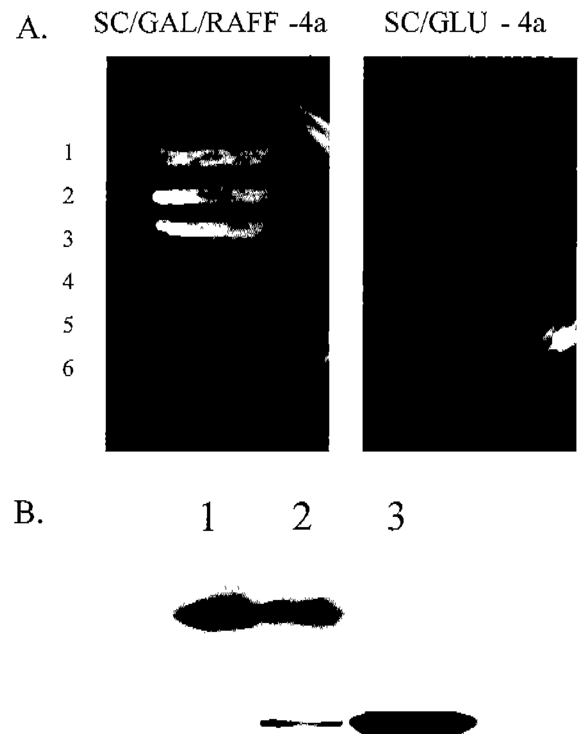
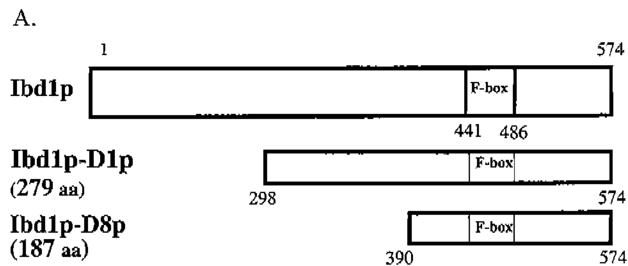


Fig. 1. Interaction of the C-terminus of Ibd1p/Bfa1p with Skp1p. (A) For a yeast two-hybrid assay, Skp1p fused to Gal4 activation domain in pJG4-5 and Ibd1-D1p fused to LexA DNA-binding domain in pLexA2-2+PL were co-transformed into EGY48. The left panel shows the interactions on galactose/raffinose and the right panel on glucose. Lane 1 for false positive (isolated in this screen), Lanes 2, 3 for Skp1p and the C-terminus of Ibd1p co-expressed, and Lanes 4, 5, 6 for negative control (N-terminal bicoid fused LexA and pJG4-5 vector only transformed cells provided by the system). Skp1p demonstrated only a positive interaction with Ibd1-D1p on galactose/raffinose but not on glucose. (B) Skp1 fused to HA domain in pJG4-5 and Ibd1-D1p fused to GST in pEMBL were co-expressed and co-precipitated. Lane 1 for the cells expressing only GST-fused Ibd1-D1p, Lane 3 for the cells expressing only HA-tagged Skp1p, and Lane 2 for the cells co-expressing GST-fused Ibd1p and HA-tagged Skp1p. The HA-tagged Skp1p was co-purified with GST-tagged Ibd1-D1p and detected with anti-HA antibody.

Search for a putative F-box motif of Ibdlp/Bfalp

The direct interaction of Ibd1-D1p with Skp1p led us to investigate the presence of a putative F-box in the C-terminus of Ibd1p, since Skp1p interacts with various proteins containing an F-box motif to recognize and degrade targets by SCF complex (9). A putative F-box motif of Ibd1p/Bfa1p was searched by comparing F-box consensus sequences with the C-terminal 275 amino acids of Ibd1p/Bfa1p and was found between the amino acid 441-485 of Ibd1p/Bfa1p (24). LPPV starting at amino acid 441 and VS of amino acids 468-469 were the most conserved residues in this putative F-box motif (Fig. 2B). The function of this putative F-box of Ibd1p was studied by mutating the essential residues in the motif. The mutants were con-

A.



B.

F-box motif Of Ibd1p ^{441}LPPVGKSMKKRSSSPFLRSKSQVNTPF^{468}VSNDNDGV YQSTAAQARL^{485}

F-box Consensus kPFPLLRLPeEILrkILekLDPiDLLrLRKVSKKWRsLVDslniw fkfIe

C.

Mutagenesis in a putative F-box of Ibd1p

1. Single mutant: ^{441}LPPV→ ^{441}DGGD (M441)
   ^{468}VS → ^{468}DA (M468)

2. Double mutant: ^{441}LPPV & ^{468}VS
   → ^{441}DGGD & ^{468}DA (DM)

Fig. 2. Structure and mutagenesis of a putative F-box motif of Ibd1p/Bfa1p. (A) Schematic illustration of Ibd1p. Full-length Ibd1p, and the C-terminal deletion mutants of Ibd1p, Ibd1-D1p and Ibd1-D8p, were illustrated including a putative F-box. (B) A putative F-box sequence of Ibd1p. A putative F-box of Ibd1p was found between amino acids 441-485 of Ibd1p by comparing with the consensus sequences of the F-box (24). In the F-box consensus sequences shown, the residues conserved in over 40% of known F-box proteins are represented as underlined capital letters, in 20-40% of known F-box proteins as non-underlined capital letters, and in 10-14% as lower case letters (10). ^{441}LPPV and ^{468}VS of Ibd1p are the most conserved residues in a putative F-box of Ibd1p. (C) Construction of Ibd1p mutants in the conserved residues of a putative F-box. The most conserved residues of a putative F-box motif of Ibd1p was mutated by site-directed mutagenesis. ^{441}LPPV were mutated to ^{441}DGGD, and ^{468}VS were mutated to ^{468}DA. ^{441}DGGD mutation was labeled as M441, the ^{468}DA mutation as M468, and both ^{441}DGGD and ^{468}DA mutations as DM.

structed in pLexA202 + PL/*IBD1-D1* to verify the interaction between Ibd1-D1p mutants and Skp1p. The mutants were also constructed in the full-length *IBD1*, *IBD1-D1*, and *IBD1-D8* under inducible *GAL1* promoter to check the overexpression phenotypes of these mutants (Fig. 2A). Since other studies in the lab showed that *IBD1-D8* containing this putative F-box motif is an essential functional domain of Ibd1p including the overexpression phenotypes and checkpoint control (J. Kim and K. Song, unpublished data), mutants were also constructed in *IBD1-D8*. ^{441}LPPV were mutated to ^{441}DGGD, and ^{468}VS were mutated to ^{468}DA by site-directed mutagenesis, as described in Materials and Methods. The ^{441}DGGD mutation was named M441, the ^{468}DA mutation M468, and both ^{441}DGGD and ^{468}DA mutations as double mutants (DM) (Fig. 2C).

Fig. 3. Interaction of Skp1p and the Ibd1p/Bfa1p mutants in a putative F-box. By a yeast two-hybrid assay, Skp1p fused to Gal4 activation domain in pJG4-5 and the Ibd1-D1p mutants in a putative F-box, M441-D1 and DM-D1, respectively fused to LexA DNA-binding domain in pLexA202+PL were co-transformed into EGY48. The left panel shows the interactions on galactose/raffinose and the right panel on glucose. Lane 1 for negative control, Lane 2 for M441-D1, Lane 3 for DM-D1, and Lane 4 for wild type Ibd1-D1p as a positive control.

Characterization of the Ibd1p/Bfa1p mutants of a putative F-box with Skp1p

The direct interactions of Skp1p and the Ibd1p/Bfa1p mutants of conserved residues in a putative F-box were analyzed by yeast two-hybrid assays. As shown in Fig. 3, EGY48 containing *SKP1* and the mutants M441 or DM grew on a galactose/raffinose plate but not on a glucose plate as EGY48 containing *SKP1* and *IBD1-D1*, demonstrating that the Ibd1p mutants of conserved residues in a putative F-box can directly interact with Skp1p regardless of the mutations. These results suggested that the putative F-box of Ibd1p/Bfa1p does not function as a real F-box for the interaction with Skp1p, since F-box proteins interact with Skp1 mainly through the F-box and mutations of the F-box lose the ability to interact with Skp1p (9).

To learn the physiological effects of the mutations of conserved residues in a putative F-box, the overexpression phenotypes of these mutations in the full-length *IBD1*, *IBD1-D1*, and *IBD1-D8* were compared with those of each wild type. As described, *IBD1-D1* showed the full functions of *IBD1* (25) and encodes for the 275 C-terminal amino acids that interact with Skp1p. *IBD1-D8* containing this putative F-box motif was elucidated as a minimal domain for the essential functions of Ibd1p/Bfa1p including overexpression phenotypes, localization, and spindle checkpoint control (J. Kim and K. Song, unpublished data). When the mutants of a putative F-box in *IBD1*, *IBD1-D1*, and *IBD1-D8* were overexpressed under *GAL1* promoter, they all showed elongated and multi-budded phenotypes just as the wild type (Fig. 4, data not shown). These results suggest that the mutated residues in a putative F-box are not essential for the function of Ibd1p/Bfa1p in mitotic exit and cytokinesis.

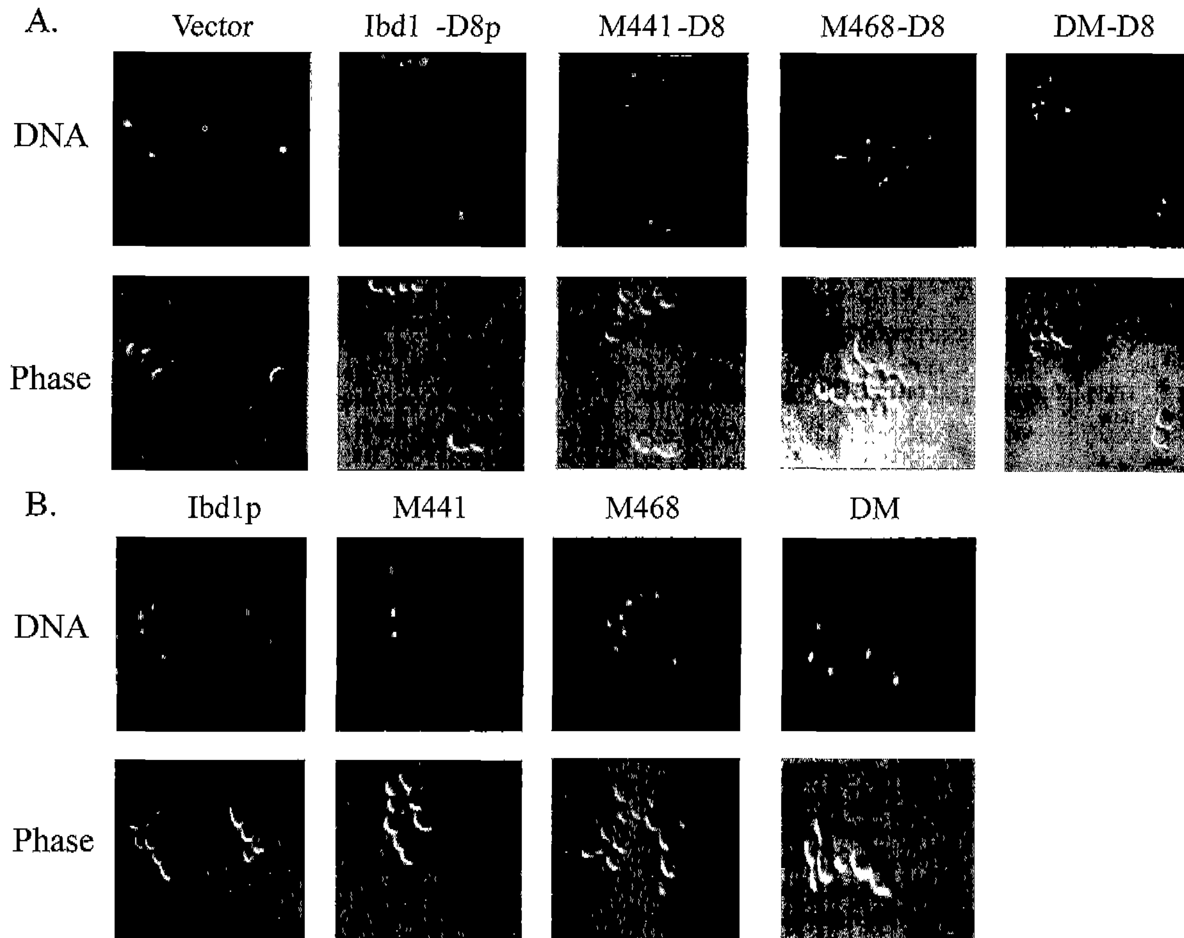


Fig. 4. Physiological effects of the Ibd1p mutations in a putative F-box. (A) The overexpression phenotypes of the Ibd1-D8p mutants in a putative F-box. The mutants and wild type Ibd1-D8p were respectively overexpressed in pMW20 where the cloned gene was induced under *GAL1* promoter. The cells overexpressed respectively with the wild type *IBD1-D8* for Ibd1-D8, M441 *mutant* of *IBD1-D8* for M441-D8, M468 mutant *of IBD1-D8* for M468-D8, and the M441 and M468 double mutant of *IBD1-D8* for DM, and with only pMW20 for negative control. Bar denotes 10 μm. (B) The overexpression phenotypes of the full-length Ibd1p mutants of a putative F-box. The full-length mutants and wild type Ibd1p were respectively overexpressed in pMW20 where the cloned gene was induced under *GAL1* promoter. The cells overexpressed respectively with the wild type *IBD1* for Ibd1p, M441 mutant of *IBD1* for M441-D8, M468 mutant of *IBD1* for M468-D8 for, and the M441 and M468 double mutant of *IBD1* for DM. For both (A) and (B), the top panels show the nuclear pattern by DAPI staining and the bottom panels show phase contrast for cell shape. Bar, 10 μm.

Possible functions of Ibd1p/Bfa1p and Skp1p interaction

Our data showed that the spindle checkpoint component Ibd1p/Bfa1p directly interacts with Skp1p but a putative F-box motif in Ibd1p is not essential for its interaction with Skp1p as well as for the regulation of mitotic exit and cytokinesis. These results suggest that a putative F-box found in Ibd1p does not function as a real F-box to regulate the cell cycle. What then is the function of the Ibd1p and Skp1 interaction in regulating the cell cycle? Apart from the function as a component of SCF complex, *SKP1* encodes a serine/thronine kinase and a conserved kinetochore protein that is essential for both the G1-S and G2-M cell cycle transitions (8). The analysis of kinetochore mutants in *S. cerevisiae* suggested that the kinetochore plays a key role in correct chromosome segregation and in checkpoint activity (16). The core of the kinetochore in yeast forms a protein complex called CBF3 that contains four essential proteins, Ndc10p, Cep3p, Ctf13p, and Skp1p, and binds to a conserved centromere DNA element (CDEIII) (24). In a recent study, Mad2p in the bifurcated spindle assembly checkpoint was shown to be associated with the kinetochore and this association is CDEIII-dependent (12). One possibility is that the interaction of Skp1 in the kinetochore complex with Ibd1p/Bfa1p in the spindle checkpoint pathway for mitotic exit and cytokinesis might transmit a signal to the Bub2p spindle checkpoint pathway to block mitotic exit and cytokinesis when there is a defect in the attachment of microtubules to kinetochores. The studies in mammalians and *Xenopus* also suggest that Skp1p localizes not only in the nucleus but also in the centrosome, and regulates the centrosome duplication cycle and its proper separation for

mitosis (14). The Bub2-dependent spindle checkpoint recognizes spindle orientation defects in order to block mitotic exit and cytokinesis, and components of the Bub2-dependent spindle checkpoint including Ibd1p/Bfa1p are localized in the spindle pole body, the microtubule-organizing center in yeast (32). Based on the localizations and functions of the Ibd1p/Bfa1p pathway and Skp1p, we could assume another possible function of the Skp1p and Ibd1p interaction apart from the function of the F-box. To verify this possibility, the interaction of Skp1 and an Ibd1p deletion mutant that does not contain the C-terminal putative F-box motif is under investigation. Since Skp1p is required for the correct segregation of the microtubule-organizing center that directly determines the mitotic spindle orientation, a signal indicating that the duplicated microtubule-organizing centers are properly separated for proper mitotic spindle formation could be transmitted to the Bub2p pathway through the direct interaction of Skp1p with Ibd1p/Bfa1p, not to block mitotic exit and cytokinesis and to proceed with the cell cycle. Further investigation of the relationships between Skp1p and the Bub2p-dependent spindle checkpoint components including Ibd1p/Bfa1p will give clues for the possible functions of the interaction of Skp1 and Ibd1p/Bfa1p in cell cycle regulation.

Acknowledgment

This work was supported by a grant from the Korean Science & Engineering Foundation [KOSEF 1999-2-207-003-3] given to K. Song.

References

1. Alexandru, G., W. Zachariae, A. Schleiffer, and K. Nasmyth. 1999 Sister chromatid separation and chromosome re-duplication are regulated by different mechanisms in response to spindle damage. *EMBO J.* 18, 2707-2721.
2. Bai, C., P. Sen, K. Hofmann, L. Ma, M. Goebl, J.W. Harper, and S.J. Elledge. 1996. *SKP1* connects cell cycle regulators to the ubiquitin proteolysis machinery through a novel motif, the F-box. *Cell* 86, 263-274.
3. Bardin, A.J., R. Visintin, and A. Amon. 2000. A mechanism for coupling exit from mitosis to partitioning of the nucleus. *Cell* 102, 21-31.
4. Bloecher, A., G.M. Venturi, and K. Tatchell, 2000. Anaphase spindle position is monitored by the *BUB2* checkpoint. *Nat. Cell Biol.* 2, 556-558.
5. Burke, D.J. 2000. Complexity in the spindle checkpoint. *Curr. Opin. Genet. Dev.* 10, 26-31.
6. Chen, R.H., A. Shevchenko, M. Mann, and A.W. Murray. 1998. Spindle checkpoint protein Xmad1 recruits Xmad2 to unattached kinetochores. *J. Cell Biol.* 143, 283-295.
7. Chen, R.H., J.C. Waters, E.D. Salmon, and A.W. Murray. 1996. Association of spindle assembly checkpoint component XMAD2 with unattached kinetochores. *Science* 274, 242-246.
8. Connelly, C. and P. Hieter. 1996. Budding yeast *SKP1* encodes an evolutionarily conserved kinetochore protein required for cell cycle progression. *Cell* 86, 275-285.
9. Craig, K.L. and M. Tyers. 1999. The F-box: a new motif for ubiquitin dependent proteolysis in cell cycle regulation and signal transduction. *Prog. Biophys. Mol. Biol.* 72, 299-328.
10. Fang, G., H. Yu, and M.W. Kirschner. 1998. Direct binding of CDC20 protein family members activates the anaphase-promoting complex in mitosis and G1. *Mol. Cell* 2, 163-171.
11. Feldman, R.M., C.C. Corell, K.B. Kaplan, and R.J. Deshaies. 1997. A complex of Cdc4, Skp1, and Cdc53/Cullin catalyze ubiquitination of the phosphorylated CDK inhibitor Sic1. *Cell* 91, 221-230.
12. Fesquet, D., P.J. Fitzpatrick, A.L. Johnson, K.M. Kramer, J.H. Toyn, and L.H. Johnston. 1999. A Bub2p-dependent spindle checkpoint pathway regulates the Dbf2p kinase in budding yeast. *EMBO J.* 18, 2424-2434.
13. Fraschini, R., E. Formenti, G. Lucchini, and S. Piatti. 1999. Budding yeast Bub2 is localized at spindle pole bodies and activates the mitotic checkpoint via a different pathway from Mad2. *J. Cell Biol.* 145, 979-991.
14. Freed, E., K.R. Lacey, P. Huie, S.A. Lyapina, R.J. Deshaies, T. Stearns, and P.K. Jackson. 1999. Components of an SCF ubiquitin ligase localize to the centrosome and regulate the centrosome duplication cycle. *Genes Dev.* 13, 2242-2257.
15. Furge, K.A., K. Wong, J. Armstrong, M. Balasubramanian, and C.F. Albright. 1998. Byr4 and Cdc16 form a two-component GTPase-activating protein for the Spg1 GTPase that controls septation in fission yeast. *Curr. Biol.* 8, 947-954.
16. Gardner, R.D., A. Poddar, C. Yellman, P.A. Tavormina, M.C. Monteagudo, and D.J. Burke. 2001. The spindle checkpoint of the yeast *Saccharomyces cerevisiae* requires kinetochore function and maps to the CBF3 domain. *Genetics* 157, 1493-1502.
17. Gyuris, J., E. Golemis, H. Chertkov, and R. Brent. 1993. Cdi1, a human G1 and S phase protein phosphatase that associates with Cdk2. *Cell* 75, 791-803.
18. He, X., D.R. Rines, C.W. Espelin, and P.K. Sorger. 2001. Molecular analysis of Kinetochore-microtubule attachment in budding yeast. *Cell* 106, 195-206.
19. Hoyt, M.A., L. Totis, and B.T. Roberts. 1991. *S. cerevisiae* genes required for cell cycle arrest in response to loss of microtubule function. *Cell* 66, 507-517.
20. Hwang, L.H., L. Lau, D. Smith, C.A. Mistrot, and K.G. Hardwick. 1998. Budding yeast Cdc20: a target of the spindle checkpoint. *Science* 279, 1041-1044
21. Jwa, M. and K. Song. 1998. Byr4, a dosage-dependent regulator of cytokinesis in *S. pombe*, interacts with a possible small GTPase pathway including Spg1 and Cdc16. *Mol. Cells* 8, 240-245.
22. Kamura, T., D.M. Koepp, M.N. Conrad, D. Skowyra, R.J. Moreland, O. Iliopoulos, W.S. Lane, W.G. Kaelin, Jr., S.J. Elledge, R.C. Conaway, J.W. Harper, and J.W. Conaway. 1999. Rbx1, A component of the YHL tumor suppressor complex and SCF ubiquitin liagase. *Science* 284, 657-661.
23. Kaplan, K.B., A.A. Hyman, and P.K. Sorger. 1997. Regulating the yeast kinetochore by ubiquitin-dependent degradation and Skp1p-mediated phosphorylation. *Cell* 91, 491-500.
24. Kipreos, E. and M. Pagano. 2000. The F-box protein family. *Genome Biology* 1, 3002.1-3002.7
25. Lee, J., H. Hwang, J. Kim, and K. Song. 1999. Ibd1p, a pos-

sible spindle pole body associated protein, regulates nuclear division and bud separation in *Saccharomyces cerevisiae*. *Biochimi. Biophysi. Acta* 1449, 239-253.

26. Li, R. 1999. Bifurcation of the mitotic checkpoint pathway in budding yeast. *Proc. Natl. Acad. Sci. U.S.A.* 96, 4989-4994
27. Li, R. and A.W. Murray. 1991. Feedback control of mitosis in budding yeast. *Cell* 66, 519-531.
28. Mathias, N., S.L. Johnson, M. Winey, A.E. Adams, L. Goetsch, J.R. Pringle, B. Byers, and M.G. Goebl. 1996. Cdc53p acts in concert with Cdc4p and Cdc34p to control the G1-to-S-phase transition and identifies a conserved family of proteins. *Mol. Cell Biol.* 16, 6634-6643.
29. Martinez-Exposito, M.J, K.B. Kaplan, J. Copeland, and P.K. Sorger. 1999. Retention of the *BUB3* checkpoint protein on lagging chromosomes. *Proc. Natl. Acad. Sci. U.S.A.* 96, 8493-8498.
30. Ohta, T., J.J. Michel, A.J. Schottelius, and Y. Xiong. 1999. ROC1, a homolog of APC11, represents a family of cullin partners with an associated ubiquitin ligase activity. *Mol. Cell* 3, 535-541.
31. Patton, E.E., A.R. Willems, D. Sa, L. Kuras, D. Thomas, K.L. Craig, and M. Tyers. 1998. Cdc53 is a scaffold protein for multiple Cdc34/Skp1/F-box protein complexes that regulate cell division and methionine biosynthesis in yeast. *Genes Dev.* 12, 692-705.
32. Pereira, G., T. Hofken, J. Grindlay, C. Manson, and E. Schiebel. 2000. The Bub2p spindle checkpoint links nuclear migration with mitotic exit. *Mol. Cell* 6, 1-10.
33. Skowyra, D., D.M. Koepp, T. Kamura, M.N. Conard, R.C. Conaway, J.W. Conaway, S.J. Elledge, and J.W. Harper. 1999. Reconstitution of G1 cyclin ubiquitination with complexes containing SCF Grr1 and Rbx1. *Science* 284, 662-665.
34. Stemmann, O. and J. Lechner. 1996. The *Saccharomyces cerevisiae* kinetochore contains a cyclin-CDK complex homologue, as identified by in vitro reconstitution. *EMBO J.* 15, 3611-3620.
35. Taylor, S.S., E. Ha, and F. McKeon. 1998. The human homologue of Bub3 is required for kinetochore localization of Bub1 and a Mad3/Bub1-related protein kinase. *J. Cell Biol.* 142, 1-11.
36. Taylor, S.S. and F. McKeon. 1997. Kinetochore localization of murine Bub1 is required for normal mitotic timing and checkpoint response to spindle damage. *Cell* 89, 727-735.
37. Toh-e, A., Asakawa, K., and S. Yoshida. 2001. Spindle checkpoint control in budding yeast. *J. Microbiol.* 39, 1-5
38. Williams, A.R., S. Lanker, E.E. Patton, K.L. Crig, T.F. Nason, N. Mathias, R. Kobayashi, C. Wittenberg, and M. Tyers. 1996. Cdc53 targets phosphorylated G1 cyclins for degradation by the ubiquitin proteolytic pathway. *Cell* 86, 453-463.
39. Zou, H., T.J. McGarry, T. Bernal, and M.W. Kirschner. 1999. Identification of a vertebrate sister chromatid separation inhibitor involved in transformation and tumorigenesis. *Science* 285, 344-345.