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Yeast Ulp1, an Smt3-Specific Protease, Associates with Nucleoporins[1]

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Yeast Smt3 is a ubiquitin-like protein similar to the mammalian SUMO-1. Cdc3, a septin component, is known to be modified by Smt3. The level of this modification was affected by Smt3-specific protease mutation *ulp1-ts* or overexpression of *ULP1*. By two-hybrid screening, we isolated 5 *UIP* (Ulp1 interacting protein) genes. *UIP1* was identical to *NUP42* encoding a component of the nuclear pore complex (NPC). Gle1, another NPC-associating component, also interacted with Ulp1 in the two-hybrid system and co-immunoprecipitation experiment. Thus Ulp1 associates with nucleoporins and may interact with septin rings in the telophase.

Key words: hydrolase, nucleoporins, Smt3, SUMO-1, Ulp1.

SMT3 of *Saccharomyces cerevisiae* is an essential gene encoding a ubiquitin-like protein, similar to the mammalian protein SUMO-1 (*1*, *2*). A heterodimer of Uba2 and Aos1 is required for activation of Smt3, and Ubc9 is an Smt3-conjugating enzyme (*1*, *3*). Both Uba2 and Ubc9 are localized at the nucleus (*4*, *5*). The only known substrate proteins are septin components such as Cdc3, localized at the mother-bud neck (*6*, *7*). Recently Smt3-specific proteases Ulp1 and Smt4/Ulp2, which remove Smt3 from its conjugates, have been discovered (*8–10*). Ulp1-mediated Smt3-protein deconjugation is essential for the G2/M phase transition of the cell cycle. Moreover, Ulp1 is required for processing the C-terminus of the Smt3 precursor to the mature form (*8*). In contrast, *SMT4/ULP2* is not essential for mitotic growth, but the *smt4* disruptant is sensitive to various stresses (*9*, *10*).

Since deconjugation of Smt3 from the septin components may play a role in disassembly of the septin ring structure during cytokinesis (*6*, *7*), we investigated which Smt3-specific protease was involved in deconjugation from the Cdc3-conjugate. First, we constructed a plasmid carrying the same kind of HA-tagged *CDC3* as used by Johnson and Blobel (*7*). The epitope-tagged gene was chromosomally integrated by replacing the wild-type *CDC3* gene (Table I). As shown in Fig. 1A, modified forms of Cdc3 were more abundant in *ulp1-ts* mutant (T-10) than *smt4* disruptant (T-11) or wild-type (T-9) cells. Furthermore, when the wild-type *ULP1* was overexpressed from a plasmid pT-13 (YEp-*ULP1*), the modified forms of Cdc3 were reduced (Fig. 1B). In contrast, when a C-terminal truncated form of *ulp1-ΔC111* (pT-14) was overexpressed, the modification remained. This truncated form lacking the UD (Ulp-domain) region lost its function, since it did not complement *ulp1-ts* (Fig. 1C). These results suggest that *ULP1* is involved in deconjugation of Smt3 from the Cdc3-conjugate.

Next, we examined subcellular localization of the tagged-Ulp1 by indirect-immunofluorescence microscopy. It was detected as punctate staining on the nuclear periphery (data not shown), which is consistent with others results (*9*, *10*). We expected there might be some factors connecting Ulp1 on the nuclear periphery and Cdc3 localized at the neck. Thus we screened Ulp1-interacting factors by the two-hybrid system. First we constructed a plasmid pT-8 (pGBDU-*ULP1*) and introduced it into the strain PJ69-4A (*11*) with pGAD-bank. Ura^+ Leu^+ transformants were checked for the His^+ Ade^+ phenotype at 26°C (Fig. 2). Five positive clones were isolated, which also gave high β-galactosidase activity (data not shown). We designated these genes *UIP1-5* (Ulp1 interacting protein). DNA sequence analysis revealed that each Gal4 *trans*-activation domain was fused in frame to Uip1/Nup42 (+N8 a.a.), Uip2/YAL-014c (ΔN6 a.a.), Uip3/YAR027w (ΔN22 a.a.), Uip4/YPL186c (ΔN89 a.a.), or Uip5/YKR044w (ΔN314 a.a.). *UIP1* was identical to *NUP42*, which encodes a protein containing multiple FG amino acid repeats typical of NPC proteins (*12*). The other *UIP* genes were novel. To determine the subcellular localization of other Uip proteins, we constructed fusion genes with *GFP* at the 3′ end of the *UIP* genes. The Uip3-GFP fusion protein was localized at the nuclear envelope region. Uip3 has strong similarity to subtelomerically-encoded proteins. Uip4-GFP was detected at the nuclear envelope and endoplasmic reticulum regions. Uip5-GFP was localized at the nuclear envelope region. These results are summarized in Table II.

To confirm that Ulp1 associates with NPCs, we tried co-immunoprecipitation of Ulp1 with Nup42, but it was unsuccessful. Since Gle1, another NPC-associated component essential for RNA export, interacts with Nup42 on the cytoplasmic side of the NPCs (*13*, *14*), we tested whether Ulp1 interacted with Gle1. As shown in Fig. 2, Ulp1 (pT-8; pGBDU-*ULP1*) interacted with Gle1 (pT-11; pGAD-*GLE1*) in the two-hybrid system. Next we constructed a plasmid (pT-12) carrying a *GLE1-GFP* fusion gene and introduced it

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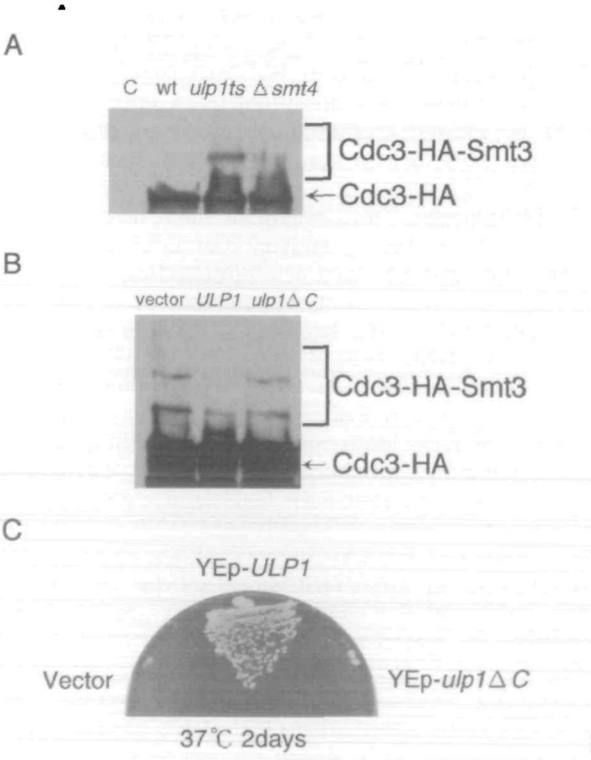
Abbreviations: a.a., amino acids; GFP, green fluorescent protein; HA, an epitope from the influenza virus hemagglutinin protein; NPC, nuclear pore complex; ORF, open reading frame.

TABLE I. **Yeast strains used in this study.**

Strain	Relevant genotype	
W303-1A	*MATa ade2 ura3 trp1 leu2 his3*	
T-8	*MATa ULP1::5XHA-LEU2*	
T-9	*MATa CDC3::5XHA-TRP1*	
MHY1488	*MATa LEU2::ulp1-ts ulp1::HIS3*	(*8*)
T-10	*MATa LEU2::ulp1-ts trp1 leu2 ulp1::HIS3 CDC3::5XHA-TRP1*	
JM-1	*MATa smt4::C.g.HIS3*	
T-11	*MATa smt4::C.g.HIS3 CDC3::5XHA-TRP1*	
PJ69-4A	*MATa trp1-901 leu2-3, 112 ura3-52 his3-200 gal4 gal80 LYS2::GAL1-HIS3 GAL2-ADE met::GAL7-lacZ*	(*11*)

To construct T-8 (*ULP1::HA*), plasmid pT-9 (YIp-*ULP1::HA*) was digested with *Msp*I and introduced into W303-1A. Deletion of *SMT4* was carried out by the PCR-based gene replacement method, where *HIS3* of *Candida glabrata* was used as a selection marker. A DNA fragment was amplified by PCR using primers and *Candida glabrata* genomic DNA as a template. The PCR product was directly used for transformation of W303-1A to generate JM-1. The deletion was confirmed by PCR. To generate T-9 (*CDC3::HA*), T-10 (*CDC3::HA ulp1-ts*), or T-11 (*CDC3::HAΔsmt4*), plasmid pT-7 (YIp-*CDC3::HA*) was digested with *Bgl*II and introduced into W303-1A (wild type), MHY1488 (*ulp1-ts*), or JM-1 (*Δsmt4*), respectively.



}-HA

ın *ulp1* and *ulp2/smt4* mutants. Cell lysates of W303-1A (no tagged gene), T-9 (*CDC3::HA*), T-10 (*ulp1-ts, CDC3::HA*), and T-11 (*smt4::C.gHIS3, CDC3::HA*) were prepared, subjected to immunoblotting and Cdc3 was detected with anti-HA (12CA5) antibody. B: Effect of overproduction of Ulp1. The strain T-9 expressing the HA-tagged Cdc3 was transformed with a control vector YEplac195, pT-13 (YEp-*ULP1*) or pT-14 (YEp-*ulp1-ΔC111*). The cell lysates were prepared and subjected to immunoblotting, as in A. C: Yeast strain T-10 (*ulp1-ts*) was transformed with a vector pTS901EU, pT-13 (YEp-*ULP1*) or pT-14 (YEp-*ulp1-ΔC111*). Ura$^+$ transformants were streaked on SD-ura medium and incubated at 37°C for 2 days.

into the strain T-8 expressing the HA-tagged Ulp1 or into the wild type strain. Cell lysates were mixed with anti-HA

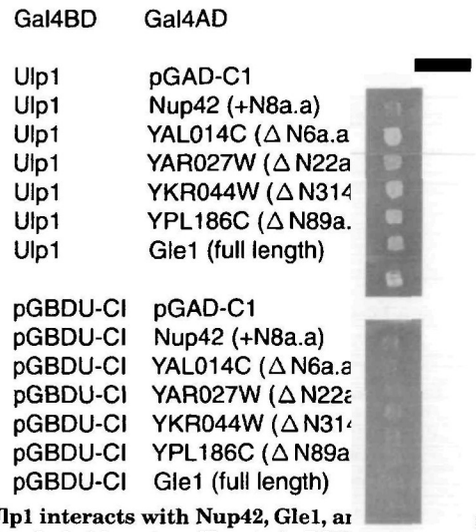


Fig. 2. **Ulp1 interacts with Nup42, Gle1, ar proteins in the two-hybrid system.** The strain PJ69-4A was co-transformed with plasmids expressing the indicated activation domain and DNA-binding domain constructs. Growth produced by different plasmid combinations is shown on SD medium lacking Ura, Leu, His, and Ade after incubation at 25°C for 4 days.

TABLE II. **Localization of Uip-GFPs.**

Gene name (ORF)	Localization of Uip-GFP[a]	Deletion phenotype
UIP1/NUP42	NPCs	Viable (*12*)
UIP2 (YAL014c)	ND[b]	Viable[c] NSP[d]
UIP3 (YAR027w)	Nuclear envelope, (plasma membrane)[e]	ND[b]
UIP4 (YPL186c)	Nuclear envelope, ER	Viable NSP[d]
UIP5 (YKR044w)	Nuclear envelope	Viable NSP[d]
UIP6/GLE1	NPCs	Inviable (*13*)

[a]*GFP* was fused to each 3′ end of *UIP* which was expressed from its own promoter. [b]Not determined. [c]Data from SGD. [d]No significant phenotype. [e]When the gene was overexpressed.

(12CA5) antibody and the precipitates were subjected to Western blotting. As shown in Fig. 3, Ulp1-HA co-immunoprecipitated with Gle1-GFP (lane 6). In contrast, little Gle1-GFP was precipitated from the lysate without Ulp1-HA (lane 5). Gle1 appeared to be modified, but the upper band did not correspond to its Smt3-conjugate (unpublished results). These results lead us to conclude that at least some fraction of Ulp1 associates with NPCs.

Ulp1 is the first known protein that associates with nucleoporins in yeast ubiquitin and ubiquitin-like protein conjugation systems. Among many interesting possibilities, we consider the following hypotheses. (i) Ulp1 processes the carboxy-terminus of the Smt3 precursor at NPCs so that only the mature form of Smt3 enters into the nucleus. (ii) Ulp1 may deconjugate Smt3 from modified proteins that physically interact with NPCs. These proteins may be factors involved in nuclear transport. In mammalian cells, SUMO-1 modification of RanGAP1 targets the otherwise cytosolic protein to NPCs, where it forms a stable complex with RanBP2 (*15*, *16*).

In the present study we have demonstrated that Ulp1 regulates the level of the Smt3-modification of Cdc3. Because no nuclear envelope breakdown occurs in yeast, the

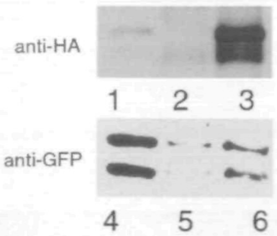


Fig. 3. **Gle1 is co-immunoprecipitated with Ulp1.** The strain T-8 (Ulp1-HA) or W303-1A was transformed with pT-12 (YEp-*GLE1::GFP*). Transformants were grown in 5 ml of selective medium to OD_{600} of 1.0. Cell lysates were prepared as described previously (*17*), except that the lysis buffer contained 0.5% Triton X-100. After cell lysates were centrifuged for 5 min at 10,000 rpm, 200 μl of the supernatant was mixed with anti-HA (12CA5) for immunoprecipitation. Samples were electrophoresed through a 10% polyacrylamide–SDS gel and subjected to Western blotting. (lanes 1 and 4) total lysate of T-8 (Ulp1-HA) containing Gle1-GFP. (lanes 2 and 5) immunoprecipitates from W303-1A containing Gle1-GFP. (lanes 3 and 6) immunoprecipitates from T-8 (Ulp1-HA) containing Gle1-GFP. The blotted membranes were treated with anti-HA (lanes 1, 2, and 3) or anti-GFP (lanes 4, 5, and 6) antibody.

cytoplasmic side of the nuclear envelope may come into contact with the septin ring during the telophase of the cell cycle. We speculate that Ulp1 at NPCs on the cytoplasmic side may interact physically with the Cdc3-Smt3 conjugate and remove Smt3 from the conjugate, which may ensure that the septin rings disassemble after the anaphase.

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REFERENCES

1. Johnson, E.S. and Blobel, G. (1997) Ubc9p is the conjugating enzyme for the ubiquitin-like protein Smt3p. *J. Biol. Chem.* **272**, 26799–26802
2. Meluh, P.B. and Koshland, D. (1995) Evidence that the *MIF2* gene of *Saccharomyces cerevisiae* encodes a centromere protein with homology to the mammalian centromere protein CENP-C. *Mol. Biol. Cell.* **6**, 793–807
3. Johnson, E.S., Schwienhorst, I., Dohmen, R.J., and Blobel, G. (1997) The ubiquitin-like protein Smt3p is activated for conjugation to other proteins by an Aos1p/Uba2p heterodimer. *EMBO J.* **16**, 5509–5519
4. Dohmen, R.J., Stappen, R., McGrath, J.P., Forrova, H., Kolarov, J., Goffeau, A., and Varshavsky, A. (1995) An essential yeast gene encoding a homolog of ubiquitin-activation enzyme. *J. Biol. Chem.* **270**, 18099–18109
5. Seufert, W., Futcher, B., and Jentsch, S. (1995) Role of a ubiquitin-conjugating enzyme in degradation of S- and M-phase cyclins. *Nature* **373**, 78–81
6. Takahashi, Y., Iwase, M., Konishi, M., Tanaka, M., Toh-e, A., and Kikuchi, Y. (1999) Smt3, a SUMO-1 homolog is conjugated to Cdc3, a component of septin fibers at mother-bud neck in budding yeast. *Biochem. Biophys. Res. Commun.* **259**, 582–587
7. Johnson, E.S. and Blobel, G. (1999) Cell cycle-regulated attachment of the ubiquitin-related protein SUMO to the yeast septins. *J. Cell. Biol.* **147**, 981–994
8. Li, S.J. and Hochstrasser, M.A. (1999) A new protease required for cell-cycle progression in yeast. *Nature* **398**, 246–251
9. Li, S.J. and Hochstrasser, M.A. (2000) The yeast *ULP2(SMT4)* gene encodes a novel protease specific for the ubiquitin-like Smt3 protein. *Mol. Cell. Biol.* **20**, 2367–2377
10. Schwienhorst, I., Johnson, E.S., and Dohmen, R.J. (2000) SUMO conjugation and deconjugation. *Mol. Gen. Genet.* **263**, 771–786
11. James, P., Halladay, J., and Craig, E.A. (1996) Genomic libraries and a host strain designed for highly efficient two-hybrid selection in yeast. *Genetics* **144**, 1425–1436
12. Stutz, F., Neville, M., and Rosbash, M. (1995) Identification of a novel nuclear pore-associated protein as a functional target of the HIV-1 Rev protein in yeast. *Cell* **82**, 495–506
13. Murphy, R. and Wente, S.R. (1996) An RNA-export mediator with an essential nuclear export signal. *Nature* **383**, 357–360
14. Strahm, Y., Fahrenkrog, B., Zenklusen, D., Rychner, E., Kantor, J., Rosbash, M., and Stutz, F. (1999) The RNA export factor Gle1p is located on the cytoplasmic fibrils of the NPC and physically interacts with the FG-nucleoporin Rip1p, the DEAD-box protein Rat8p/Dbp5p and a new protein Ymr255p. *EMBO J.* **18**, 5761–5777
15. Matunis, M.J., Coutavas, E., and Blobel, G. (1996) A novel ubiquitin-like modification modulates the partitioning of the Ran-GTPase-activating protein RanGAP1 between the cytosol and the nuclear pore complex. *J. Cell Biol.* **35**, 1457–1470
16. Mahajan, R., Delphin, C., Guan, T., Gerace, L., and Melchior, F. (1997) A small ubiquitin-related polypeptide involved in targeting RanGAP1 to nuclear pore complex protein RanBP2. *Cell* **88**, 97–107
17. Yashiroda, H., Oguchi, T., Yasuda, Y., Toh-e, A., and Kikuchi, Y. (1996) Bul1, a new protein that binds to the Rsp5 ubiquitin ligase in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **16**, 3255–3263