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The ubiquitin-like proteins SMT3 and SUMO-1 are conjugated by the UBC9 E2 enzyme

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ABSTRACT **The ubiquitin-like protein SMT3 from *Saccharomyces cerevisiae* and SUMO-1, its mammalian homolog, can be covalently attached to other proteins posttranslationally. Conjugation of ubiquitin requires the activities of ubiquitin-activating (E1) and -conjugating (E2) enzymes and proceeds via thioester-linked enzyme-ubiquitin intermediates. Herein we show that UBC9, one of the 13 different E2 enzymes from yeast, is required for SMT3 conjugation *in vivo*. Moreover, recombinant yeast and mammalian UBC9 enzymes were found to form thioester complexes with SMT3 and SUMO-1, respectively. This suggests that UBC9 functions as an E2 in a SMT3/SUMO-1 conjugation pathway analogous to ubiquitin-conjugating enzymes. The role of yeast UBC9 in cell cycle progression may thus be mediated through its SMT3 conjugation activity.**

Ubiquitin, a highly conserved protein of 76 amino acid residues, can be attached to other proteins posttranslationally (for reviews, see refs. 1–3). This reaction involves the formation of an isopeptide bond between the carboxyl-terminal glycine residue of ubiquitin and the ε-amino group of a lysine residue of an acceptor protein. All known functions of ubiquitin, including its role in selective protein degradation, are thought to be mediated through this reaction. Conjugation of ubiquitin proceeds via a reaction cascade involving ubiquitin-activating (E1) and ubiquitin-conjugating (E2) enzymes and, at least in some cases, ubiquitin–protein ligases (E3). The E1 enzyme hydrolyzes ATP and forms a high-energy thioester intermediate between a cysteine residue of its active site and the carboxyl terminus of ubiquitin. Ubiquitin is then passed on to E2 enzymes that form thioesters with ubiquitin in a similar fashion. Finally, ubiquitin is covalently attached to a substrate protein by the E2 enzymes or, alternatively, by E3 enzymes as indicated by the observation that at least some E3 enzymes are loaded with ubiquitin by E2 enzymes via thioester formation (4).

The yeast *Saccharomyces cerevisiae* genome contains several homologous genes encoding putative E1 enzymes (*UBA* genes). *UBA1* is essential for viability and encodes the activating enzyme required for ubiquitin conjugation (5). *UBA2*, also required for viability, encodes a smaller protein that bears a cysteine residue at a position similar to the active-site cysteine of UBA1 (6). A *uba2* mutant, expressing a protein that lacks this cysteine, is inviable, suggesting that this conserved residue is involved in thioester formation as well. Surprisingly, however, purified UBA2 failed to form a thioester complex with ubiquitin, suggesting that it may function in a different pathway (6). Indeed, UBA2 was recently shown to cooperate with another protein termed AOS1 in the activation of SMT3, a protein of 98 amino acid residues with 17% sequence similarity to ubiquitin (7). Interestingly, AOS1, also encoded by an essential gene, and UBA2 show sequence similarity to the amino- and the carboxyl-terminal domains of the UBA1 protein, respectively. This suggests that upon heterodimerization UBA2 and AOS1 resemble an E1 enzyme in the activation of SMT3 (7).

Similar to ubiquitin, SMT3 is found either free or covalently attached to other proteins (7). Because null mutants in *smt3*, *uba2*, or *aos1* are inviable, conjugation of this ubiquitin-like molecule is expected to fulfill essential cellular roles. The function of SMT3 conjugation is presently unknown, but recent evidence derived from studies on a related mammalian protein termed SUMO-1 [ref. 8, also known as GMP1 (9), PIC1 (10), UBL1 (11), or sentrin (12, 13); for review, see ref. 14] suggests that it may play a role in protein localization. SUMO-1, which displays 50% sequence identity to SMT3, was found covalently linked to RanGAP1, the activating protein of the Ran GTPase involved in the regulation of nucleocytoplasmic trafficking (8, 9). Conjugation of SUMO-1 to RanGAP1 targets the otherwise cytosolic protein to the nuclear pore complex. Thus SUMO-1, and possibly its yeast homolog SMT3, may function as posttranslationally added targeting devices that direct conjugated substrates to distinct cellular compartments.

Thirteen different genes for putative E2 enzymes (*UBC* genes) have been identified in the yeast genome (3, 15). The encoded enzymes are related proteins bearing a (~160 amino acid) highly conserved so-called UBC domain (15). Within this domain E2 enzymes possess a specific cysteine residue, thought to function in ubiquitin-E2 enzyme thioester formation. Yeast E2 enzymes are involved in a variety of cellular functions, including bulk protein degradation, stress resistance, cadmium tolerance, DNA repair, peroxisome biogenesis, and cell cycle progression (15). Conjugation of ubiquitin via these enzymes often targets the protein substrates for proteasomal degradation. Genetic studies have indicated that of the 13 yeast *UBC* genes only two are essential for viability: *UBC3* and *UBC9*. The UBC3 protein, also known as CDC34, is required for G_1–S cell cycle transition and targets (among other substrates) the cell cycle regulator SIC1 and the G_1 cyclins CLN1 and CLN2 for ubiquitin/proteasome-dependent degradation (3). Conversely, repression of UBC9 synthesis prevents cell cycle progression at the G_2 or early M phase, causing the accumulation of large budded cells with a single nucleus, a short spindle, and replicated DNA (16). In *ubc9*

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Abbreviations: HA, influenza virus hemagglutinin epitope; WT, wild type.

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mutants both CLB5, an S-phase cyclin, and CLB2, an M-phase cyclin, were found to be stabilized (16).

We now report that UBC9 and its mammalian homolog are E2 enzymes for SMT3/SUMO-1 conjugation. Yeast cells mutant for *UBC9* fail to form SMT3 conjugates *in vivo*. Moreover, we show that yeast and mammalian UBC9 can form thioester complexes with SMT3 and SUMO-1, respectively. UBC9 is thus part of the SMT3 pathway and functions together with the UBA2–AOS1 enzyme heterodimer in SMT3 conjugation. The data further suggest that the observed defects of *ubc9* mutants in cell cycle progression and cyclin degradation may be consequences of a deficiency in SMT3–protein conjugation and possibly nucleocytoplasmic trafficking.

MATERIALS AND METHODS

Yeast Strains and Media. *S. cerevisiae* strains were grown in rich (YP) or synthetic (S) medium containing 2% glucose, 2% raffinose, or 2% galactose as carbon sources. Yeast transformation was done by standard protocols (17). Selection of transformants was on selective medium lacking uracil (SD, −ura). Wild-type (WT; DF5) and the *ubc9–1* temperature-sensitive mutant were as described (16). Strain YWO61 (*MATα, his3, leu2, lys2, trp1, ura3, ubc2*(*rad6*)::*HIS3*) is a *ubc2*(*rad6*) null mutant.

Plasmids and Protein Expression. The *SMT3* gene (GenBank accession no. U27233) was cloned via PCR using gene-specific primers and genomic yeast DNA as template. The resulting 322-bp product was cloned after *Kpn*I and *Pst*I restriction endonuclease digestion into a derivative of YIplac211 (18) containing the *GAL1–10* promoter in front of a triple hemagglutinin (HA) tag and the *ADH1* transcription terminator. The resulting plasmid pKM092 was sequenced on both strands and linearized within the *URA3* gene by *Eco*RV digestion to allow integration into the *ura3* locus upon transformation. For bacterial expression the *SMT3* cDNA (amino acids 1–98) was cloned into pGEX-2TK (5′ *Bam*HI and 3′ *Sma*I; see below). By using cytoplasmic RNA derived from HeLa cells, a cDNA encoding SUMO-1 [amino acids 1–97 of the ORF (8, 9)] was cloned by reverse transcription followed by PCR amplification. The PCR product was cloned into pGEX-2TK (5′ *Bam*HI and 3′ *Sma*I).

Glutathione *S*-transferase fusion proteins (SMT3, SUMO-1, and ubiquitin) were expressed in *Escherichia coli* DH5α, affinity-purified on glutathione-Sepharose (Pharmacia), and radioactively labeled while bound to glutathione-Sepharose by using protein kinase A (Sigma) in the presence of [γ-^{32}P]ATP (19). The radiolabeled fusion proteins were eluted with 10 mM glutathione and cleaved with thrombin (Novagen) to yield free SMT3, SUMO-1, and ubiquitin, respectively. Upon cleavage, thrombin was inactivated by incubation at 75°C for 15 min. The radiolabeled cleavage products (SMT3, SUMO-1, and ubiquitin with an amino-terminal protein kinase A phosphorylation site) were used for thioester experiments.

For bacterial expression, cDNAs encoding *S. cerevisiae* UBC9, the mutant protein ubc9-C93S, and murine mUbc9, respectively, were amplified by PCR, cut with *Nde*I and *Bam*HI, and cloned into pET-3a. The plasmids used for PCR have been described (16, 20). *S. cerevisiae* UBC9 and ubc9-C93S, mUbc9, and human UbcH5 were expressed in *E. coli* BL21(DE3) (21). The *S. cerevisiae UBC2*(*RAD6*) expression plasmid has been described (22). Crude bacterial extracts were used as a source of the different E2 enzymes in thioester assays.

Protein Procedures. Preparation of yeast protein extracts was performed essentially as described (22). The crude extracts were used in thioester assays as a source of the SMT3 activating enzyme. For Western blot analysis, equal amounts of protein were separated on a 12% SDS/PAGE gel followed by blotting onto a poly(vinylidene difluoride) membrane. HA-tagged proteins were detected by incubation with anti-HA mAb (12CA5) tissue culture supernatant diluted 1:300 and subsequent incubation with peroxidase-coupled anti-mouse IgG by following the standard ECL protocol. As a source of SUMO-1 activating enzyme activity, as well as of the ubiquitin activating enzyme, protein extracts were prepared from confluent NIH 3T3 cells in 1% Nonidet P-40/20 mM Tris·HCl, pH 8.0/100 mM NaCl/1 mM DTT/aprotinin (1 μg/ml)/leupeptin (1 μg/ml)/0.01% phenylmethylsulfonyl fluoride. Extract prepared from ten 10-cm plates of cells was chromatographed on a 1-ml Mono Q column. The column was washed with 25 mM Tris·HCl, pH 7.6/50 mM NaCl/1 mM DTT, and bound proteins were eluted with 400 mM NaCl in 25 mM Tris·HCl, pH 7.6/1 mM DTT.

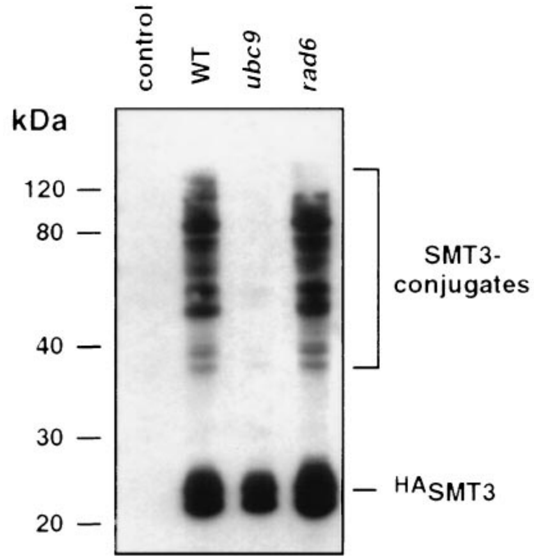

FIG. 1. SMT3-protein conjugation requires UBC9 *in vivo*. WT, *ubc9–1*, and *ubc2*(*rad6*) cells were transformed with an integrating plasmid carrying the gene for HA-tagged SMT3 (^{HA}SMT3) under the control of the inducible *GAL1–10* promoter. The strains were pregrown in YPRaf medium and shifted to YPGal medium inducing the ^{HA}SMT3 gene expression for 12 hr at 28°C. Protein extracts were prepared and subjected to Western blot analysis with anti-HA antibodies (12CA5). ^{HA}SMT3 monomer appears as a doublet. High molecular mass SMT3-protein conjugates are indicated by a bracket. Hardly any ^{HA}SMT3 conjugates can be detected in the *ubc9–1* mutant cells already at its permissive temperature (23°C).

Thioester Assays. Formation of thioester adducts of the different E2 enzymes was determined as described (21). Reaction mixtures contained either 20 μg of yeast extract or 10 μg of the Mono Q fraction of NIH 3T3 cell extracts, 500 ng of the respective E2, and 300 ng of ^{32}P-labeled SMT3, SUMO-1, or ubiquitin, in 20 mM Tris·HCl, pH 7.6/50 mM NaCl/4 mM ATP/10 mM MgCl$_2$/0.2 mM DTT. After 5 min at 25°C, reactions were terminated by incubating the mixtures for 15 min at 30°C in 50 mM Tris·HCl, pH 6.8/4 M urea/2% SDS/10% glycerol or by boiling the mixtures in the buffer above containing 100 mM DTT instead of urea. Reaction mixtures were separated on 14% SDS/PAGE gels at 4°C and radioactively labeled bands were visualized by autoradiography.

RESULTS

Yeast UBC9 Is Required for SMT3 Conjugation *in Vivo*. To study the conjugation of yeast SMT3 to cellular proteins *in vivo*, the SMT3 protein was amino-terminally tagged with three copies of the HA epitope (^{HA}SMT3). The gene encoding this fusion protein was placed under control of the inducible *GAL1–10* promoter and integrated into the yeast genome. When expressed in WT yeast, Western blot analysis of total cell

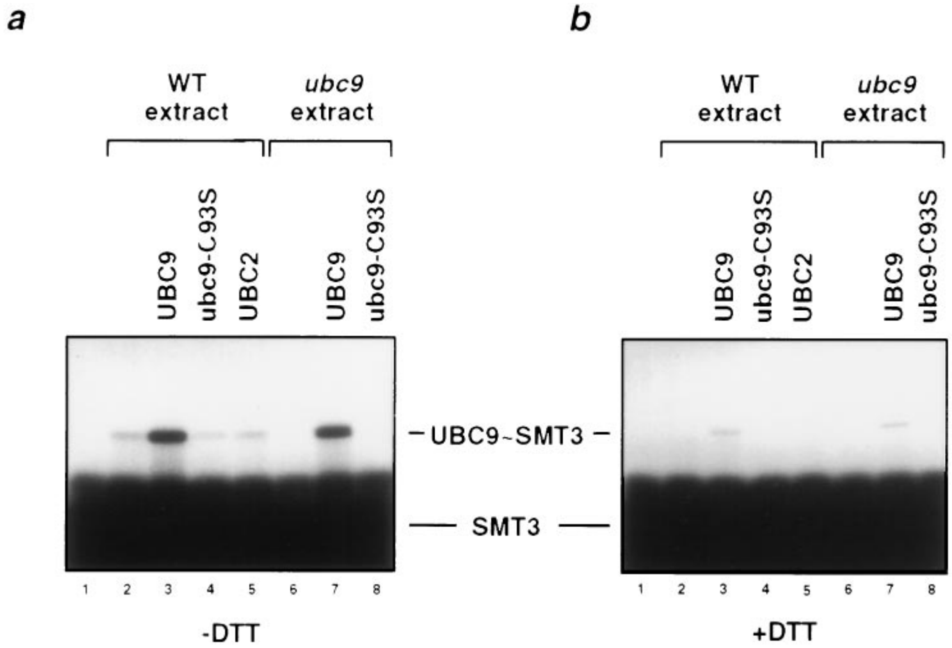

FIG. 2. Thioester complex formation between SMT3 and *S. cerevisiae* UBC9. Thioester reactions contained ^{32}P-labeled SMT3, protein extracts from yeast WT (lanes 2–5) or from *ubc9–1* mutant strain (lanes 6–8) (16), and crude extracts from bacteria expressing various E2 enzymes as indicated (lane 1 shows SMT3 only; lanes 2 and 6, without bacterially expressed E2 enzymes). The ubc9-C93S enzyme is a mutant of UBC9 in which the presumed catalytic-site cysteine residue at position 93 was altered to serine. Similar amounts of the respective E2 enzymes were used as determined by staining with Coomassie blue (data not shown). After 5 min at 25°C, reactions were stopped in the absence (*a*) or presence (*b*) of a reducing agent, and the products were subjected to SDS/PAGE followed by autoradiography. Positions of free ^{32}P-labeled SMT3 and of the UBC9 thioester complex are indicated. Note that in the presence of protein extract derived from WT *S. cerevisiae* a band is detected (lane 2, 4, and 5) that comigrates with the SMT3-thioester complex of recombinant UBC9 (lane 3). Because this band is absent in the reactions with protein extracts derived from the *ubc9–1* mutant (lane 6), this band is likely to represent the thioester adduct of SMT3 with endogenous UBC9 present in the WT strain extract.

extract using an anti-HA antibody revealed the expression of a protein with a size corresponding to ^{HA}SMT3. In addition, several other anti-HA-reactive proteins with sizes from ~40 to more than 100 kDa were observed (Fig. 1). These bands most likely represent conjugates of SMT3 with different cellular proteins (7). In striking contrast to the large number of ubiquitin-protein conjugates that are detected by anti-ubiquitin Western blots (data not shown), it appears that there is only a limited number of substrates for SMT3 conjugation.

Similar to ubiquitin conjugation, it seems likely that, in addition to the E1 activity of the heterodimeric UBA2–AOS1 complex, SMT3 conjugation requires an E2 enzyme that transfers activated SMT3 to cellular proteins. Obvious candidates for such enzymes are members of the large yeast UBC family. To test this possibility, ^{HA}SMT3 was expressed in different yeast cells mutant for distinct yeast E2 enzymes (*ubc* mutants). No difference in SMT3 conjugation was found in strains deficient in major ubiquitin-conjugating enzymes, including UBC2(RAD6) (Fig. 1) and UBC4 (data not shown). However, SMT3-protein conjugates were virtually absent in a conditional *ubc9* mutant although ^{HA}SMT3 was expressed at similar levels in these cells (Fig. 1). From these data we conclude that the yeast UBC9 E2 is required for SMT3 conjugation *in vivo.* Consistent with a function in a SMT3 conjugation pathway, the UBC9 enzyme is required for cell viability (see *Discussion*).

Yeast UBC9 Forms a Thioester with SMT3 *in Vitro*. Yeast UBC9 shows 35% sequence identity to other E2 enzymes, including a conserved putative active site cysteine residue. Substitution of this residue by other amino acids results in a complete loss of UBC9 function *in vivo* (16). Because radiolabeled UBC9 incubated with a total yeast cell extract led to the ATP-dependent formation of a thioester-linked UBC9 protein adduct, we previously concluded that UBC9 can form a thioester with ubiquitin *in vitro* (16). However, this assay does not distinguish between UBC9 thioesters formed with ubiquitin or ubiquitin-like proteins. On the basis of the results presented above, therefore, it seemed likely that the observed adduct represented a complex of UBC9 with SMT3, rather than with ubiquitin. To test this possibility, UBC9 was expressed in *E. coli* and the bacterial lysate was incubated with radiolabeled SMT3 and ATP in the presence of a yeast cell extract (Fig. 2). As controls, extracts from bacteria expressing UBC2(RAD6) or a *ubc9* mutant (*ubc9-C93S*) in which the putative catalytic cysteine residue was altered to serine were used. As shown in Fig. 2*a*, only bacterial extracts containing WT UBC9 mediated the formation of a radiolabeled complex of ~30 kDa, consistent with the size of an adduct between SMT3 and UBC9. This complex was sensitive to boiling under reducing conditions, indicating that the adduct is indeed linked via a thioester bond (Fig. 2*b*). Furthermore, thioester formation was dependent on the presence of yeast extract that supplies the SMT3 activating activity (UBA2 and AOS1) (data not shown). These findings indicate that yeast UBC9 can form a thioester with SMT3 in a reaction that depends on the UBA2/AOS1 activating enzyme. In agreement with the central role of UBC9 in SMT3 conjugation *in vivo*, this E2 enzyme was the only endogenous protein in a total yeast cell extract that could form a thioester complex with SMT3 (Fig. 2; compare lanes 2 and 6).

Mouse UBC9 Forms a Thioester with SUMO-1 *in Vitro*. It has recently been shown that a close murine homolog of yeast UBC9, termed mUBC9, can complement the cell cycle defect of the conditional yeast *ubc9* mutant mentioned above (20). Furthermore, it was reported that SUMO-1, a mammalian homolog of SMT3, can be covalently linked to cellular proteins (8, 9, 13). Thus, this indicated that, similar to yeast UBC9, mUBC9 may be involved in SUMO-1 conjugation. To test this hypothesis, mUbc9 was expressed in *E. coli* and tested in thioester formation assays with SUMO-1 and, as a control,

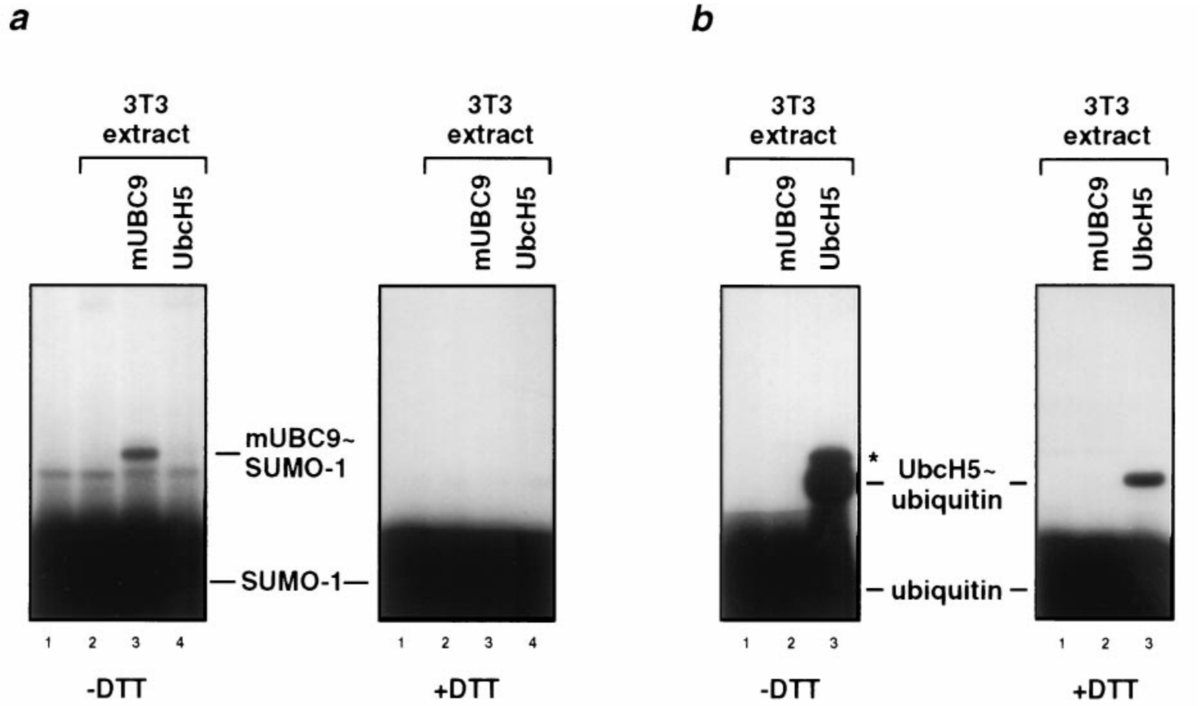

FIG. 3. Thioester complex formation of murine mUBC9 with SUMO-1 but not with ubiquitin. (*a*) ^{32}P-labeled SUMO-1 was incubated with a Mono Q fraction derived from NIH 3T3 cell extracts and crude extracts from bacteria expressing mUBC9 (lane 3) or UbcH5 (lane 4) as indicated (lane 1 shows SUMO-1 only; lane 2 is without bacterially expressed E2 enzymes). Similar amounts of the respective E2 enzymes were used as determined by Coomassie blue staining (data not shown). After 5 min at 25°C, reactions were stopped in the absence or presence of DTT and subjected to SDS/PAGE followed by autoradiography. Positions of free ^{32}P-labeled SUMO-1 and of the respective E2 thioester adducts are indicated. (*b*) Similar to *a* but ^{32}P-labeled ubiquitin was incubated with NIH 3T3 cell extracts and extracts from bacteria expressing mUBC9 (lane 2) or UbcH5 (lane 3; lane 1 is without bacterially expressed E2 enzymes). The band marked by an asterisk presumably represents ubiquitin that is covalently linked to UbcH5 via an isopeptide bond. Note that the NIH 3T3 Mono Q fraction contained both the enzyme activities needed to activate ubiquitin and SUMO-1. However, the SUMO-1 activating enzyme activity is distinct from the well-characterized ubiquitin-activating enzyme (data not shown).

with ubiquitin. As shown in Fig. 3, mUBC9 was indeed able to form thioester complexes with SUMO-1 but not with ubiquitin under the conditions used. In contrast, thioester complex formation of UbcH5, the human homolog of yeast UBC4/UBC5 (21), was observed only with ubiquitin but not with SUMO-1, indicating the specificity of the respective reactions. Furthermore, the ability of mUBC9 to form thioester complexes with SUMO-1 was dependent on the presence of a mouse cell extract (data not shown) that presumably provides the SUMO-1 activating activity. Although the mouse SUMO-1 activating enzyme has not been identified so far, biochemical studies indicate that, similar to the SMT3 activating enzyme of yeast (7), it is distinct from the well-characterized ubiquitin-activating enzyme (S.E.S. and M.S., unpublished results).

DISCUSSION

Attachment of ubiquitin to cellular proteins targets these substrates for proteasome-mediated degradation. Recently, however, alternative destinations for ubiquitin-protein conjugates have been described. Ubiquitination of certain cell surface proteins appears to direct these substrates to lysosomal proteolysis via the endocytic route (23). It can therefore be assumed that ubiquitin functions primarily as a posttranslationally added targeting module, directing substrate proteins for proteolytic destruction. It has been suggested (23) that the different fates (proteasome- versus lysosome-mediated degradation) are controlled by the nature of the modification (i.e., multiubiquitination versus monoubiquitination), but the discriminating molecular mechanism remains enigmatic.

The recent discoveries of ubiquitin-like proteins in apparently all eukaryotes suggest that posttranslational modification of proteins by the covalent attachment of other proteins appears to be a more general phenomenon than previously assumed. The interferon-inducible protein UCRP, possibly restricted to mammalian cells, can be covalently linked to other proteins and appears to direct them to the cytoskeleton (24). More recently, yeast SMT3 and its apparent mammalian homolog SUMO-1 have been found to form conjugates with a unique set of cellular proteins. In mammalian cells, RanGAP1 appears to be a major substrate for SUMO-1 conjugation (8, 9). Attachment of SUMO-1 targets this otherwise cytosolic protein to the RanBP2 protein of the nuclear pore complex, a reaction crucial for nuclear protein import. However, an epitope-tagged form of RNA1, the yeast homolog of RanGAP1, does not seem to be modified by SMT3 conjugation (D.L., K.M., and S.J., unpublished data). The function of SMT3 conjugation in yeast is currently unknown, but a function similar to SUMO-1 conjugation is expected as SUMO-1 can complement yeast *smt3* null mutants (14).

In this paper we identify UBC9 and its mammalian homolog mUBC9 as conjugating enzymes (E2) for SMT3 from yeast and SUMO-1 from mammalian cells, respectively. We show that these enzymes can form thioester complexes with these ubiquitin-like proteins *in vitro*. Importantly, in yeast *ubc9* mutants conjugation of SMT3 to other cellular proteins is apparently abolished, indicating that UBC9 plays a pivotal role in SMT3 conjugation *in vivo*. Like other proteins of the SMT3 conjugating pathway (i.e., SMT3, UBA2, and AOS1), the UBC9 enzyme is essential for viability. Conditional mutants in *smt3* or *ubc9* accumulate cells at G_2/M phase of the cell cycle at their nonpermissive temperature (14, 16). Moreover, UBC9 depletion leads to a stabilization of yeast B type cyclins (16). This phenotype is strikingly similar to mutants in the genes for

SRP1, the yeast α-importin (25), and CSE1, identified in a screen for cyclin stabilizing mutants (26), which bears a putative Ran binding motif (27). It is thus reasonable to speculate that B type cyclin stability in yeast is controlled by a SRP1/CSE1-dependent protein import pathway that in turn may be controlled by a SMT3/UBC9-dependent protein modification event. B type cyclin destruction proceeds via the ubiquitin/proteasome system and is controlled by a large protein complex known as anaphase promoting complex or cyclosome (26). Thus, an attractive but speculative hypothesis is that cell cycle-regulated cyclin destruction requires the specific import of components or activators of the anaphase promoting complex/cyclosome complex.

Indirect evidence that the UBC9 homolog from higher eukaryotes is a SUMO-1-conjugating enzyme came from recent reports, demonstrating physical interactions of UBC9 with SUMO-1 (11) and RanBP2, the nuclear pore complex protein bound by SUMO-1 conjugated RanGAP1 (28). UBC9 has also been identified in numerous two-hybrid interaction assays with other proteins. These include conspicuously interesting molecules such as adenovirus E1A (20), human papillomavirus type 16 E1 protein (29), IκB (30), poly(ADP ribose) polymerase (31), p53 (11), E2A (32), Jun (33), the glucocorticoid receptor (33), Fas/CD95 (34), RAD51, and RAD52 (11, 35). Whether all these interactions are of biological relevance remains to be shown, however. The interaction of UBC9 with RAD51 may be of particular interest (11, 35) because RAD51 interacts with the breast cancer susceptibility gene products BRCA1 and BRCA2. Furthermore, RAD51, BRCA1, and BRCA2 colocalize with UBC9 on synaptonemal complexes of paired meiotic chromosomes (35), suggesting the intriguing possibility that certain steps in meiosis are controlled by UBC9-mediated SUMO-1 conjugation. However, the full picture of UBC9 function has to await the identification and characterization of the entire set of UBC9 targets.

Note added in proof. While this work was under review Johnson and Blobel (36) came to a similar conclusion by showing that UBC9 can conjugate SMT3 *in vitro*.

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1. Ciechanover, A. (1994) *Cell* **79,** 13–21.
2. Jentsch, S. & Schlenker, S. (1995) *Cell* **82,** 881–884.
3. Hochstrasser, M. (1996) *Annu. Rev. Genet.* **30,** 405–439.
4. Scheffner, M., Nuber, U. & Huibregtse, J. M. (1995) *Nature (London)* **373,** 81–83.
5. McGrath, J. P., Jentsch, S. & Varshavsky, A. (1991) *EMBO J.* **10,** 227–236.
6. Dohmen, R. J., Stappen, R., McGrath, J. P., Forrová, H., Kolarov, J., Goffeau, A. & Varshavsky, A. (1995) *J. Biol. Chem.* **270,** 18099–18109.
7. Johnson, E. S., Schwienhorst, I., Dohmen, R. J. & Blobel, G. (1997) *EMBO J.* **16,** 5509–5519.
8. Mahajan, R., Delphin, C., Guan, T., Gerace, L. & Melchior, F. (1997) *Cell* **88,** 97–107.
9. Matunis, M. J., Coutavas, E. & Blobel, G. (1996) *J. Cell. Biol.* **135,** 1457–1470.
10. Boddy, M. N., Howe, K., Etkin, L. D., Solomon, E. & Freemont, P. S. (1996) *Oncogene* **13,** 971–982.
11. Shen, Z., Pardington-Purtymun, P. E., Comeaux, L. C., Moyzis, R. K. & Chen, D. J. (1996) *Genomics* **37,** 183–186.
12. Okura, T., Gong, L., Kamitani, T. Wada, T., Okura, I., Wei, C. F., Chang, H. M. & Yeh, E. T. (1996) *J. Immunol.* **157,** 4277–4281.
13. Kamitani, T., Nguyen, H. P. & Yeh, E. T. H. (1997) *J. Biol. Chem.* **272,** 14001–14004.
14. Johnson, P. R. & Hochstrasser, M. (1997) *Trends Cell. Biol.* **7,** 408–413.
15. Smith, S. E., Koegl, M. & Jentsch, S. (1996) *Biol. Chem.* **377,** 437–446.
16. Seufert, W., Futcher, B. & Jentsch, S. (1995) *Nature (London)* **373,** 78–81.
17. Ausubel, F. J., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (1992) *Current Protocols in Molecular Biology* (Wiley Interscience, New York).
18. Gietz, R. D. & Sugino, A. (1988) *Gene* **74,** 527–534.
19. Scheffner, M., Huibregtse, J. M., Vierstra, R. D. & Howley, P. M. (1993) *Cell* **75,** 495–505.
20. Hateboer, G., Hijmans, E. M., Nooij, J. B. D., Schlenker, S., Jentsch, S. & Bernards, R. (1996) *J. Biol. Chem.* **271,** 25906–25911.
21. Scheffner, M., Huibregtse, J. M. & Howley, P. M. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 8797–8801.
22. Jentsch, S., McGrath, J. P. & Varshavsky, A. (1987) *Nature (London)* **329,** 131–134.
23. Hicke, L. & Riezman, H. (1996) *Cell* **84,** 277–287.
24. Loeb, K. R. & Haas, A. L. (1994) *Mol. Cell. Biol.* **14,** 8408–8419.
25. Loeb, J. D. J., Schlenstedt, G., Pellman, D., Kornitzer, D., Silver, P. A. & Fink, G. R. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 7647–7651.
26. Irniger, S., Piatti, S., Michaelis, C. & Nasmyth, K. (1995) *Cell* **81,** 269–277.
27. Görlich, D., Dabrovski, M., Bischoff, F. R., Kutay, U., Bork., P., Hartmann, E., Prehn, S. & Izaurralde, E. (1997) *J. Cell Biol.* **138,** 65–80.
28. Saitoh, H., Pu, R., Cavenagh, M. & Dasso, M. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 3736–3741.
29. Yasugi, T. & Howley, P. M. (1996) *Nucleic Acids Res.* **24,** 2005–2010.
30. Tashiro, K., Pando, M. P., Kanegae, Y., Wamsley, P. M., Inoue, S. & Verma, I. M. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 7862–7867.
31. Masson, M., Menissier de Murcia, J., Mattei, M. G. & Niedergang, C. P. (1997) *Gene* **190,** 287–296.
32. Kho, C. J., Huggins, G. S., Endege, W. O., Hsieh, C. M., Lee, M. E. & Haber, E. (1997) *J. Biol. Chem.* **272,** 3845–3851.
33. Gottlicher, M., Heck, S., Doucas, V., Wade, E., Kullmann, M., Cato, A. C., Evans, R. M. & Herrlich, P. (1996) *Steroids* **61,** 257–262.
34. Wright, D. A., Futcher, B., Gosh, P. & Geha, R. S. (1996) *J. Biol. Chem.* **271,** 31037–31043.
35. Kovalenko, O. V., Plug, A. W., Haaf, T., Gonda, K. D., Ashley, T., Ward, D. C., Radding, C. M. & Golub, E. I. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 2958–2963.
36. Johnson, E. S. & Blobel, G. (1997) *J. Biol. Chem.* **272**, 26799–26802.