SUMO (small ubiquitin-like modifier)/Smt3 (suppressor of mif two) is a member of the ubiquitin-related protein family and is known to conjugate with many proteins. In the sumoylation pathway, SUMO/Smt3 is transferred to substrate lysine residues through the thioester cascade of E1 (activating enzyme) and E2 (conjugating enzyme), and E3 (SUMO ligase) functions as an adaptor between E2 and each substrate. Yeast Ull1 (ubiquitin-like protein ligase 1)/Siz1, a PIAS (protein inhibitor of activated STAT)-type SUMO ligase, modifies both cytoplasmic and nuclear proteins. In this paper, we performed a domain analysis of Ull1/Siz1 by constructing various deletion mutants. A novel conserved N-terminal domain, called PINIT, as well as the RING-like domain (SP-RING) required for the SUMO ligase activity in the in vitro conjugation system and for interaction with Smt3 in an in vitro binding assay. The most distal C-terminal region, which contains a putative DNA-binding SAF-A/B, Acinus, and PIAS (SAP) motif, was not required for the ligase activity but was involved in nuclear localization. A strong SUMO-binding motif was identified, which interacted with Smt3 in the two-hybrid system but was not necessary for the ligase activity. The most distal C-terminal domain was important for stable localization at the bud neck region and thereby for the substrate recognition of septins. Furthermore, the C-terminal half conferred protein instability on Ull1/Siz1. Taken together, we conclude that the SP-RING and PINIT of Ull1/Siz1 are core domains of the SUMO ligase, and the other domains are regulatory for protein stability and subcellular localization.

Small ubiquitin-like modifier (SUMO)$^3$/Smt3 (suppressor of mif two) is a member of a family of ubiquitin-related proteins and is known to conjugate with RanGAP1, promyelocytic leukemia protein, IkBα, p53 (reviewed in Refs. 3–5), yeast septin components, etc. (6, 7). In budding yeast, Smt3 is the only member of the SUMO family, and the Smt3 conjugation system is essential for mitotic growth (8). The lethal-ity of the smt3 deletion mutant can be suppressed by expressing human SUMO-1, suggesting that SUMO-1 is a functional homologue of yeast Smt3 (6).

For the protein conjugation of all of the SUMO family members, common E1 and E2 enzymes are required. The E1 enzymes are heterodimers known as Uba2-Aos1 for the yeast Smt3 system and form a thioester bond between the C-terminal glycine residue of SUMO and the cysteine residue of Uba2 (9, 10). SUMO is then transferred to E2 enzyme, known as the Ubc9-conjugating enzyme (11, 12). Eventually, SUMO1/Smt3 ligases (E3s) were discovered and found to be required for both substrate recognition and promoting conjugation. Yeast Ull1/Siz1, a member of the PIAS (protein inhibitor of activated STAT) family, was identified as an E3 factor specific for septin components (13–15). In higher eukaryotes, PIAS proteins have been identified as SUMO ligases (16, 17). These PIAS family proteins contain SP-RING (18), a putative zinc-binding RING-like domain with an octet of ordered cysteine and histidine residues essential for the ligase activity in vivo and in vitro, just like certain ubiquitin-ligase components (19, 20).

Ull1/Siz1 exhibits cell cycle-dependent subcellular localization; Ull1/Siz1 localizes in the nucleus before the G2/M phase and accumulates in the M phase at the bud neck region, a junction between a mother cell and its bud (13–15). Consistent with this result, both cytoplasmic (Cdc3, Cdc11, and Shs1) and nuclear proteins, such as proliferating cell nuclear antigen (Pol30), have been identified as target proteins of Ull1/Siz1 (21). In addition, Ull1ΔC440, a C-terminal truncated form of Ull1/Siz1 always localizes in the nucleus but functions as a SUMO ligase for Cdc3 in the in vitro conjugation system (22). These results raise the question of how Ull1/Siz1 is regulated in both cellular compartments.

The Ull1 protein (we use Ull1 (ubiquitin-like protein ligase 1) in this report) is composed of 904 amino acids and carries two domains and two motifs (see Fig. 1). The SAF-A/B, Acinus, and PIAS (SAP) domain (aa 34–68) with the LXXL signature in the N-terminal region may interact with DNA, because PIAS-SAP binds to DNA (23). A newly identified PINIT motif has been defined as a well conserved region of 180 residues in the PIAS family and is involved in nuclear retention (24). This motif may be located between aa 181 and 349, which is adjacent to the SP-RING domain (aa 352–409). A putative SXS motif, originally reported as a SUMO-interacting motif (25), may be located around 490 of the Ull1 sequence. However, it has been reported recently that a true SUMO binding motif was identified as SBM, which resides adjacent to the SXS motif of PIAS proteins (26). It is not clear whether Ull1 contains the SBM in the equivalent region.

Here we present our in vitro and in vivo assay findings, using various truncated forms of Ull1, and demonstrate that Ull1, a SUMO ligase, is regulated through subcellular localization and protein stability.

**EXPERIMENTAL PROCEDURES**

**Strains and Genetic Manipulations**—*Escherichia coli* strains, DH5α, BL21 (DE3), and M15 (pREP4), were used for plasmid propagation and protein purification. Strains of *Saccharomyces cerevisiae,* KY8029 (MATa cdc20–3 ura3 trp1 his3 leu2 ade2) (27), and KN1389 (MATa cdc28–1N ura3 trp1 his3 leu2 ade2) (28) were gifts from K. Nasmyth,
and YAT1908 (MATa cdcl28-1N) was a clone obtained by crossing KN1389 with W303-1B (29). T-13 (ull1::XGH33) and T-20 (ull1::XGH33 CDC3-HA) were isogenic to W303-1A (15). The F69g-4A (MATa ura3 trp1 leu2 his3 gal4 gal180 lys2-gal1-HIS3 gal2-ade2 met2::gal7-lac2) used for the two-hybrid system was given by E. Craig (30).

Plasmids—Plasmids pT-17 (pTS901CL-ULL1-HA), pT-23 (pTS910CU-ULL1-GFP), pT-32 (pTS901CL-ull1gly400CA-HA), pT-35 (pGEX-KG-SMT3gq), pT-39 (pGAD-SMT3), pT-60 (pGEX-KG-UBC9), pT-77 (pET21b-ull1CA440), and pT-81 (pTS910CU-ull1C440-GFP) were described previously (14, 15, 22). YCUP4-CDC28 contained the 3.5 kb XbaI-Sall fragment carrying CDC28 on YCUP4 vector (31). To construct the plasmid pT-82 (pET21a-ull1-N1), pT-83 (pET21a-ull1-N), or pT-84 (pET21a-ull1-RING), DNA fragments carrying each amino acid sequence of the ULL1 open reading frame from position 112 to 465, 223 to 465, or 318 to 465, were amplified by PCR, using genomic DNA of W303-1A as template and the following primers, ULL1-N ATG-SalI (5′-CCGCGTCGACATGCTGTAATACTTTTTGCAGG-3′) primers, cut with EcoRI and Sall, and inserted into pET21a vector (Novagen). To construct pT-85 (GFP-tagged ull1-N2), pT-86 (GFP-tagged ull1-C1), pT-87 (GFP-tagged ull1-C3), and pT-88 (GFP-tagged ull1-N), DNA fragments of C-terminal truncated forms of ull1 were amplified by PCR using the following primer pairs, ULL1-N PstI (GCAACTGCAGGCTTGTTCTCGTCCTGACA) and one of ULL1-C222-SalI (GCGCGTCGACTGGCGTTGGGGATGATA-ATAGCAAACACAGGCTATACT-3′), ULL1-N222-EcoRI-a (5′-CCGCGTTCGACTGGCGTTGGGGATGATA-ATAGCAAACACAGGCTATACT-3′), ULL1-C222-SalI (GCGCGTCGACTGGCGTTGGGGATGATA-ATAGCAAACACAGGCTATACT-3′), and ULL1-N317-EcoRI-a (5′-CCGCGTTCGACTGGCGTTGGGGATGATA-ATAGCAAACACAGGCTATACT-3′), respectively.

In Vitro Conjugation Assay—All of the components, Smt3gg, T7-Cdc3-His, GST-Uba2, GST-Aos1, Ubc9, and Ull1 derivatives, were purified from E. coli lysates, as described above. Reaction mixtures (20 μl) of Cdc3, GST-Uba2, GST-Aos1, Ubc9, and Smt3gg were incubated with Ull1 derivatives (Ull1ΔC440, Ull1-N1, Ull1-N2, and Ull1-RING) in the presence of 10 mM ATP, 50 mM Tris-HCl (pH 7.4), 5 mM MgCl2, and 2 mM diethiothreitol at 37 °C for 30 min, as described previously (22).

Preparation of Yeast Lysates and Immunoblot Analysis—Cell lysates for immunoblotting were prepared as described previously (14) or by an alkaline method (33). For the experiment shown in Fig. 6B, cells of T-13 (Δull1) carrying pT-115 (pGAL-ULL1-HA) or pT-110 (pGAL-ULL1C440-HA) were grown in S-raffinose medium at 25 °C and arrested with hydroxyurea or nocodazole for 2 h. One percent galactose was added to the cultures to express the Ull1 proteins, and 1 h later, 2% glucose was added to the cultures to shut off the transcription (0′). Samples were taken at 15 min (15′) and 30 min (30′). The cell lysates were subjected to immunoblotting.

In Vitro Binding Assay—GFP-tagged proteins were purified from E. coli cells carrying pGAL-GFP-ull1 or pGAL-GFP-ull1ΔC440 by glutathione-Sepharose 4B (Amersham Biosciences) at 4 °C for 2 h. After the beads were washed three times with lysis buffer 2, each protein was eluted with 10 μl glutathione in 50 mM Tris-HCl (pH 8.0), and the samples were dialyzed against 10 mM Tris-HCl (pH 7.5) buffer containing 3 mM MgCl2. For the preparation of the untagged mature forms of Smt3 and Ubc9, the GST-Smt3gg and GST-Ubc9 bound to beads were treated with thrombin, and the supernatants were dialyzed to remove glutathione, as described previously (22).
Domain Analysis of Yeast PIAS-type Ull1/Siz1 SUMO Ligase

FIGURE 1. Schematic representation of Ull1 and its various truncated forms. Ull1-(1–904) contains several domains, including the SAP-(34–68), PINIT-(181–349), SP-RING-(352–409), and a putative SKS motif around aa 490, which are shown as black boxes. Ull1C440, Ull1-N1, Ull1-N2, and Ull1-RING proteins were expressed in $E. coli$ from the plasmids, pT-77, pT-82, pT-83, and pT-84, respectively, and purified from the cell lysates. A summary of the results in Fig. 2 is shown on the right.

RESULTS

Essential Domains for the SUMO Ligase Activity—We have previously shown that the SP-RING of Ull1 is essential to the ligase activity for septin sumoylation in vivo and in vitro (14, 15) and that a C-terminal truncated form of Ull1 (Ull1ΔC440) possesses SUMO ligase activity in vitro (22). To narrow down the region essential for the in vitro ligase activity, we prepared the various truncated forms of Ull1 shown in Fig. 1 and assayed them in the in vitro conjugation system (Fig. 2A). All of the components, Smt3gg (a mature form of Smt3), E1, E2, the T7-tagged Ull1 derivatives, and Cdc3, were prepared from $E. coli$ lysates, as described under “Experimental Procedures.”

Both Ull1-N1-(112–465) and Ull1ΔC440-(1–465) promoted the sumoylation of Cdc3 (Fig. 2A). In contrast, neither Ull1-RING-(318–465) nor Ull1-N2-(223–465) possessed the SUMO ligase activity. These results indicate that a region containing the PINIT motif (residues 181–349) in addition to SP-RING-(352–409) is required for the sumoylation of Cdc3 in vitro and that the most distal N-terminal region containing the SAP-domain (residues 34–68) is not necessary for the SUMO ligase activity. It should be noted that the primary structure of Ull1-N1 is weakly similar to that of Mms21, a recently identified component of the SUMO ligase complex (35).

Without any additional substrate proteins in this in vitro system, T7-tagged Ull1ΔC440 was found to be modified. New bands corresponding to the Smt3 conjugates of Ull1ΔC440 were detected after incubation (Fig. 2B, lane 11). When GST-Smt3 was used in place of Ull1-RING, a putative sumoylation site(s) exists within the N-terminal 111-amino acid sequence containing the SAP domain. These results suggest that in the in vitro system Ull1ΔC440 is sumoylated and an autosumoylation site(s) exists within the N-terminal 111-amino acid sequence containing the SAP domain. There are eight lysine residues in this region, but none are consensus acceptor lysine residues (IV/L)KXD(/E) for sumoylation (13).

Characterization of the N-terminal Domains—To examine which protein directly interacts with these N-terminal domains, we performed a binding assay. GST, GST-Ubc9, and GST-Smt3 proteins were prepared from $E. coli$ lysates and bound to Sepharose beads. T7-Ull1 derivatives were prepared from $E. coli$ lysates and mixed with the beads. After washing, the bound proteins were subjected to immunoblotting using an anti-T7 antibody to detect Ull1 derivatives. As shown in Fig. 3, Ubc9 strongly bound to Ull1-RING (318–465) (lane 9), which is consistent with the result of the two-hybrid interaction (15). Ubc9 bound to Ull1-N1-(112–465) (lane 7) and only weakly to Ull1-N2-(223–465) (lane 8).

In contrast, GST-Smt3 bound to Ull1-N1 (lane 10) but not to Ull1-N2 (lane 11) or Ull1-RING (lane 12). These results suggest that the N-terminal region of Ull1-N1-(112–465) is necessary for direct interaction with Smt3.

Next, we examined the localization of various GFP-tagged truncated forms of Ull1 expressed from the GAL1 promoter (Fig. 4). As shown in Fig. 4B, GFP-Ull1-N10-(1–103), containing the SAP domain, was localized in the nucleus and also diffusely in the cytoplasm. GFP-Ull1-N1-(1–326) was localized as distinct dots in the nucleus.

The C-terminal Domain of Ull1 Is Required for Bud Neck Localization—Our previous study suggested that the C-terminal half of Ull1 is required...
for the bud neck localization (22). To examine this further, we constructed various plasmids carrying each C-terminal truncated form of Ull1-GFP and expressed from its own promoter (Fig. 5A). The wild-type Ull1-(1–904)-GFP was localized at the neck region in the M phase, when the cell cycle was arrested with nocodazole (Fig. 5B). Ull1-C1-(1–793)-GFP, which lacks the C-terminal 111 amino acids, was diffusely localized in both the cytoplasm and nucleus. In contrast, Ull1-C2-(1–682)-GFP, Ull1-C3-(1–571)-GFP, Ull1ΔC440-(1–465)-GFP and Ull1-N-(1–326)-GFP were exclusively accumulated in the nucleus.

In accordance with these results, the proportion of sumoylated forms of HA-Cdc3 was 36% in cells expressing Ull1-C1-GFP (Fig. 5C, lane 2) and 15% in Ull1-C2-GFP (lane 3), when compared with the sumoylated forms of HA-Cdc3 in the wild type Ull1-GFP (lane 1). HA-tagged Cdc3 was not modified with Smt3 in the presence of Ull1-C3-GFP (6%), Ull1ΔC440-GFP (4%), or Ull1-N-GFP (3%) (Fig. 5C). Thus, the C-terminal 111-amino acid sequence of Ull1 is required for the stable localization at the neck region to recognize substrate. And the C-terminal 222-amino acid sequence of Ull1 is responsible for cytoplasmic localization in the M phase.

The Instability of the Ull1 Protein—The inability to promote the sumoylation of septin components by the Ull1 derivatives could be due to the instability of the proteins. To examine the protein level of each Ull1 derivative, immunoblotting analysis was performed, as shown in Fig. 6A. The steady state level of GFP-Ull1 (lanes 1 and 2), GFP-Ull1-C1 (lanes 3 and 4), or GFP-Ull1-C2 (lanes 5 and 6) was lower than that of the smaller proteins, GFP-Ull1-C3 (lanes 7 and 8), GFP-Ull1ΔC440 (lanes 9 and 10), or GFP-Ull1-N (lanes 11 and 12) when cells were arrested in either the S phase with hydroxyurea (HU) (odd lanes) or M phase with nocodazole (NZ) (even lanes).

To confirm that the C-terminal region is involved in protein instability, Ull1-HA and Ull1ΔC440-(1–465)-HA were expressed from the GAL1 promoter in the presence of HU or NZ, the transcription was shut off by adding glucose, and samples were taken at various time points to examine the protein decay. As shown in Fig. 6B, the protein level of Ull1-HA decreased in either the S or M phase. In contrast, the protein...
lysates were subjected to immunoblotting by probing with anti-HA. Samples were taken at 15 min (lane 2), 30 min (lane 3), and 1 h later, 2% glucose was added to shut off the transcription (lane 4). The cell lysates were subjected to immunoblotting, and anti-HA and anti-Cdc11 were used to detect Ull1-HA and Cdc11, respectively. Various mutants involved in the M phase. HA-tagged Ull1 was introduced into a temperature-sensitive mutant, particularly in the cells arrested at the restrictive temperature (lanes 3–4). Cell lysates were subjected to immunoblotting, and Ull1 was probed with anti-HA, and Cdc11 was probed with anti-Cdc11. Equal loading of each sample was confirmed by anti-PSTAIR staining. Lanes 1 and 7, Ull1-HA; lanes 2 and 8, Ull1 vector; lanes 3 and 9, Ull1S460C + CDC28; lanes 4 and 10, Ull1S460C + vector; lanes 5 and 11, vector + CDC28; lanes 6 and 12, vector.

level of Ull1ΔC440 did not change in either, indicating that Ull1ΔC440 is a stable protein. Thus, the instability of the C-terminal region of the Ull1 protein evidently influences the steady state level of the protein.

Cell Cycle-dependent Regulation of Ull1—To search for factors to affect the cell cycle dependence of the Ull1 protein level, we tested various mutants involved in the M phase. HA-tagged Ull1 was introduced into a temperature-sensitive cdc20-3 mutant. The CDC20 gene encodes an activator of anaphase-promoting complex, and a temperature-sensitive cdc20-3 mutant induces arrest at the G2/M phase with a high cyclin-dependent protein kinase (CDK) activity at the restrictive temperature. As shown in Fig. 7A, Ull1 was highly phosphorylated in the cdc20 mutant, particularly in the cells arrested at the restrictive temperature (lane 10). At the same time, Cdc11 was sumoylated in the cdc20 mutant (lower panel).

Next, we introduced HA-tagged Ull1 into cdc28-1N cells carrying an allele that specifically arrests at the G2/M phase at high temperatures. When cell extracts were prepared from random cultures, arrested in the presence of nocodazole in the M phase at 25 °C or the M phase at 37 °C, the Ull1 protein level was almost undetectable (Fig. 7A, lanes 2–4). Consistent with these results, the Smt3 conjugates of Cdc11 decreased in the cdc28-1N mutant (Fig. 7A, bottom). To confirm whether this phenotype was really CDC28-dependent, we tested another cdc28-1N strain with a different genetic background. The protein level of Ull1-HA in the mutant was very low when cells were arrested in the G2/M phase in the presence of NZ (Fig. 7B, lane 2) or at high temperatures (lane 8), and the sumoylation of Cdc11 was not detected. On the other hand, if CDC28 was supplied from a plasmid in this strain, a large amount of Ull1-HA was detected and partially phosphorylated in the presence of NZ (lane 1–6), and without NZ at 37 °C for 2 h (lanes 7–12). Cell lysates were subjected to immunoblotting, Ull1 was probed with anti-HA, and Cdc11 was probed with anti-Cdc11. Equal loading of each sample was confirmed by anti-PSTAIR staining. Lanes 1 and 7, Ull1-HA; lanes 2 and 8, Ull1 vector; lanes 3 and 9, Ull1S460C + CDC28; lanes 4 and 10, Ull1S460C + vector; lanes 5 and 11, vector + CDC28; lanes 6 and 12, vector.

FIGURE 7. Cell cycle dependence of the Ull1 protein. A, cells of KN1389 (cdc28–1N), W303–1A (wild type [WT]), and KY8029 (cdc20–3) were transformed with pT-17 (YCP-ULL1-HA) or mock vector, and the transformants were grown at 25 °C (random cultures), arrested at G2/M with NZ at 25 °C, or arrested at restrictive temperature (37 °C) for 3 h. Extracts were subjected to immunoblotting, and anti-HA and anti-Cdc11 were used to detect Ull1-HA and Cdc11, respectively. Non-specific bands are marked by an asterisk.

DISCUSSION

We characterized various truncated forms of Ull1 and identified certain critical domains. It has been established that SP-RING is essential for the ligase activity in vivo and in vitro (13–15, 22). The present study showed that the most distal N-terminal domain containing a putative
DNA binding SAP domain was not necessary for the ligase activity (Fig. 2A), but the adjacent region (the PINIT motif) of SP-RING was necessary for the ligase activity when Cdc3 was used as a substrate in the in vitro reaction, because Ull1-N1-(112–465) was functional, but Ull1-N2-(223–465) was not (Fig. 2A).

What is a role of the PINIT motif? This domain appears to be required for the direct interaction with Smt3, as shown in the binding assay (Fig. 3). Recently, a SUMO-interacting domain was identified as the SBM motif (VX(V/I/Y)YX) found in the adjacent region of the SXS motif of PIASX-N and PIASX-P, in PML-P, SAE2-P, and the IR1 region of RanBP2 (26). Ull1, a PIAS family member, contains the sequence 480PPIINL near the putative SBM motif, which could be an SBM, and the two-hybrid interaction, shown in Fig. 8, may support this notion. On the other hand, a sequence like192LVMNVEV within the Ull1 PINIT motif is a receptor for Smt3 bound to Ubc9 (27), and the two-hybrid interaction, shown in Fig. 8, may support this notion. On the other hand, a sequence like 192LVMNVEV within the PINIT motif of Ull1 might be an Smt3 binding motif, because a weak binding region of Smt3 may be located within the sequence from aa 112 to 222 (Fig. 3). Furthermore, Reverter and Lima (36) have suggested that RanBP2 acts as E3 by binding both SUMO and Ubc9 so as to position the SUMO-E2-thioester in the optimal orientation to enhance conjugation. We speculate that a putative SBM within the Ull1 PINIT motif is a receptor for Smt3 bound to Ubc9 through a thioester bond, which would be crucial for the ligase activity. In contrast, the other strong Smt3 binding region, including the putative SXS motif, shown in Fig. 8, could exert functions other than the accommodation of the SUMO-E2 complex, because it is not required for the SUMO ligase activity.

The most distal N-terminal domain seems to carry an acceptor site for autosumoylation in vitro (Fig. 2B). This domain contains a putative DNA binding SAP domain (23). Siz1/Ull1 may sumoylate a nuclear Top2 (topoisomerase II), and an excessive sumoylation of Top2 in an smt4-SUMO hydrolase mutant is reported to cause temperature-sensitive cellular growth (37). It is possible that the autosumoylation may disturb the DNA binding, which might in turn facilitate release from the chromosomes.

Although some of the truncated forms of Ull1 did not sumoylate septin components in vivo, they did possess the ligase activity in vitro (22). Thus, the localization of the E3 components is crucial for the substrate determination in vivo. The C-terminal 111-amino acid sequence was required for the stable bud neck localization of Ull1, thereby recognizing the septin components as substrates, and the C-terminal 222 amino acid sequence may be necessary for its nuclear export (Fig. 5). We tested which factor was involved in altering the subcellular localization. The result was not clear; the subcellular localization of Ull1 only partially depended on Msn5, an export carrier, and not on Xpo1.4 Another redundant carrier may export Ull1. Alternatively, it is possible that Ull1 is not a direct cargo of Msn5, and unknown Msn5-dependent factors may help Ull1 to facilitate the bud neck localization indirectly. Consistent with this hypothesis, Msn5 failed to bind to Ull1 in a binding assay using bacterially expressed Msn5 protein.4 The C-terminal domain seems to confer the protein instability on Ull1 (Fig. 6). The longer the C-terminal tail Ull1 possesses, the less stable it becomes, which suggests that multiple domains are involved. When the M phase CDK activity was low in the cdc28–1N mutant, the Ull1 protein level was also low (Fig. 7). In contrast, when the CDK activity was high in the cdc20 mutant, the protein level was high. Ull1 contains many potential CDK target sites. We found that the mutation of the classical CDK target site (serine 460) did not affect the function of ULL1 (Fig. 7B). It remains to be determined whether Ull1 is phosphorylated directly by CDK.

What is the biological significance of the long C-terminal extension of Ull1? Among the known SUMO ligases in yeast (Ull1/Siz1, Nfi1, and Msn21), Ull1 is the only protein that changes its subcellular localization in a cell cycle-dependent manner. The other yeast PIAS family proteins are always localized in the nucleus. One may speculate that this is needed to reduce the level of nuclear sumoylation in the M phase by exporting a SUMO ligase to the cytoplasm. In this case, the septin ring functions as a reservoir of the ligase. Alternatively, Ull1 might be required at the bud neck region to sumoylate septins, etc., for as yet unidentified purposes.

In summary, Ull1/Siz1, a PIAS family protein, contains multiple domains, and both the SP-RING and PINIT motifs are essential for the SUMO ligase activity. The other domains are involved in its own regulation for the protein stability and subcellular localization.

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