Pli1 \textsuperscript{PIAS1} SUMO Ligase Protected by the Nuclear Pore-associated SUMO Protease Ulp1 \textsuperscript{SENP1/2}\textsuperscript{*}

Received for publication, June 17, 2015, and in revised form, July 21, 2015 Published, JBC Papers in Press, July 28, 2015, DOI 10.1074/jbc.M115.673038

Minghua Nie and Michael N. Boddy \textsuperscript{1}
From the Department of Cell and Molecular Biology, The Scripps Research Institute, La Jolla, California 92037

**Background:** SUMO conjugation to the proteome critically controls cell growth, but mechanisms for regulating SUMO ligases are poorly defined.

**Results:** Desumoylation of the major fission yeast SUMO ligase by a nuclear pore-associated protease protects it from proteolysis.

**Conclusion:** Desumoylation of a SUMO ligase antagonizes autoinhibition of the SUMO pathway.

**Significance:** These data demonstrate cooperation between STUbL and Cdc48\(\text{p97}\) in proteasome-mediated degradation of SUMO conjugates.

Covalent modification of the proteome by SUMO is critical for genetic stability and cell growth. Equally crucial to these processes is the removal of SUMO from its targets by the Ulp1 (HuSENP1/2) family of SUMO proteases. Ulp1 activity is normally spatially restricted, because it is localized to the nuclear periphery via interactions with the nuclear pore. Delocalization of Ulp1 causes DNA damage and cell cycle defects, phenotypes thought to be caused by inappropriate desumoylation of nucleoplasmic targets that are normally spatially protected from Ulp1. Here, we define a novel consequence of Ulp1 deregulation, with a major impact on SUMO pathway function. In fission yeast lacking Nup132 (Sc/HuNUP133), Ulp1 is delocalized and can no longer antagonize sumoylation of the PIAS family SUMO E3 ligase, Pli1. Consequently, SUMO chain-modified Pli1 is targeted for proteasomal degradation by the concerted action of a SUMO-targeted ubiquitin ligase (STUbL) and Cdc48\(\text{p97}\)-Ufd1\(\text{Np4}\). Pli1 degradation causes the profound SUMO pathway defects and associated centromere dysfunction in cells lacking Nup132. Thus, perhaps counterintuitively, Ulp1-mediated desumoylation can promote SUMO modification by stabilizing a SUMO E3 ligase.

SUMO is removed by one of a small family of Ubl-specific proteases (11, 12). In yeasts there are two Ubl-specific proteases, Ulp1 and Ulp2, of which Ulp1 processes SUMO into a mature form by removing a C-terminal peptide to reveal a diglycine motif. Both Ulp1 and Ulp2 desumoylate a subset of SUMO conjugates, with specificity likely driven in large part by their spatial separation (12). Ulp1 localizes to the nuclear rim by associating with nuclear pores, whereas Ulp2 is nucleoplasmic (12–18). In higher eukaryotes, SENP1/2 localize to nuclear pores like Ulp1 (19–21), and SENP6/7, like Ulp2, are nucleoplasmic (22, 23).

Sumoylated proteins can also be ubiquitinated by a SUMO-targeted ubiquitin ligase (STUbL) to promote their degradation at the proteasome (24–26). Correlative evidence suggests that SUMO chains act as targeting signals for STUbLs. Consistent with this, high molecular weight SUMO chains accumulate in STUbL mutant cells (24–26), a phenotype also caused by Ulp2 inactivation (10, 27). Moreover, in fission yeast, the growth and genome stability defects caused by both STUbL and Ulp2 inactivation are suppressed by blocking SUMO chain formation (10, 28). In contrast, preventing SUMO chain formation in budding yeast is lethal to STUbL mutants but suppresses some ulp2\(\Delta\) defects (27, 29, 30). These findings highlight the importance of SUMO pathway homeostasis and that the “wiring” of the STUbL–SUMO interface differs notably between these yeasts (see above and Refs. 10 and 27–30).

The nuclear pore has emerged as a broadly conserved hub of SUMO-mediated signaling, impacting key processes such as transcription, chromosome segregation, and DNA repair (31, 32). However, despite functional overlap, mutants of orthologous nuclear pore components cause different phenotypes in fission and budding yeast, the latter of which has been used in

\textsuperscript{*} This work was supported, in whole or in part, by National Institutes of Health Grants GM068608 and GM081840 (to M. N. B.). The authors declare that they have no conflicts of interest with the contents of this article.

\textsuperscript{1} To whom correspondence should be addressed: Dept. of Cell and Molecular Biology, The Scripps Research Institute, 10550 N. Torrey Pines Rd., La Jolla, CA 92037. Tel.: 858-784-7042; Fax: 858-784-2265; E-mail: nboddy@scripps.edu.

\textsuperscript{2} The abbreviations used are: SUMO, small ubiquitin-like modifier; HMW, high molecular weight; STUbL, SUMO-targeted ubiquitin ligase.
most studies (33, 34). Therefore, to robustly model how the nuclear pore could impact SUMO pathway homeostasis in higher eukaryotes, it is important to determine how the nuclear pore impacts sumoylation in fission yeast.

Herein we reveal that sumoylation is globally reduced in fission yeast deleted for Nup132, which contrasts with the relatively mild and selective sumoylation defects of analogous budding yeast mutants (16, 35, 36). Nevertheless, we show that as in budding yeast (36), Ulp1 is both delocalized and less abundant in nup132Δ (Sc nup133Δ) cells. What then causes the profound SUMO conjugation defect in nup132Δ cells? Intriguingly, we found that Ulp1 dysfunction in nup132Δ cells allows an accumulation of SUMO chains on the major SUMO E3 ligase Pli1, which promote its degradation in a STUbL-, Cdc48-Ufd1-Npl4-, and proteasome-dependent manner. Moreover, this novel phenomenon allowed us to execute a detailed mechanistic dissection of SUMO pathway and cofactor requirements in the turnover of a specific STUbL substrate. Overall, our data define how the activity of a PIAS family SUMO ligase, and thus its physiological impact, e.g. centromere function, hang in the balance between its autosumoylation and desumoylation by a nuclear pore localized SUMO protease.

### Experimental Procedures

#### Yeast Strains and Growth Conditions

Standard yeast methods were performed as described previously (37). The strains used in this study are listed in Table 1.

#### Spot Assays

The cells were grown at 25 °C to logarithmic phase (A600 of 0.6–0.8), spotted on agar plates in 5-fold dilutions from a starting A600 of 0.5 and then incubated at 25–35 °C for 3–5 days.

#### Fluorescence Microscopy

GFP fusion proteins and DAPI staining were imaged in live cells using an Eclipse E800 microscope (Nikon Metrology) with a 100× Plan Differential Interference Contrast H oil immersion objective. The images were captured through an INFINITY 3 charge-coupled device camera using the INFINITY ANALYZE software (Lumenera Corporation).

#### Western Blotting

Exponentially growing cells (~15 A600 units) were washed with STOP buffer (10 mM EDTA, 50 mM NaF, 150 mM NaCl, 1 mM NaN3). The pellets were flash-frozen in liquid nitrogen, and the cells were lysed by beating twice at 5.0 m/s for 20 s in a FastPrep-24 instrument (MP Biochemicals) in 200 μl of 20% TCA supplied with 100 ml silica-zirconia beads (BioSpec Products). After bead beating, 400 μl of 5% TCA was

---

**TABLE 1**

List of yeast strains used in this study

| Strain       | Genotype*          | Source   |
|--------------|--------------------|----------|
| NBY752       | imr1R (dg-glu)NcoI::ura4 orl ade6-210/ade6-216 | Ref. 31  |
| NBY5460      | nup132::ura4*      | Ref. 33  |
| NBY780       | h*                 |          |
| NBY781       | h*                 |          |
| NBY1493      | pli1::kanMX6       |          |
| NBY1820      | pli1::TAPkanMX6    |          |
| NBY1967      | pli1::hphMX6 imr1R (dg-glu)NcoI::ura4 orl ade6-210/ade6-216 |          |
| NBY2471      | slo8-29::kanMX6    |          |
| NBY2754      | pREP2::ura4*       |          |
| NBY2756      | slo8-29::kanMX6 pREP2::ura4* integrated at ars1 |          |
| NBY2927      | ulp2::kanMX6       |          |
| NBY3063      | ulp1::GFP-kanMX6   |          |
| NBY3172      | ulp2::hphMX6       |          |
| NBY5159      | ulp1-myc13::kanMX6 |          |
| NBY5428      | nup132::ura4*      |          |
| NBY5467      | nup132::ura4* ulp2::kanMX6 |          |
| NBY5468      | nup132::ura4* ulp1::GFP-kanMX6 |          |
| NBY5471      | pREP41::SUMOFL::LEU2 pREP2::ura4* integrated at ars1 |          |
| NBY5472      | pREP41::SUMOGL::LEU2 pREP2::ura4* integrated at ars1 |          |
| NBY5473      | slo8-29::kanMX6 pREP41::SUMOFL::LEU2 pREP2::ura4* integrated at ars1 |          |
| NBY5474      | slo8-29::kanMX6 pREP41::SUMOGL::LEU2 pREP2::ura4* integrated at ars1 |          |
| NBY5475      | nup132::ura4*      |          |
| NBY5476      | nup132::ura4* pREP41::SUMOGL::LEU2 |          |
| NBY5477      | nup132::ura4* slo8-29::kanMX6 pREP41::SUMOGL::LEU2 |          |
| NBY5478      | nup132::ura4* slo8-29::kanMX6 pREP41::SUMOFL::LEU2 |          |
| NBY5506      | pli1::TAPkanMX6 nup132::ura4* |          |
| NBY5528      | ulp1::kanMX6       |          |
| NBY5544      | pli1::TAPkanMX6 nup132::ura4* pREP41::ulp1::LEU2 |          |
| NBY5546      | pli1::TAPkanMX6 nup132::ura4* slo8-29::hphMX6 |          |
| NBY5547      | pli1::TAPkanMX6 slo8-29::hphMX6 |          |
| NBY5548      | pli1::TAPkanMX6 slo8-29::ura4* ufd1-1::natMX6 |          |
| NBY5549      | pli1::TAPkanMX6 ufd1-1::natMX6 |          |
| NBY5550      | pli1::TAPkanMX6 nup132::ura4* mts3-1 |          |
| NBY5551      | pli1::TAPkanMX6 mts3-1 |          |
| NBY5569      | pli1::TAPkanMX6 nup132::ura4* slo8-29::hphMX6 pREP41::HIS-SUMOFL::LEU2 |          |
| NBY5573      | pli1::TAPkanMX6 ulp1::kanMX6 |          |
| NBY5577      | pli1::kanMX6 pREP41::pli1::LEU2 |          |
| NBY5579      | pli1::kanMX6 pREP41::ura4* |          |
| NBY5582      | pli1::TAPkanMX6 nup132::ura4* SUMO1443X |          |
| NBY5593      | pli1::TAPkanMX6 nup132::ura4* SUMO1443X |          |
| NBY5620      | pli1::TAPkanMX6 nup132::ura4* imr1R (dg-glu)NcoI::ura4 orl ade6-210/ade6-216 |          |
| NBY5621      | pli1::TAPkanMX6 nup132::ura4* SUMO1443X |          |
| NBY5633      | pli1::kanMX6 ulp2::hphMX6 |          |
| NBY5665      | ulp1-myc13::kanMX6 nup132::hphMX6 |          |

*All strains are of *ura4-D18 leu1-32* background genotype, unless otherwise stated. Double colons represent knockouts; single colons represent tagging. A reference is given for strains not generated in this study.
added, and the total cell lysate was centrifuged at 16,000 × g for 5 min at 4 °C. The pellet was washed twice with 0.1% TCA. The precipitated proteins were resuspended in 8 M urea, 50 mM Tris, pH 8.5, 150 mM NaCl. Protein was quantitated by measuring absorbance at A280, and 60 µg of proteins were resolved on a gradient SDS-PAGE gel and transferred to a nitrocellulose membrane. The membrane was blocked in 1% (w/v) nonfat milk in phosphate-buffered saline solution with 0.1% (v/v) Tween 20, probed with peroxidase anti-peroxidase and then detected using an ECL Dura system (Pierce) or probed with antibodies against α-tubulin, Pmt3 (SUMO) and then an IRDye-conjugated secondary antibody and imaged on an ODYSSEY scanner (Li-Cor).

Nickel-Nitrilotriacetic Acid Pulldown—Proteins from cell pellets were extracted by TCA precipitation as described above, and 20 mg of proteins were incubated with 50 µl of nickel-nitrilotriacetic acid beads (Qiagen) in the presence of 15 mM imidazole at room temperature for 1 h. The beads were then washed three times with 8 M urea, 50 mM Tris, pH 8, 150 mM NaCl, and 20 mM imidazole and eluted with 2× LDS sample loading buffer (Invitrogen) at 70 °C for 10 min. Input, flow through, and one-third of the nickel pulldown were resolved by SDS-PAGE and detected by peroxidase anti-peroxidase Western blotting.

RNA Extraction and RT-Quantitative PCR—Total RNA was extracted from ~5 A600 units of exponentially growing cells. The cells were lysed by bead beating in 0.8 ml of TRizol (Invitrogen), using the equipment and settings described above. RNA extraction with TRizol was performed by following the manufacturer’s instructions. The extracted RNA was treated with DNase I (New England Biolabs) and cleaned up with the RNeasy Plus minikit (Qiagen). A total of 1 µg of DNase-treated RNA was reverse transcribed using the ProtoScript® II first strand cDNA synthesis kit (New England Biolabs). The completed reactions were diluted 10-fold, and 5 µl of the dilutions was used as the template in 20-µl quantitative PCR mixtures using the SensiFAST SYBR No-ROX kit (Bioline), and the quantitative PCRs were carried out using the Chromo4 system (Bio-Rad).

Plasmid Construction—Unless otherwise stated, cDNAs were cloned by PCR amplification using specific primers and PrimeStar DNA polymerase (Clontech) followed by ligation into the pREP series of yeast shuttle vectors (38). Details regarding plasmid construction are available on request.

Results

Nuclear Pore Mutant Suppresses Phenotypes of STUbL Dysfunction—To assess roles of fission yeast Nup132 in STUbL pathway homeostasis and genome stability, we analyzed genetic interactions between its deletion (nup132Δ) and alleles of STUbL (slx8-29) and Ulp2 (ulp2Δ). As in previous reports, nup132Δ cells grew similarly to wild type and exhibited little to no sensitivity to genotoxins or elevated temperatures (Fig. 1A) (33, 34). These subtle phenotypes are consistent with the fact that protein import/export and RNA export pathways are intact in nup132Δ cells and that Nup132 has an expressed paralog called Nup131 (33, 34).

Intriguingly, deletion of Nup132 strongly suppressed the growth defects and genotoxin sensitivity of slx8-29 (Fig. 1A). This was surprising, because combined deletions of the analogous factors in budding yeast are synthetically sick (36). As observed for slx8-29, we found that ulp2Δ phenotypes were also suppressed by deleting Nup132 (Fig. 1A). Because an accumulation of SUMO conjugates, particularly chains, drives slx8-29 and ulp2Δ phenotypes (10, 28, 39), we analyzed global sumoylation in Nup132 deleted cells.

At permissive temperature, hypomorphic slx8-29 cells have increased levels of high molecular weight (HMW) SUMO conjugates as compared with wild type (Fig. 1B) (28). In contrast, both nup132Δ and slx8-29 nup132Δ cells had less HMW SUMO conjugates than wild type (Fig. 1B). Moreover, at restrictive temperature, the strong accumulation of HMW SUMO conjugates in slx8-29 cells was absent in slx8-29 nup132Δ cells (Fig. 1B). There was also a lower level of HMW SUMO conjugates in ulp2Δ nup132Δ double mutant versus ulp2Δ single mutant cells (Fig. 1B). Therefore, for as yet undefined reasons, nup132Δ cells exhibit reduced global sumoylation that correlates with the rescue of STUbL and Ulp2 SUMO protease dysfunction.

Altered Ulp1 Activity in nup132Δ Cells—Ulp1 endogenously tagged with GFP (Ulp1-GFP) gives a weak fluorescence signal but shows that fission yeast Ulp1 exhibits a perinuclear localization in live as well as fixed cells (Fig. 2A) (14). Based on
analyses of Ulp1 orthologs in other species, this likely reflects its nuclear pore association (32). Budding yeast Ulp1 is anchored at the nuclear pore via a complex set of interactions between the Ulp1 N terminus and several nuclear pore-associated factors, and both its anchoring and protein stability are disrupted in cells lacking Nup133 (fission yeast Nup132; reviewed in Ref. 32). Similarly, we found that both the perinuclear signal of Ulp1 and the level of full-length protein were reduced in nup132Δ cells (Fig. 2, A and B). Thus, the anchoring mechanism of Ulp1 at the nuclear pore is broadly conserved, but the consequences of Ulp1 delocalization and destabilization for SUMO pathway homeostasis are different.

Because Ulp1 functions in SUMO maturation, we next tested whether reduced sumoylation in nup132Δ cells was due to inefficient SUMO maturation. We ectopically expressed either full-length (SUMOFL) or the mature form (SUMOGG) of SUMO, which both ran at the size of mature SUMO (Fig. 2C, black arrowhead) in wild type cells, indicating that normal Ulp1 activity can fully process overexpressed full-length SUMO. Ulp1 maturation activity is slightly compromised in nup132Δ cells, because they show a small amount of residual immature full-length SUMO (Fig. 2C, white arrowhead). However, because SUMO conjugation in nup132Δ cells is low and unchanged whether they express full-length or mature SUMO (Fig. 2C), we conclude that reduced sumoylation in nup132Δ cells is not caused by a SUMO maturation defect.

Interestingly, nup132Δ slx8-29 cells exhibited a major increase in SUMO conjugates, to a level exceeding that of slx8-29 single mutant cells (Fig. 2C). Therefore, the inactivation of STUbL, when combined with higher levels of SUMO, restores global sumoylation in nup132Δ cells. Enhanced global sumoylation in nup132Δ slx8-29 as compared with slx8-29 cells is consistent with reduced Ulp1-mediated desumoylation in nup132Δ mutants.

The PIAS Family Ligase Pli1 Is Destabilized in Cells Lacking Nup132—In fission yeast, the sole PIAS family ligase called Pli1 is responsible for generating the majority (>90%) of SUMO conjugates detectable by Western blotting (8, 10, 28, 40). Although Pli1 deletion does not overtly affect cell growth, it strongly suppresses STUbL mutants, disrupts centromere function, and causes telomere elongation (8, 10, 28, 40, 41). Because nup132Δ cells share the pli1Δ phenotypes of global hyposumoylation, elongated telomeres, and suppression of STUbL dysfunction (Fig. 1, A and B) (28, 42, 43), we analyzed Pli1 levels in nup132Δ cells.

The stability of endogenous Pli1 was monitored over time in cells treated with cycloheximide, an inhibitor of protein synthesis. Intriguingly, we found that Pli1 was both less abundant and more rapidly degraded in nup132Δ versus wild type cells (Fig. 3A). Given that STUbL inactivation can restore global sumoylation in nup132Δ cells (Fig. 2C), we tested whether the slx8-29 mutation affected Pli1 stability. Strikingly, whereas Pli1 was only weakly detectable in nup132Δ cells, Pli1 abundance and a series of lower mobility species were dramatically increased in slx8-29 nup132Δ cells (Fig. 3B). Neither Pli1 levels nor its gel migration were affected in slx8-29 single mutant cells (Fig. 3B).

We and others recently proposed a cooperative role for STUbL and Cdc48 (p97) AAA+ ATPase in the proteasomal degradation of SUMO conjugates, at a global level (44, 45). Consistent with this proposal, mutations in the Cdc48 cofactor Ufd1 (ufd1-1) or in the 19S proteasome regulatory subunit Mts3 (mts3-1) also stabilized Pli1 in nup132Δ cells (Fig. 3B).

Direct Evidence that SUMO Chains Are Required for STUbL Targeting—The role of STUbL in targeting SUMO-conjugated proteins is now well established, and notably, slower migrating forms of Pli1 accumulate in slx8-29 nup132Δ cells (Fig. 3B). To confirm that these species are SUMO-conjugated Pli1, we purified SUMO conjugates under denaturing conditions from slx8-29 nup132Δ cells and probed for Pli1 (Fig. 4A). This approach enriched only the slower migrating Pli1 species and revealed higher order polysumoylation of Pli1 (Fig. 4A, white arrowheads).

STUbLs are thought to target proteins that are modified by SUMO chains, and strong correlative data have been published (see Refs. 25, 26, and 46–48). To test the role of SUMO chains more directly, we used two SUMO mutants that we previously showed block SUMO chain formation in different ways (10). SUMO chains form through Lys14 and Lys30 in fission yeast SUMO and are therefore absent in SUMO<sup>K14,30R</sup> mutant cells (10, 49). Strikingly, Pli1 was stabilized in nup132Δ SUMO<sup>K14,30R</sup> cells (Fig. 4B), to an extent similar to
that seen in nup132Δ slx8-29 cells (Fig. 3B). Moreover, the SUMOΔ22682 mutation, which abolishes the noncovalent SUMO:Ubc9 complex required for SUMO chain formation (10), also stabilized Pli1 in nup132Δ cells (Fig. 4A).

In light of the above data, it is interesting to note that the absence of SUMO chains in SUMOΔ14,30Δ, SUMOΔ22682, or pli1Δ cells suppresses all tested STUbL mutant phenotypes (10, 28). Explaining this epistatic relationship, as demonstrated for Pli1, STUbL is only engaged when SUMO chains are present. Overall, these data provide direct mechanistic insight into the role of SUMO chains in STUbL targeting.

**Ulp1 Protects Pli1 from Sumoylation and STUbL-mediated Degradation**—Given that the Ulp1 SUMO protease is deregulated in nup132Δ/H9004 cells, we tested whether increased Ulp1 dosage would impact the SUMO conjugation state and stability of Pli1. Strikingly, overexpression of Ulp1 in nup132Δ cells restored normal Pli1 levels (Fig. 4C). Moreover, Pli1 was restored in a hyposumoylated form, consistent with Ulp1 desumoylating and protecting it against STUbL-dependent degradation (Fig. 4C). Because budding yeast Ulp1 modulates transcription of the GAL1 gene (50), we monitored transcript levels of Pli1 upon Ulp1 overexpression. Whereas Ulp1 overexpression yields the anticipated increase in Ulp1 transcript levels, no difference in Pli1 transcription was detected (Fig. 4D). These results suggest that Ulp1 is normally optimally positioned to desumoylate and stabilize Pli1 and that increased Ulp1 dosage can compensate for this loss of spatial regulation.

**Pli1 Overexpression Reverses SUMO-related Phenotypes of nup132Δ Cells**—Pli1 acts at the end of the SUMO conjugation pathway to attach activated SUMO to its substrates. Therefore, if mature SUMO or E1/E2 factors were limiting, this would render the SUMO pathway unresponsive to the presence of Pli1. Therefore, to test whether Pli1 is the major or sole SUMO pathway factor deregulated by deleting Nup132, we overexpressed Pli1 in pli1Δ, nup132Δ, and nup132Δ slx8-29 cells. As anticipated, expression of Pli1 in pli1Δ control cells restored global SUMO conjugates to a level higher than that seen in wild type (Fig. 5A). Increased Pli1 dosage also restored SUMO con-
Ulp1 Desumoylates Pli1

Compromised Centromere Function in Cells Lacking Nup132—Centromere function is disrupted in pli1Δ cells, whereby a ura4+ marker inserted at the heterochromatic inner repeats (imr) is constitutively expressed, versus the variegating phenotype in wild type cells (40). We therefore assessed chromatin function at imr in nup132Δ cells. As expected, wild type cells carrying the ura4+ insertion at imr (imrura4+) exhibited variegated expression, being able to grow both in the absence of uracil (leucine adenine histidine) and in the presence of 5-fluoroorotic acid (51). However, both pli1Δ imrura4+ and nup132Δ imrura4+ cells grew poorly in the presence of FOA but robustly in the absence of uracil (Fig. 5D). This result is consistent with the constitutive degradation of Pli1 in nup132Δ cells, causing hyposumoylation of key epigenetic regulators.

Discussion

Herein, we reveal a key novel consequence of nuclear pore dysfunction and subsequent spatial deregulation of Ulp1, which has a major impact on global sumoylation and centromere function. In budding yeast that lack Nup133 (SpNup132), Nup60, or Nup120, Ulp1 is destabilized and mislocalized in the nucleoplasm, causing altered global SUMO conjugate patterns and associated defects in genetic stability (16, 31, 35, 36, 52). A gain of Ulp1 function that promotes the desumoylation of normally spatially protected nucleoplasmic SUMO conjugates is proposed to cause these phenotypes. Such promiscuous desumoylation of nucleoplasmic SUMO conjugates by delocalized Ulp1 in nup132Δ fission yeast is also likely.

Interestingly, however, our results demonstrate that the residual Ulp1 activity in nup132Δ cells is unable to counteract Pli1 sumoylation. This leads to Pli1 degradation and associated hyposumoylation phenotypes. It will be interesting to determine whether this novel mechanism contributes to the SUMO conjugation defects reported for certain budding yeast nucleoporin mutants (16, 31, 35, 36, 52). In this regard, STUbL degrades the nuclear pool of a budding yeast PIAS family ligase, Siz1, particularly when its nuclear export is inhibited (53). Therefore, although untested, it is possible that nuclear pore-associated Ulp1 desumoylates Siz1 and protects it from degradation. If so, given the evolutionary divergence of fission and budding yeast, conservation of this mechanism in human cells seems likely.

How Ulp1, which is normally tethered at the nuclear periphery, accesses nucleoplasmic Pli1 is an interesting question for the future. We determined that increasing Ulp1 dosage in nup132Δ cells stabilized Pli1. This is consistent with increased Ulp1 activity compensating for the loss of a normally more coordinated interaction with Pli1. For example, fission yeast centromeres cluster at the nuclear periphery and are subject to functionally critical Pli1-dependent sumoylation (40, 54).
Therefore, Pli1 acting at centromeres is also spatially organized at the nuclear periphery, more proximal to Ulp1.

In addition, budding yeast Ulp1 was recently shown to desumoylate transcription factors on chromatin to facilitate transcriptional activation, providing an example of recruitment of Ulp1 targets to the nuclear pore (50). Interestingly, a subcomplex of the human nuclear pore including Nup133 transiently localizes to centromeres and contributes to their mitotic function (55). Therefore, it will be interesting to determine whether Nup133-associated SENP2 (56) also modulates centromere/kinetochore sumoylation to support its function. Relocalization of the human pore complex and SUMO protease, rather than the target, may be a result of the open mitosis in human cells versus the closed mitosis of yeast.

Based on high throughput analysis, nup132Δ cells share another phenotype of piliΔ cells, that is, highly elongated telomeres (40, 43). Notably, like centromeres, telomeres are also clustered at the nuclear periphery and are subject to Pli1-dependent regulation (40, 41). Pli1 sumoylates the telomere protein Tpz1 to support a mechanism for inhibiting excessive telomerase activity at chromosome ends (57). Therefore, based on the synonymous centromere silencing roles of Pli1 and Nup132, we envisage that Tpz1 will be hypusinomylated in cells lacking Nup132, leading to telomere elongation.

Identification of Pli1 as a STUBL substrate has enabled a comprehensive analysis of SUMO pathway and ancillary factor requirements. The role of SUMO chains in STUBL targeting has been largely inferred from (i) the accumulation of SUMO chain species when STUbL activity is compromised, (ii) the multivalent SUMO-interacting motif arrangement in STUbLs, and (iii) SUMO chain-induced autoubiquitination/degradation of RNFL (25, 47, 58). We now demonstrate directly that the STUbL pathway is dependent on the chain forming lysine residues of SUMO, as well as the noncovalent SUMO-Ubc9 interface that promotes SUMO chain formation. These data fit well with the epistatic relationship of SUMO chain and STUbL mutants in fission yeast (10).

In addition, Cdc48-Ufd1-Npl4 was recently identified as a cofactor for STUbL in managing global SUMO conjugate levels (44, 45). However, evidence for a cooperative role in degrading a specific substrate was lacking. Herein, we show that both STUbL and Ufd1 are indeed involved in the proteasome-dependent turnover of Pli1 in nup132Δ cells.

As in fission yeast (44, 45), budding yeast Cdc48 also acts as a SUMO-targeted segregase that removes sumoylated DNA repair factors from chromatin (59). Given this degree of functional conservation, it seems likely that the human STUbL RNFL and p97 cooperate in the remodeling of chromatin e.g. at DNA repair sites, as reported independently for each factor (60–63).

**Author Contributions**—M. N. and M. N. B. were both involved in study design, experimental execution, and writing of the manuscript.

**Acknowledgments**—We thank Dr. Takegawa (Kyushu University) for the nup132::ura4+ strain and Dr. Felicity Watts (University of Sussex) for the upl1-1/myc3kanMX6 strain. We thank Emily Arner for technical assistance. We also thank members of the Cell Cycle Groups at the Scripps Research Institute for support.

**References**

1. Cuberas-Potts, C., and Matunis, M. I. (2013) SUMO: a multifaceted modifier of chromatin structure and function. *Dev. Cell* 24, 1–12
2. Girdwood, D. W., Tatham, M. H., and Hay, R. T. (2004) SUMO and transcriptional regulation. *Semin. Cell Dev. Biol.* 15, 201–210
3. Jackson, S. P., and Durocher, D. (2013) Regulation of DNA damage responses by ubiquitin and SUMO. *Mol. Cell* 49, 795–807
4. Kerscher, O., Felberbaum, R., and Hochstrasser, M. (2006) Modification of proteins by ubiquitin and ubiquitin-like proteins. *Annu. Rev. Cell Dev. Biol.* 22, 159–180
5. Felberbaum, R., and Hochstrasser, M. (2008) Ulp2 and the DNA damage response: desumoylation enables safe passage through mitosis. *Cell Cycle* 7, 52–56
6. Flotho, A., and Melchior, F. (2013) Sumoylation: a regulatory protein modification in health and disease. *Annu. Rev. Biochem.* 82, 357–385
7. Bawa-Khalfe, T., and Yeh, E. T. (2010) SUMOylation: a regulatory protein modification in health and disease. *Annu. Rev. Biochem.* 82, 357–385
8. Watts, F. Z., Skilton, A., Ho, J. C., Boyd, L. K., Trickey, M. A., Gardner, L., Ogi, F. X., and Outwin, E. A. (2007) The role of Schizosaccharomyces pombe SUMO ligases in genome stability. *Biochem. Soc. Trans.* 35, 1379–1384
9. Rytinki, M. M., Kaikkonen, S., Pekhonen, P., Jääskeläinen, T., and Palvimo, J. J. (2009) PIAS proteins: pleiotropic interactors associated with SUMO. *Cell. Mol. Life Sci.* 66, 3029–3041
10. Prudden, J., Perry, I. J., Nie, M., Vashi, A. A., Arvai, A. S., Hitomi, C., Guenther, G., Wohlschlegel, J. A., Tainer, J. A., and Boddy, M. N. (2011) DNA repair and global sumoylation are regulated by distinct Ubc9 noncovalent complexes. *Mol. Cell Biol.* 31, 2299–2310
11. Mukhopadhyay, D., and Dasso, M. (2007) Modification in reverse: the SUMO-specific protease, SENP1.
12. Mukhopadhyay, D., Ayaydin, F., Kolli, N., Tan, S. H., Anan, T., Kametaka, A., Azuma, Y., Wilkinson, K. D., and Dasso, M. (2006) SUSP1 antagonizes protein Tpz1 to support a mechanism for inhibiting excessive telomerase activity at chromosome ends (57).
13. Jongjitwimol, J., Feng, M., Zhou, L., Wilkinson, O., Small, L., Baldock, R., Taylor, D. L., Smith, D., Bowler, L. D., Morley, S. J., and Watts, F. Z. (2014) The S. pombe translation initiation factor eIF4G is Sumoylated and associates with the SUMO protease Ulp2. *PLoS One* 9, e94182
14. Taylor, D. L., Ho, J. C., Oliver, A., and Watts, F. Z. (2002) Cell-cycle-dependent localisation of Ulp1, a Schizosaccharomyces pombe Pmt3 (SUMO)-specific protease. *J. Cell Sci.* 115, 1113–1122
15. Li, S. J., and Hochstrasser, M. (2000) The yeast ULP2 (SMT4) gene encodes a novel protease specific for the ubiquitin-like Smt3 protein. *Mol. Cell Biol.* 20, 2367–2377
16. Zhao, X., Wu, C. Y., and Blobel, G. (2004) Mlp-dependent anchorage and stabilization of a desumoylating enzyme is required to prevent clonal lethality. *J. Cell Biol.* 167, 605–611
17. Panse, V. G., Küster, B., Gerstberger, T., and Hurt, E. (2003) Unconventional tethering of Ulp1 to the transport channel of the nuclear pore complex by karyopherins. *Nat. Cell Biol.* 5, 1113–1122
18. Li, S. J., and Hochstrasser, M. (2003) The Ulp1 SUMO isopeptidase: distinct domains required for viability, nuclear envelope localization, and progression. *Dev. Cell* 7, 52–56
19. Panse, V. G., Küster, B., Gerstberger, T., and Hurt, E. (2003) Unconventional tethering of Ulp1 to the transport channel of the nuclear pore complex by karyopherins. *Nat. Cell Biol.* 5, 1113–1122
20. Panse, V. G., Küster, B., Gerstberger, T., and Hurt, E. (2003) Unconventional tethering of Ulp1 to the transport channel of the nuclear pore complex by karyopherins. *Nat. Cell Biol.* 5, 1113–1122
21. Panse, V. G., Küster, B., Gerstberger, T., and Hurt, E. (2003) Unconventional tethering of Ulp1 to the transport channel of the nuclear pore complex by karyopherins. *Nat. Cell Biol.* 5, 1113–1122
22. Panse, V. G., Küster, B., Gerstberger, T., and Hurt, E. (2003) Unconventional tethering of Ulp1 to the transport channel of the nuclear pore complex by karyopherins. *Nat. Cell Biol.* 5, 1113–1122
23. Panse, V. G., Küster, B., Gerstberger, T., and Hurt, E. (2003) Unconventional tethering of Ulp1 to the transport channel of the nuclear pore complex by karyopherins. *Nat. Cell Biol.* 5, 1113–1122
gel, J. A., Yates, J. R., 3rd, and Boddy, M. N. (2012) Dual Recruitment of Cdc6/4 (p97)-Ufd1-Npl4 Ubiquitin-selective segrege by small ubiquitin-like modifier protein (SUMO) and ubiquitin in SUMO-targeted ubiquitin ligase-mediated genome stability functions. J. Biol. Chem. 287, 29610–29619

46. Hunter, T., and Sun, H. (2008) Crosstalk between the SUMO and ubiquitin pathways. Ernst Schering Found Symp Proc 1–16

47. Rojas-Fernandez, A., Plechanovova, A., Hattersley, N., Jaffrey, E., Tatham, M. H., and Hay, R. T. (2014) SUMO chain-induced dimerization activates RNF4. Mol. Cell 53, 880–892

48. Praefcke, G. J., Hofmann, K., and Dohmen, R. J. (2012) SUMO playing tag with ubiquitin. Trends Biochem. Sci. 37, 23–31

49. Skiltton, A., Ho, J. C., Mercer, B., Outwin, E., and Watts, F. Z. (2009) SUMO chain formation is required for response to replication arrest in S. pombe. PLoS One 4, e7670

50. Texari, L., Dieppois, G., Vinciguerra, P., Contreras, M. P., Groner, A., Letourneau, A., and Stutz, F. (2013) The nuclear pore regulates GAL1 gene transcription by controlling the localization of the SUMO protease Ulp1. Mol. Cell 51, 807–818

51. Allshire, R. C., Nimmoh, E. R., Ekwall, K., Javerzat, J. P., and Cranston, G. (1995) Mutations deregressing silent centromeric domains in fission yeast disrupt chromosome segregation. Genes Dev. 9, 218–233

52. Bennett, C. B., Lewis, L. K., Karhikyan, G., Bolachev, K. S., Jin, Y. H., Sterling, J. F., Snape, J. R., and Resnick, M. A. (2001) Genes required for ionizing radiation resistance in yeast. Nat. Genet. 29, 426–434

53. Westerbeck, J. W., Pasupala, N., Guillotte, M., Szymanski, E., Matson, B. C., Esteban, C., and Kerscher, O. (2014) A SUMO-targeted ubiquitin ligase is involved in the degradation of the nuclear pool of the SUMO E3 ligase Siz1. Mol. Biol. Cell 25, 1–16

54. Funabiki, H., Haban, I., Zawada, S., and Yanagida, M. (1993) Cell cycle-dependent specific positioning and clustering of centromeres and telomeres in fission yeast. J. Cell Biol. 121, 961–976

55. Zuccolo, M., Alves, A., Galy, V., Bolhney, S., Formstecker, E., Racine, V., Sibarita, J. B., Fukagawa, T., Shiekhattar, R., Yen, T., and Doye, V. (2007) The human Nup107–160 nuclear pore complex subcomponents to proper kinetochore functions. EMBO J. 26, 1853–1864

56. Goeres, J., Chan, P. K., Mukhopadhay, D., Zhang, H., Raught, B., and Matunis, M. I. (2011) The SUMO-specific isopeptidase SENP2 associates dynamically with nuclear pore complexes through interactions with karyopherins and the Nup107–160 nucleoporin subcomplex. Mol. Biol. Cell 22, 4868–4882

57. Miyagawa, K., Low, R. S., Santosa, V., Tsuji, H., Moser, B. A., Fujisawa, S., Harland, J. L., Raguinou, O. N., Go, A., Ueno, M., Matsuyama, A., Yoshiba, M., Nakamura, T. M., and Tanaka, K. (2014) SUMOylation regulates telomere length by targeting the shelterin subunit Tpz1(Tpp1) to modulate shelterin-Sirt1 interaction in fission yeast. Proc. Natl. Acad. Sci. U.S.A. 111, 5950–5955

58. Heideker, J., Perry, J. J., and Boddy, M. N. (2009) Genome stability roles of SUMO-targeted ubiquitin ligases. DNA Repair 8, 517–524

59. Bergink, S., Ammon, T., Kern, M., Schermelleh, L., Leonhardt, H., and Jentsch, S. (2013) Role of Cdc6/4(p97) as a SUMO-targeted segregase cing Rad51-Rad52 interaction. Nat. Cell Biol. 15, 526–532

60. Acx, S., Lijsterburg, M., Ackermann, L., Salomons, F. A., Hoppe, T., and Dantuma, N. P. (2011) The AAA-ATPase VCP/p97 promotes 53BP1 interaction. Nat. Struct. Mol. Biol. 18, 1345–1350

61. Meerang, M., Ritz, D., Paliwal, S., Garajova, Z., Bosshard, M., Mailand, N., Janscak, P., Hübscher, U., Meyer, H., and Ramadan, K. (2011) The ubiquitin-fusion degradation protein 1 (Ufd1) and Sumo-targeted ubiquitin ligases (STUbLs) in the DNA-damage response. PLoS One 8, e80442

62. Nie, M., Aslanian, A., Prudden, J., Heideker, J., Vashisht, A. A., Wohlschle-