

TORC1-dependent sumoylation of Rpc82 promotes RNA polymerase III assembly and activity

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Maintaining cellular homeostasis under changing nutrient conditions is essential for the growth and development of all organisms. The mechanisms that maintain homeostasis upon loss of nutrient supply are not well understood. By mapping the SUMO proteome in *Saccharomyces cerevisiae*, we discovered a specific set of differentially sumoylated proteins mainly involved in transcription. RNA polymerase III (RNAPIII) components, including Rpc53, Rpc82, and Ret1, are particularly prominent nutrient-dependent SUMO targets. Nitrogen starvation, as well as direct inhibition of the master nutrient response regulator target of rapamycin complex 1 (TORC1), results in rapid desumoylation of these proteins, which is reflected by loss of SUMO at *tRNA* genes. TORC1-dependent sumoylation of Rpc82 in particular is required for robust *tRNA* transcription. Mechanistically, sumoylation of Rpc82 is important for assembly of the RNAPIII holoenzyme and recruitment of Rpc82 to *tRNA* genes. In conclusion, our data show that TORC1-dependent sumoylation of Rpc82 bolsters the transcriptional capacity of RNAPIII under optimal growth conditions.

Sumo | transcription | RNA polymerase III | *tRNA* | TORC1

In yeast and in more complex eukaryotes, cell growth is restricted by the rate of mRNA translation and ribosome biogenesis, which depend on the transcription of ribosomal protein genes (RPGs), *tRNAs* and *rRNAs*. Synthesis of *rRNA*, *tRNAs*, and 5S *rRNA* represents 75% of total cellular transcription, whereas transcription of RPGs corresponds to 50% of RNA polymerase II (RNAPII) initiation events (1). These processes consume a significant portion of the cell's resources, making nutrient availability a limiting factor to cell growth and proliferation (2). The conserved rapamycin-sensitive target of rapamycin complex 1 (TORC1) is a master regulator of the cellular nutrient response (2, 3). Under nitrogen-rich conditions, TORC1 promotes growth-related processes, like protein synthesis, ribosome biogenesis, and *tRNA* synthesis, while inhibiting catabolic processes, like autophagy (2). Conversely, inhibition of TORC1 activity by nitrogen depletion (or addition of the TORC1 inhibitor rapamycin) results in a metabolic switch from anabolism to catabolism, which involves many cellular processes, including down-regulation of transcription of RPGs, *rRNA* and *tRNA* genes (2, 3).

A key downstream target of TORC1 in regulation of *tRNA* transcription is the conserved RNAPIII inhibitor Maf1, which is phosphorylated and maintained in the cytoplasm under nitrogen-rich conditions (2, 3). Maf1 becomes hypophosphorylated under conditions that inhibit TORC1, allowing it to enter the nucleus where it associates with TFIIB. The interaction between Maf1 and TFIIB prevents the recruitment of RNAPIII and precludes transcription reinitiation at 5S *rRNA* and *tRNA* genes (4, 5). However, expression of an unphosphorylatable *maf1* mutant does not completely repress *tRNA* expression in nutrient-replete cells (6), suggesting that dephosphorylation of Maf1 alone is not sufficient to fully inhibit RNAPIII. Indeed, inhibition of TORC1 also results in phosphorylation of the RNAPIII subunit Rpc53 by the kinases Kns1 and Mck1, which inhibits RNAPIII activity (7).

Nonetheless, mutation of the Kns1/Mck1 phosphorylation sites on Rpc53 is not sufficient to completely prevent inactivation of RNAPIII by rapamycin (7), indicating that additional post-translational modifications may exist that regulate RNAPIII under changing nutrient conditions.

The ubiquitin family member SUMO has important functions in maintaining cell homeostasis (8). For instance, we recently discovered that SUMO is important for transcription of nutrient-dependent genes, such as RPGs (9, 10). In the present study, we analyzed the plasticity of the SUMO proteome and the genome-wide localization of SUMO on chromatin in nutrient-rich and nutrient-poor conditions. We demonstrate that TORC1-dependent sumoylation of the RNAPIII subunit Rpc82 promotes assembly of the RNAPIII complex to stimulate *tRNA* transcription under optimal growth conditions.

Results

Starvation-Induced Remodeling of the SUMO Proteome. To better understand the function of SUMO in regulation of nutrient

Significance

How organisms maintain homeostasis when confronted with environmental stress is an important question in biology. The master nutrient response regulator target of rapamycin complex 1 (TORC1) regulates many progrowth cellular processes, including transcription of genes required for protein synthesis. RNA polymerase III (RNAPIII) plays a crucial role in regulation of protein synthesis by transcribing *tRNA* genes. RNAPIII activity is known to be dependent on TORC1, but the underlying molecular mechanisms remain to be fully elucidated. Here, we show that TORC1 promotes sumoylation of a specific set of proteins mainly involved in transcription. Subunits of RNAPIII, particularly Rpc82, are among the most prominent TORC1-dependent SUMO substrates. Mechanistically, we demonstrate that TORC1-dependent sumoylation of Rpc82 is required for efficient assembly and full activity of the RNAPIII holoenzyme.

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Data deposition: All ChIP sequencing data have been submitted to the European Nucleotide Archive (ENA), www.ebi.ac.uk/ena (accession no. PRJEB7579); all RNA sequencing data have been submitted to the Gene Expression Omnibus database (GEO), www.ncbi.nlm.nih.gov/geo/ (accession no. GSE85622); and all mass spectrometry datasets have been submitted to the ProteomeXchange consortium via the PRIDE database, www.ebi.ac.uk/pride (accession no. PXD005142).

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responses, we incubated cells in nitrogen-rich and nitrogen-limiting media and purified SUMO under denaturing conditions. We found that nitrogen starvation resulted in an apparent increase in several SUMO conjugates (Fig. 1A), suggesting that nitrogen starvation alters the SUMO proteome. To gain further insight into starvation-induced SUMO proteome remodeling, we analyzed SUMO conjugates by mass spectrometry (MS). We found that 226 proteins were significantly enriched (group 1) and 76 proteins were significantly depleted (group 2) from SUMO pull-downs upon starvation (Dataset S1). Gene ontology (GO) analysis revealed that the proteins belonging to group 1 are enriched for transcriptional regulators (Fig. S1A and Dataset S1), such as the transcriptional activators Gcn4 and Rap1 and the transcriptional repressors Tup1 and Cyc8 (Fig. 1B), which we and others have previously shown to be SUMO targets (10–12). The vast majority of proteins in group 2 are involved in cellular processes that ultimately impinge upon mRNA translation (Fig. 1C and Dataset S1), including several subunits of RNAPIII, such as Rpc53, Rpc82, and Ret1 (Fig. 1B). These data show that nutritional status has a major impact on the SUMO proteome, particularly on sumoylation of proteins that regulate the cell's translational capacity.

Nitrogen starvation is well known to inhibit TORC1 (13). To directly test whether rewiring of the SUMO proteome depends on TORC1 activity, we treated cells with the TORC1 inhibitor rapamycin. Interestingly, this resulted in a similar change in global sumoylation as observed with nitrogen starvation (Fig. S1B). To identify the proteins that are differentially sumoylated upon inhibition of TORC1, we performed a quantitative MS experiment using stable isotope labeling with amino acids in cell culture (SILAC). Consistent with the nitrogen starvation data, Rpc82, Rpc53, and Ret1 were all depleted from the SUMO fraction upon rapamycin treatment (Fig. 1D and Dataset S2). Validation of the MS data by Western blotting confirmed that sumoylation of Rpc82 and Ret1, and to a lesser degree also Rpc53, was substantially decreased upon nitrogen starvation (Fig. 1E). Similar results were obtained with the TORC1 inhibitor rapamycin (Fig. 1F).

Taken together, these data show that TORC1 has an important function in shaping the SUMO proteome and that several proteins involved in transcription are sumoylated in a TORC1-dependent manner, including subunits of RNAPIII.

TORC1 Promotes Recruitment of SUMO to tRNA Genes. To begin unraveling the physiological relevance of TORC1-dependent sumoylation in regulation of transcription, we first studied the global chromatin localization of SUMO by reanalyzing our previously published ChIP-seq dataset (10). Consistent with our finding that RNAPIII is a TORC1-dependent SUMO target, we found that SUMO is present at nearly all RNAPIII-transcribed genes, including the 275 genes encoding tRNAs (*tDNA*) as well as noncoding RNAs such as *SCR1* and *SNR52* (Fig. 2A, Upper, gray) (9). Meta-analysis of this dataset showed that SUMO overlaps the transcription unit of *tRNA* genes, forming a peak right at the transcription start site (TSS; Fig. 2B), resembling previously published meta-analyses of the chromatin localization of RNAPIII and its nascentome (14, 15). These high-throughput data were validated by ChIP followed by quantitative PCR (ChIP-qPCR) using primers specific for a panel of RNAPIII-transcribed genes (Fig. S2A).

To further validate our data, we reanalyzed our previous SUMO ChIP-seq experiments that were performed with a temperature-sensitive *ubc9-1* mutant (10). The *ubc9-1* allele encodes a destabilized form of Ubc9 (16), resulting in a strong reduction in sumoylated protein levels even at the permissive temperature of 30 °C (Fig. S2B). Importantly, the levels of SUMO at RNAPIII-transcribed genes were significantly reduced in *ubc9-1* cells compared with wild-type (WT) cells (Fig. 2A and B and Fig. S2A).

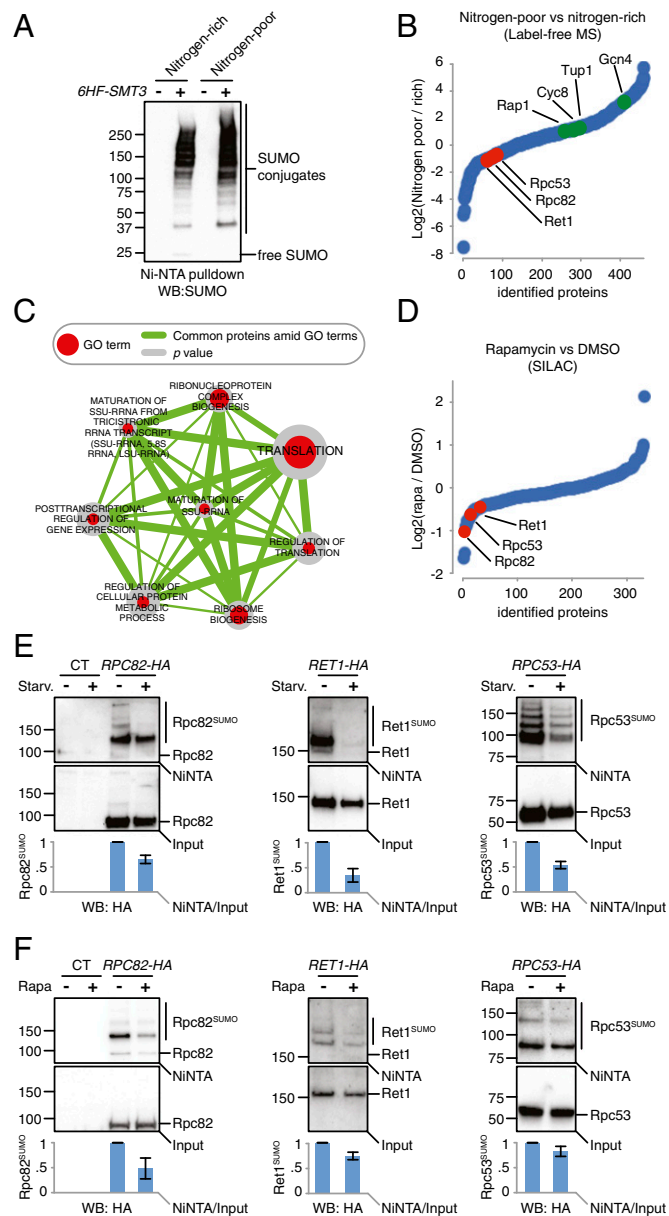


Fig. 1. Inhibition of TORC1 alters the sumoylated protein landscape. (A) Nitrogen starvation alters the profile of protein sumoylation. Sumoylated proteins were purified under denaturing conditions from cells expressing HIS₆-FLAG-tagged SUMO (6HFS-SMT3, which encodes yeast SUMO) and analyzed by Western blotting using SUMO antibodies. (B) Differential sumoylation of proteins in response to nitrogen starvation. Sumoylation of Cyc8, Tup1, and Rap1 was increased, whereas sumoylation of RNAPIII components Rpc53, Rpc82, and Ret1 was decreased upon nitrogen starvation. (C) GO analysis showing that proteins that are desumoylated following nitrogen stress and are primarily involved in mRNA translation. The size of the nodes indicates the relative number of proteins associated with that specific GO term; the width of the edges indicates the relative number of proteins in common between the GO terms. (D) Differential sumoylation of proteins in response to inhibition of TORC1. Sumoylation of RNAPIII components Rpc53, Rpc82, and Ret1 was decreased upon rapamycin treatment. (E) Validation of the MS data. SUMO pull-downs were performed under denaturing conditions using cell lysates of cells grown under nitrogen-rich or nitrogen-starved conditions (Starv.), and the levels of copurifying Rpc82-HA, Rpc53-HA, and Ret1-HA were analyzed by Western blotting with anti-HA antibodies. Rpc82-HA signals (Lower graphs) were quantified using ImageJ. Error bars indicate the deviation from the average of two experiments. (F) Validation of MS data. Cells were treated with rapamycin (Rapa), and cell lysates were analyzed as in E.

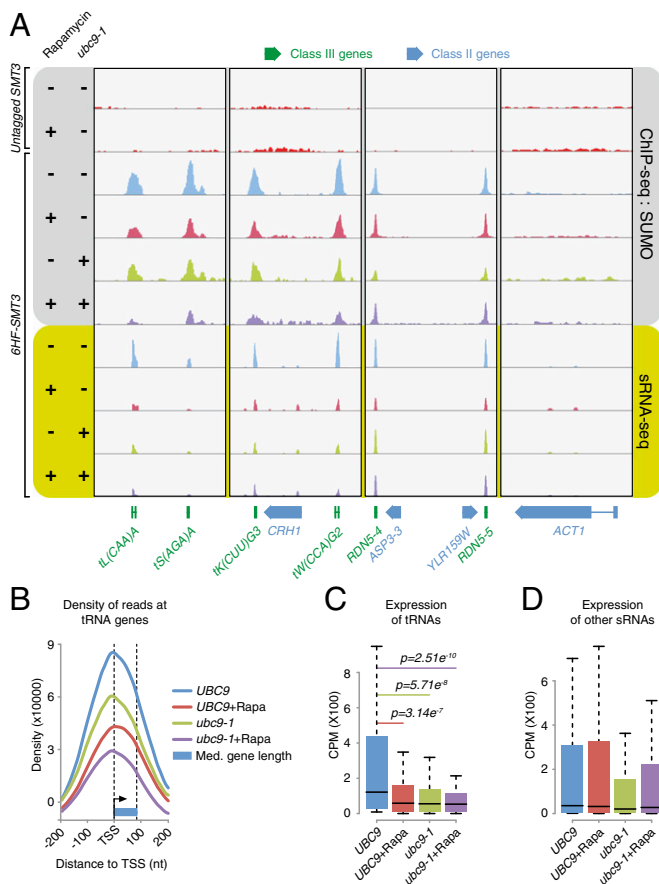


Fig. 2. SUMO is enriched at *tRNA* genes and regulates *tRNA* transcription. (A) Snapshots of SUMO ChIP-seq experiments (Upper, gray) and sRNA-seq experiments (Lower, yellow) in WT and *ubc9-1* cells grown at permissive temperature and treated with either DMSO or rapamycin. “Class II genes” indicates genes that are transcribed by RNAPII, whereas “class III genes” indicates genes transcribed by RNAPIII. (B) Meta-analysis of SUMO levels at *tRNA* genes. Rapa, rapamycin; TSS, TSS. (C and D) Global expression levels of *tRNAs* (C) and other short RNAs (D) in WT and *ubc9-1* cells grown at permissive temperature and treated with either DMSO or 100 nM of rapamycin (Rapa) for 30 min.

This shows that the SUMO ChIP-seq data are specific and not caused by a hyper-ChIPable chromatin artifact (17).

Our MS data showed that several subunits of RNAPIII are desumoylated upon nitrogen starvation or TORC1 inhibition, strongly suggesting that RNAPIII is a critical TORC1-dependent SUMO target at *tRNA* genes. Consistent with this hypothesis, SUMO ChIP-seq experiments using WT and *ubc9-1* cells treated with rapamycin revealed that the level of SUMO at *tRNA* genes was reduced by ~30% in *ubc9-1* mutants compared with WT cells (Fig. 2A and B). Treatment with rapamycin further reduced SUMO levels at *tRNA* genes, particularly in the *ubc9-1* mutant. These data were confirmed by ChIP-qPCR (Fig. S2A). Nitrogen starvation also resulted in a reduction in SUMO levels at RNAPIII-transcribed genes (Fig. S2C).

Paradoxically, our MS data also showed that Brf1 and Tfc4 (subunits of TFIIB and TFIIC, respectively) are hypersumoylated upon rapamycin treatment (Dataset S1). This finding appeared to be contradictory to the weaker binding of SUMO to the *tDNA* detected in rapamycin-treated cells. However, it has been recently shown that hypersumoylation of the transcriptional regulator Gcn4 prevents its binding to the chromatin (18), and hypersumoylation of Brf1 and Tfc4 may similarly reduce their binding to chromatin. To test this, we analyzed the association of SUMO, Rpc82, Ret1, Brf1, and Tfc4 to class III genes by ChIP-qPCR after rapamycin

treatment. We found not only that the level of SUMO was lower at *tRNA^W*, *tRNA^L*, and *SCR1* but that the recruitment of Rpc82, Ret1, Brf1, and Tfc4 was also lower after inhibition of TORC1 (Fig. S2D–F). These data suggest that SUMO regulates the recruitment of the RNAPIII transcriptional complex to regulate *tRNA* transcription.

To study the effect of TORC1 and the Ubc9–SUMO pathway on transcriptional output of RNAPIII, we performed transcriptome analysis using short-RNA sequencing (sRNA-seq; <200 nt) (Fig. 2A, Lower, yellow and Dataset S3). We extracted *tRNA* genes from the sRNA expression dataset and found that global *tRNA* transcription was much lower in *ubc9-1* cells than in WT cells (Fig. 2C), whereas the expression of the remaining sRNAs was not significantly affected (Fig. 2D). These data show that sumoylation is required for efficient *tRNA* transcription under optimal growth conditions. Interestingly, the strong decrease in *tRNA* transcription observed in the *ubc9-1* mutant was not further reduced by treatment with rapamycin (Fig. 2C), which supports our finding that Ubc9–SUMO and TORC1 function in the same pathway. RT-qPCR using primers specific for the immature forms of *tRNA^L* and *tRNA^W* confirmed these data, whereas the expression of *ACT1*, which is regulated by neither SUMO nor TORC1 (10), was unchanged (Fig. S2G).

Together, these data show that both TORC1 and Ubc9–SUMO promote *tRNA* transcription and suggest that they ultimately act on the same downstream target.

Sumoylation of Rpc82 Promotes Transcription of *tRNA* Genes. To gain insight into the regulation of RNAPIII by SUMO, we first mapped the lysines in Rpc82, Ret1, and Rpc53 to which SUMO is conjugated (sumoylation of the RNAPIII basal transcription factors Tfc4 and Brf1 will be the topic of future studies). Using GPS-SUMO (19), we identified multiple potential SUMO acceptor sites (Table S1). We mutated these lysines into non-sumoylatable arginine residues and determined the sumoylation status of these mutants in vivo. Interestingly, lysine-to-arginine substitution of K403, K406, K591, and K596 in Rpc82 (Rpc82-4KR; Fig. 3A) completely abolished Rpc82 sumoylation (Fig. 3B), with a migration profile that was identical to WT Rpc82 expressed in a *ubc9-1* mutant (Fig. 3C). Importantly, transcription of *tRNA* genes was reduced by ~50% in *rpc82-4KR* cells compared with *RPC82* cells (Fig. 3D), showing that sumoylation of Rpc82 is important for RNAPIII activity. Treatment with rapamycin did not further reduce *tRNA* transcription in the *rpc82-4KR* mutant (Fig. 3D), indicating that Ubc9–SUMO acts on Rpc82 either downstream of or in parallel to TORC1 to control *tRNA* expression. This interpretation is supported by our finding that deletion of *MAF1* did not restore *tRNA* transcription in the *rpc82-4KR* mutant (Fig. 3E), strongly suggesting that SUMO exerts its effect on *tRNA* transcription downstream of Maf1.

In contrast to our findings with Rpc82, mutating the predicted SUMO sites in Rpc53 and Ret1 did not prevent, or only partially reduced, sumoylation of these proteins in vivo (Fig. S3A and B). Accordingly, *tRNA* transcription in these mutants was either unaffected (*rpc53-K24,51,115,216,236,322,325*; “*rpc53-7KR*” in Fig. S3C) or apparently increased (*ret1-K17,448,927R*; “*ret1-3KR*” in Fig. S3D) compared with WT cells. The critical SUMO sites and the physiological significance of sumoylation of Rpc53 and Ret1 remain to be characterized, which is the aim of future studies.

Together, these data show that sumoylation of Rpc82 is important for *tRNA* transcription. We decided to focus on Rpc82 sumoylation for the remainder of this study, because the *rpc82-4KR* phenotype most closely resembled the severe *tRNA* transcription defect observed in the *ubc9-1* mutant.

Sumoylation of Rpc82 Stabilizes the RNAPIII Transcriptional Complex.

One emerging function of SUMO is to promote the cohesion of entire protein complexes (9, 20). We therefore hypothesized that

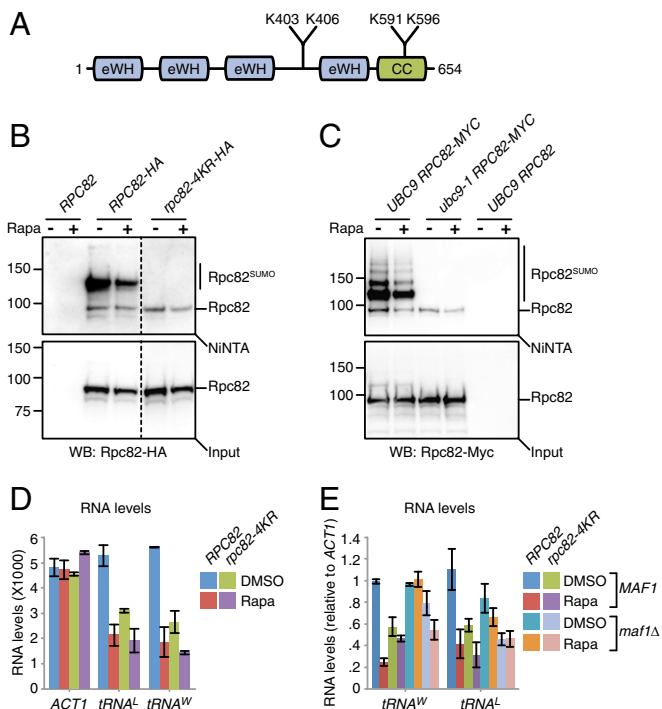


Fig. 3. Sumoylation of Rpc82 promotes *tRNA* transcription. (A) Location of the putative SUMO sites in Rpc82. CC, coiled-coil domain; eWH, extended winged helix domain. Protein domain organization was derived from the structure of human RPC3 (31). (B) Rpc82-4KR is not sumoylated *in vivo*. Cells expressing *RPC82-HA* or *rpc82-4KR-HA* as well as HIS₆-FLAG-tagged SUMO were treated with DMSO or 100 nM rapamycin (Rapa) for 30 min, after which SUMO was purified and eluates were analyzed by Western blotting with HA antibodies. Dashed line indicates where the image was cropped to remove irrelevant data. (C) Rpc82 sumoylation is abolished in the *ubc9-1* mutant. WT cells or *ubc9-1* mutants (both expressing normal *RPC82-HA* and *6Hf-SMT3*) were grown to log phase at permissive temperature and treated with either DMSO or 100 nM rapamycin (Rapa) for 30 min. SUMO was then purified and eluates were analyzed as in B. (D) *tRNA* transcription is significantly reduced in *rpc82-4KR* mutants. WT cells or *rpc82-4KR* mutants were grown and treated as in B, after which RNA was purified and analyzed by qPCR using primer pairs against the indicated genes. Error bars, SEM of three independent experiments. (E) Deletion of *MAF1* does not restore *tRNA* transcription in *rpc82-4KR* mutants. The indicated strains were grown to log phase and treated with either DMSO or 100 nM rapamycin (Rapa), after which RNA was purified and analyzed by qPCR as in D. Error bars, SEM of three independent experiments.

sumoylation of Rpc82 promotes stability of the RNAPIII complex. To test this hypothesis, we first purified WT Rpc82 and Rpc82-4KR from cells grown under nutrient-rich conditions and analyzed the associated proteins by quantitative MS. Interestingly, this experiment revealed that the interactions between Rpc82-4KR and other components of the RNAPIII enzyme, as well as the basal transcription factor TFIIB, were strongly reduced (Fig. 4A and [Dataset S4](#)), suggesting that sumoylation of Rpc82 is important for integrity of the RNAPIII complex. These MS data were confirmed by coimmunoprecipitation experiments, which showed a substantial reduction in the interaction of Rpc82-4KR with Brf1-GFP and with Ret1-GFP compared with WT Rpc82 (Fig. 4B and C). Furthermore, ChIP-qPCR experiments demonstrated that Rpc82-4KR was specifically lost from the chromatin at *tRNA^L* and *tRNA^W* genes, whereas the presence of other components of the RNAPIII complex, such as Ret1, was unaffected in the *rpc82-4KR* mutant (Fig. 4D and E); this specific reduction in chromatin-bound Rpc82-4KR was not due to a nuclear entry defect or a defect in Rpc82-4KR protein stability, because Rpc82-4KR entered the nucleus normally ([Fig. S4A](#)).

and the stability of Rpc82-4KR was comparable to that of WT Rpc82 (Fig. S4 B and C). These data show that the weaker interaction of Rpc82-4KR with the RNAPIII holoenzyme is not due to a trafficking defect or a defect with Rpc82-4KR protein stability.

Together, these data indicate that Rpc82 sumoylation is important for efficient incorporation of Rpc82 into the RNAPIII holoenzyme.

Discussion

RNAPIII activity is tightly regulated by the cell. Compared with RNAPII, which is regulated by a plethora of modifications in its C-terminal domain (21), little is known about regulation of RNAPIII activity. Several types of stress signals result in inhibition of RNAPIII activity, including nutrient stress and DNA damage, and most of these signals appear to converge on Maf1 (3). However, it has recently become clear that RNAPIII activity is also directly regulated by the cell; for instance, TORC1 regulates Rpc53 activity by preventing the direct, inhibitory phosphorylation of Rpc53 by Kns1 and Mck1 (7). Our finding that the Ubc9–SUMO pathway directly controls RNAPIII activity, which occurs in parallel to or downstream of Maf1 (Fig. 3E), reveals a layer of complexity of RNAPIII regulation. Our findings are supported by previous high-throughput proteomic studies that showed that Rpc82 and Rpc53, as well as subunits of TFIIB, are SUMO targets (22, 23), although the function of SUMO in regulation of these proteins was not characterized in these studies. Here, we have demonstrated that TORC1-dependent sumoylation of Rpc82 is required for efficient *tRNA* transcription under optimal growth conditions.

We found that sumoylation of Rpc82 is important for its incorporation into the RNAPIII holoenzyme. Interestingly, K403 and K406 lie in an 80 residue-long flexible loop of Rpc82 (Fig. 4F), which could serve as a platform that stabilizes interactions either within RNAPIII or with the basal transcription factors of RNAPIII. Furthermore, K591 lies in the coiled-coil domain of Rpc82 and faces outward toward a cavity that is formed by multiple RNAPIII components, including C160, C25, C31, and C34 (Fig. 4G). It is possible that sumoylation of K591 in this multidomain cavity stabilizes the interaction of Rpc82 with the RNAPIII complex to promote the closed clamp conformation of RNAPIII, which may be important for promoter opening and subsequent elongation (24). K596 is situated in the coiled-coil region of Rpc82, which in structural analyses is surrounded by a low-resolution density of Rpc31, which occludes the precise position of SUMO, making it difficult to predict how sumoylation of this residue might affect the interaction of Rpc82 with RNAPIII (Fig. S4D). Finally, whereas K403, K406, and K591 are highly conserved in fungi, K596 is not, which suggests that sumoylation of K596 may not be important for regulation of RNAPIII. Our model in which sumoylation of Rpc82 promotes the cohesion of the RNAPIII holoenzyme is supported by the fact that *rpc82-4KR* cells are sensitive to elevated temperatures, which are known to destabilize protein complexes (Fig. 4H).

Although we have shown here that sumoylation of Rpc82 promotes RNAPIII activity, we cannot exclude the possibility that sumoylation of other RNAPIII components may have differential effects on RNAPIII activity. Indeed, we found that mutation of the putative SUMO-targeted lysines in Ret1 appeared to promote rather than reduce the transcriptional activity of RNAPIII. Therefore, it is possible that different RNAPIII components may be modified by SUMO in response to specific intracellular and environmental signals and that the balance of sumoylation of different RNAPIII subunits ultimately determines its transcriptional output. Nonetheless, under optimal growth conditions SUMO has a net positive effect on RNAPIII, as evidenced by our finding that RNAPIII activity is strongly reduced in sumoylation-defective *ubc9-1* mutants.

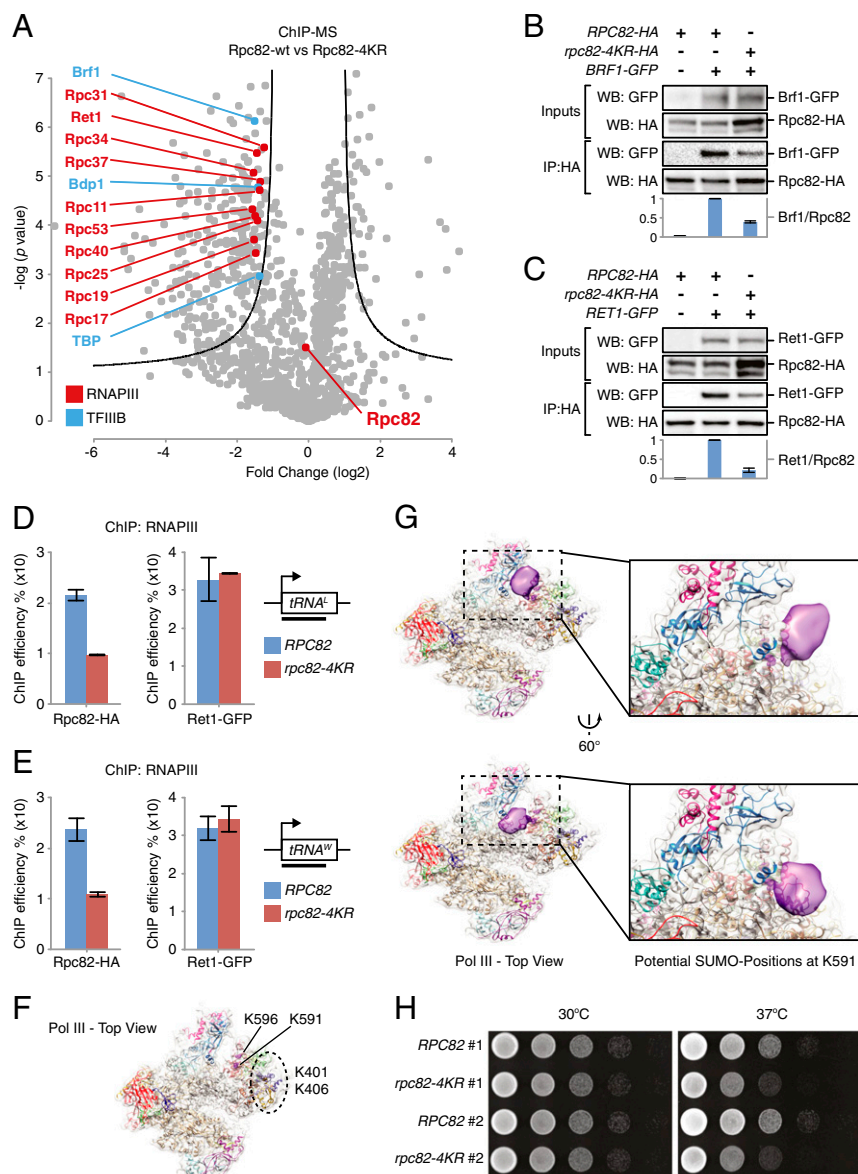


Fig. 4. Sumoylation of Rpc82 promotes its interaction with RNAPIII and TFIIIB. (A) Reduced binding of Rpc82-4KR to RNAPIII and TFIIIB. Cells were grown to log phase, and equal amounts of Rpc82-HA and Rpc82-4KR-HA were purified with HA antibodies in the presence of NEM to prevent desumoylation. Copurifying proteins were analyzed by quantitative MS. (B) Sumoylation of Rpc82 is important for the interaction with Brf1. Rpc82-HA and Rpc82-4KR-HA were purified with HA antibodies under native conditions in the presence of NEM, and the amount of copurifying Brf1-GFP was analyzed by Western blotting with GFP antibodies. GFP over HA ratios were determined by quantifying Western blot signals with ImageJ. Error bars indicate the deviation from the mean of two independent experiments. (C) Sumoylation of Rpc82 promotes the interaction with Ret1. Rpc82-HA and Rpc82-4KR-HA were purified from cells as described in B, and the amount of copurifying Ret1-GFP was analyzed by Western blotting with GFP antibodies. GFP over HA ratios were determined as in B. Error bars indicate the deviation from the mean of two independent experiments. (D and E) Sumoylation of Rpc82 promotes its recruitment to *tRNA*^L. *RPC82-HA RET1-GFP* cells and *rpc82-4KR-HA RET1-GFP* mutants were grown to log phase, and the relative level of Rpc82 or Rpc82-4KR and Ret1 at *tRNA*^L (D) or *tRNA*^W (E) was determined by ChIP-qPCR using antibodies against HA and GFP, respectively. Error bars, SEM of three independent experiments. (F) Rpc82 SUMO sites in the context of the structure of RNAPIII. K401 and K406 lie in a low-density flexible loop of Rpc82 WH3 domain. K591 lies in the coiled-coil domain of Rpc82 and faces outwards in a cavity formed by Rpc160, Rpc82, Rpc34, Rpc31, and Rpc25. K596 lies under a low-density (flexible) domain of Rpc31. (G) The putative positioning of SUMO density at K591 (purple) suggests that SUMO may stabilize the active conformation of RNAPIII. SUMO may either coordinate or stabilize the multidomain cavity formed by Rpc160, Rpc82, Rpc34, Rpc31, and Rpc25, potentially favoring the closed-clamp (active) conformation of RNAPIII. (H) *rpc82-4KR* cells are sensitive to heat stress. WT and *rpc82-4KR* cells were spotted on plates supplemented and were incubated at 30 °C or 37 °C until colonies appeared.

Taken together, we have discovered a mechanism for regulation of RNAPIII during changing nutrient conditions.

Methods

Yeast Strains and Plasmids. *S. cerevisiae* strains were grown in appropriate media, depending on the experiment/genotype. Strains were derived directly from either the S288c strains RDKY3615 (25) or BY4741 using

standard gene-replacement methods or intercrossing (see Table S2 for strains and plasmids).

ChIP, ChIP-Seq, and Small RNA-Seq. ChIP and short RNA-seq were performed as described previously (10, 26) with minor modifications (SI Methods). The original SUMO ChIP-seq dataset has been reported previously (10). For primer sequences, see Table S3.

Analysis of Sequencing Data. ChIP-seq data were analyzed as previously described (10). Short RNA-seq was performed in duplicates by BGI (www.genomics.cn/en/). Raw reads were filtered for low-quality reads, adapters, and contamination before reads were mapped to the R64-1-1 genome assembly using TopHat2 (v. 2.0.13) (27) using default options. The number of reads per gene in the Ensemble 75 were counted using featureCounts (v. 1.4.6-p3) (28) in paired-end mode and with $-t$ «gene» and $-M$ options. The counts were then normalized for sequencing depth by dividing each value by the total number of reads in million (generating counts per million, cpm). Raw data and expression values before and after normalization are available in [Datasets S3](#) and [S5](#). Expression values of RNAPIII-transcribed genes were extracted based on the gene list from the yeast database (www.yeastgenome.org/).

RNA Preparation and Reverse Transcription. Total RNA purification and reverse transcription were performed as previously described (26).

SUMO Pull-Down Under Denaturing Conditions. Pull-downs in denaturing conditions were performed as previously described (10, 11, 29) with minor modifications ([SI Methods](#)).

MS. Label-free MS samples were processed at the Proteomics Core Facility at Oslo University Hospital-Rikshospitalet (www.med.uio.no/cir/english/services/proteomics/) ([SI Methods](#)). Briefly, cells were cultured until log phase in complete synthetic medium, washed, and incubated for 3 h in either complete synthetic medium or in synthetic drop-out medium lacking nitrogen and amino acids. Cells were harvested, washed with ice-cold water, and processed for purification of SUMO conjugates under denaturing conditions ([SI Methods](#)).

Modified SILAC experiments were performed and analyzed at the proteomic core facility at the EMBL in Heidelberg (<https://www.embl.de/proteomics/>).

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[proteomics_services/index.html](#)). Briefly, protein extracts from DMSO- and rapamycin-treated cells were prepared in denaturing conditions ([SI Methods](#)). After digestion with LysC and trypsin, DMSO-treated samples were labeled light and rapamycin-treated samples were labeled heavy. Both samples were mixed and submitted to MALDI-TOF MS/MS ([Dataset S2](#)).

ChIP-MS experiments were performed as previously described (10) with minor modifications ([SI Methods](#)).

Cell Lysis, Immunoprecipitation, and Western Blotting. IP and Western blotting was performed as previously described (26, 29, 30). All buffers contained 10 mM of fresh *N*-ethylmaleimide (Sigma) to prevent desumoylation by SUMO proteases.

For coimmunoprecipitations, log phase cells were collected and washed in lysis buffer [50 mM Hepes-KOH (pH 7.5), 150 mM NaCl, 1 mM EDTA, 0.05% Nonidet P-40, 0.5 mM DTT, 20% (vol/vol) glycerol, 10 mM NEM, protease inhibitors]. Pellets were resuspended in lysis buffer, and cells were lysed by vortexing with glass beads followed by centrifugation to remove cell debris. Equal amounts of proteins were used for immunoprecipitation using magnetic beads conjugated covalently to relevant antibodies. After extensive washing with lysis buffer, coimmunoprecipitated proteins were resolved by SDS/PAGE and analyzed by Western blotting with the indicated antibodies.

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