Desumoylation of RNA polymerase III lies at the core of the Sumo stress response in yeast

Received for publication, June 7, 2019, and in revised form, October 30, 2019 Published, Papers in Press, November 1, 2019, DOI 10.1074/jbc.RA119.009721

Aurélie Nguéa P[‡§¶1], Joseph Robertson[‡§], Maria Carmen Herrera[§¶], Pierre Chymkowitch[‖2], and Jorrit M. Enserink[‡§¶3]

From the [‡]Department of Molecular Cell Biology, Institute for Cancer Research, Norwegian Radium Hospital, Montebello, N-0379 Oslo, Norway, the [§]Centre for Cancer Cell Reprogramming, Institute of Clinical Medicine, Faculty of Medicine and the [¶]Department of Biosciences, Faculty of Mathematics and Natural Sciences, University of Oslo, 0371 Oslo, Norway, and the [‖]Department of Microbiology, Oslo University Hospital, Rikshospitalet, 0372 Oslo, Norway

Edited by Karin Musier-Forsyth

Post-translational modification by small ubiquitin-like modifier (Sumo) regulates many cellular processes, including the adaptive response to various types of stress, referred to as the Sumo stress response (SSR). However, it remains unclear whether the SSR involves a common set of core proteins regardless of the type of stress or whether each particular type of stress induces a stress-specific SSR that targets a unique, largely nonoverlapping set of Sumo substrates. In this study, we used MS and a Gene Ontology approach to identify differentially sumoylated proteins during heat stress, hyperosmotic stress, oxidative stress, nitrogen starvation, and DNA alkylation in *Saccharomyces cerevisiae* cells. Our results indicate that each stress triggers a specific SSR signature centered on proteins involved in transcription, translation, and chromatin regulation. Strikingly, whereas the various stress-specific SSRs were largely nonoverlapping, all types of stress tested here resulted in desumoylation of subunits of RNA polymerase III, which correlated with a decrease in tRNA synthesis. We conclude that desumoylation and subsequent inhibition of RNA polymerase III constitutes the core of all stress-specific SSRs in yeast.

Rapidly sensing and relaying stimuli is a key aspect of normal cellular physiology and is of great importance for maintaining cellular homeostasis during environmental stress. To survive stress, cells rewire their transcriptome to promote the transcription of key genes whose products will help to adapt to the stressful environment. Rewiring of transcriptional programs typically involves rapid post-translational modification (PTM)[4] of signaling proteins and transcription factors.

Sumoylation is a conserved PTM involving the covalent attachment of the small ubiquitin-like modifier (Sumo) to target proteins and plays a critical role in cellular responses to stress. Plants express eight versions of Sumo, whereas mammals express four Sumo isoforms (Sumo-1, -2, -3, and -4) (1). In contrast, the budding yeast *Saccharomyces cerevisiae* expresses one Sumo isoform encoded by the essential gene *SMT3* (2). Protein sumoylation is a reversible process that follows an ordered series of events. Sumo is initially synthesized as an inactive precursor, which is proteolytically processed by the yeast Sumo proteases Ulp1 and Ulp2 (SENP1–SENP6 in mammals) at the C-terminal tail to yield an active conjugable form (3, 4). Subsequently, an E1-activating enzyme consisting of the Aos1/Uba2 heterodimer (Sae1/Sae2 in mammals) (5, 6) transfers Sumo to the sole E2-conjugating enzyme Ubc9, which is conserved from yeast to humans (5, 6). Ubc9 then transfers Sumo to one of its substrates, either with or without the help of E3 enzymes (7, 8). Finally, the Sumo proteases Ulp1 and Ulp2 can remove Sumo to complete the sumoylation cycle (3).

Sumoylation of a protein may influence its fate and activity. For instance, Sumo conjugation can induce conformational changes (9, 10); hide or reveal interacting motifs, thereby altering protein–protein or protein–DNA interactions (11–14); affect cellular localization (15); compete with other PTMs (16, 17); alter enzymatic activity (18); or affect protein stability (19). Sumo is often simultaneously conjugated to a group of proteins that belong to the same complex or act within the same pathway, a phenomenon referred to as *waves of sumoylation* or *Sumo spray*, which is assumed to reinforce the activation of a given biological process, such as DNA damage checkpoint activation and DNA repair (11, 20).

Under homeostatic conditions, a large portion of Sumo substrates is involved in the process of transcription. It was long thought that sumoylation generally had an inhibitory effect on

This work was supported by the Norwegian Research Council Grant 261936 (to J. M. E.), Norwegian Cancer Society Project 182524 (to J. M. E.), and Helse Sør-Øst Grant 2017065 (to P. C.). This work was supported in part by the Research Council of Norway through its Centers of Excellence funding scheme Project 262652. The authors declare that they have no conflicts of interest with the contents of this article.

This article contains Tables S1–S3 and Figs. S1 and S2.

The mass spectrometric raw data and spectral libraries associated with this manuscript are available from ProteomeXchange with the accession number PXD013884.

[1] Present address: Dept. of Microbiology, Oslo University Hospital, Rikshospitalet, 0372 Oslo, Norway.

[2] To whom correspondence may be addressed: Dept. of Microbiology, Oslo University Hospital, Rikshospitalet, 0372 Oslo, Norway. Tel.: 0047-91-63-12-31; E-mail: pierre.chymkowitch@rr-research.no.

[3] To whom correspondence may be addressed: Dept. of Molecular Cell Biology, Institute for Cancer Research, Norwegian Radium Hospital, Montebello, N-0379 Oslo, Norway. Tel.: 0047-22-78-19-82; E-mail: jorrit.enserink@rr-research.no.

[4] The abbreviations used are: PTM, post-translational modification; Sumo, small ubiquitin-like modifier; SSR, Sumo stress response; SST, Sumo stress target; tRNA, transfer RNA; RNAP, RNA polymerase; GO, Gene Ontology; qPCR, quantitative PCR; CSM, complete synthetic medium; Ni–NTA, nickel–nitrilotriacetic acid.

transcription, an assumption that has since been challenged (reviewed in Ref. 21). Indeed, we have shown that chromatin-bound Sumo promotes the expression of progrowth genes (22, 23). More specifically, we found that sumoylation of the transcription factor Rap1 promotes the interaction between Rap1 and the RNA polymerase (RNAP) II transcriptional machinery, thereby stimulating transcription of ribosomal protein genes (22). We have also recently shown that sumoylation of RNAPIII promotes tRNA synthesis (23).

Interestingly, the Ubc9-Sumo pathway also has important functions in the response to environmental stress, a phenomenon often referred to as the Sumo stress response (SSR) (24). For example, it has been observed that proteins involved in transcriptional regulation, chromatin remodeling, or DNA repair become highly sumoylated upon environmental stress, including hyperosmotic and heat stress (25). Conversely, the SSR can also involve desumoylation of certain substrates. For instance, we have shown that nitrogen starvation, as well as treatment of cells with the TORC1 inhibitor rapamycin, results in desumoylation of components of RNAPIII, leading to a strong reduction in RNAPIII activity (23).

Although several studies have analyzed stress-induced changes in cellular sumoylation patterns, it remains unclear whether the SSR involves a common set of core Sumo substrates that are altered regardless of the type of stress or whether the SSR rather targets a unique set of proteins depending on each particular type of stress. In this study, we used a proteomic approach to gain more insight into the SSR. We found only limited overlap between SSRs induced by different types of stress, suggesting that each form of stress triggers a unique SSR. However, we did find that desumoylation and inactivation of the RNAPIII machinery were a common response to all forms of stress. We conclude that desumoylation and inactivation of the RNAPIII machinery constitute a common core component of the SSR.

Results

A comparative analysis of Sumo stress targets in various stress conditions

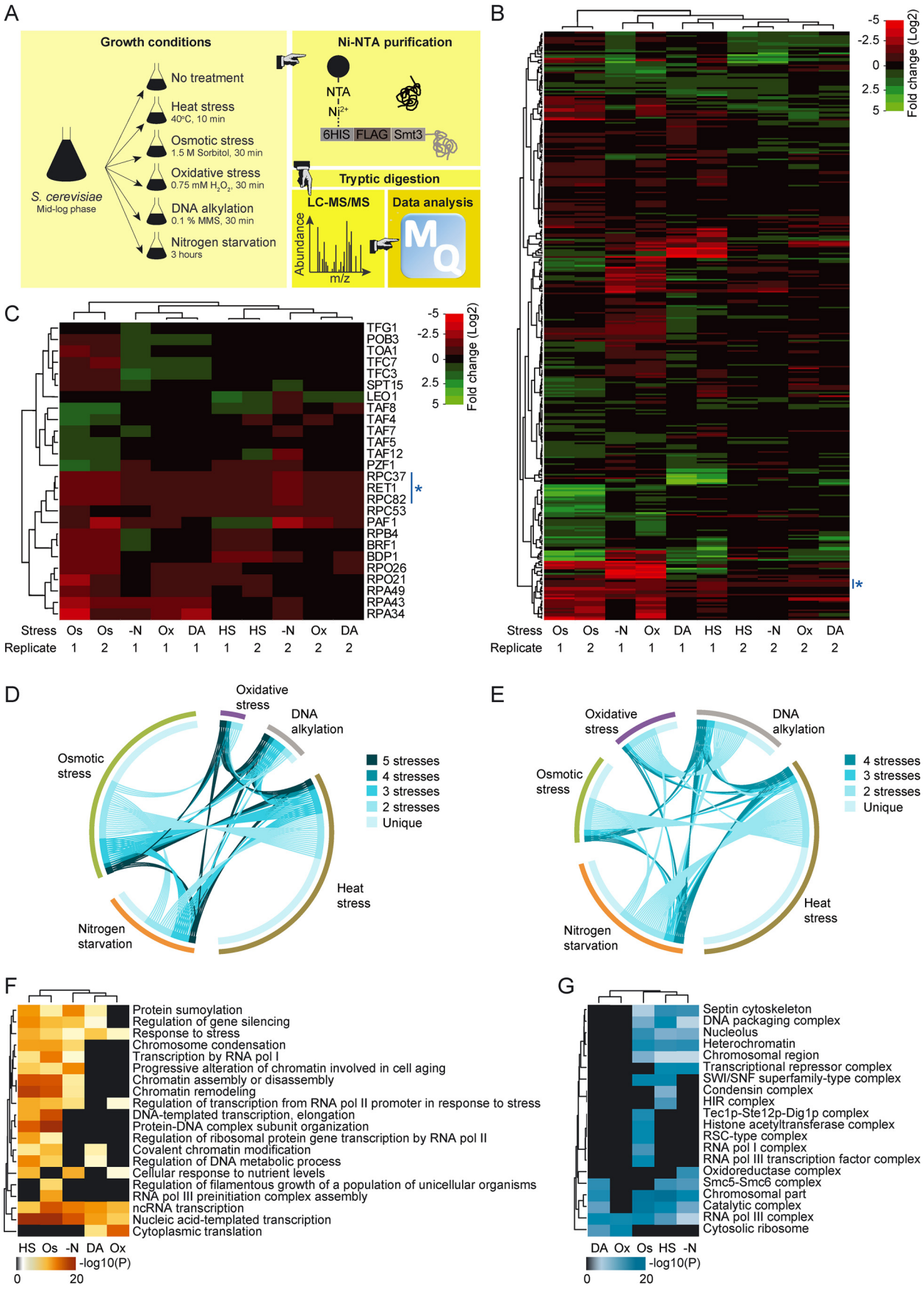
To better define the *S. cerevisiae* SSR, we performed affinity purification combined with MS to identify Sumo stress targets (SSTs), *i.e.* sets of differentially sumoylated proteins in various stress conditions. Cells expressing His_6-FLAG-tagged Sumo were exposed to heat, hyperosmotic and oxidative stresses, DNA alkylation, and nitrogen starvation, followed by purification of Sumo under denaturing conditions (Fig. 1*A*). As assessed by Western blotting, all five stresses correlated with an overall increase in Sumo conjugation, which is usually referred to as the SSR (24) (Fig. S1). A comparative analysis of differentially sumoylated proteins was performed in these five stress conditions (*i.e.* increased or decreased sumoylation compared with normal growth conditions). Differentially sumoylated proteins were then reproducibly identified by MS in two biological repeats, although fold changes varied to some degree (Fig. 1, *B* and *C*, Fig. S2, and Table S1). This revealed little overlap between the various treatments (Fig. 1, *B–E*, and Tables S1 and S2), suggesting that each stress triggers a stress-specific SSR. However, a small set of proteins was globally desumoylated independently of the type of cellular injury (Fig. 1, *B–D*, Fig. S2, and Tables S1 and S2). This cluster included subunits of the three RNA polymerases, as well as subunits of their general transcription machineries (Fig. 1*C* and Table S2). Strikingly, only three proteins were desumoylated upon all five stresses: Rpc37, Ret1, and Rpc82 (Fig. 1*C*, Fig. S2, and Table S1), which are three subunits of RNAPIII. Furthermore, a fourth RNAPIII subunit, Rpc53, was desumoylated in all stresses with the exception of one nitrogen starvation biological replicate (Fig. 1*C* and Fig. S2) where it fell just below the cutoff. These data indicate that different stresses trigger stress-specific SSRs, and that RNAPIII subunits are desumoylated upon each stress tested here (also see below).

Additionally, three proteins showed increased sumoylation upon four stresses but not oxidative stress: the rRNA processing element–binding protein Stb3 and two TCA cycle enzymes Sdh1 and Lpd1 (Tables S1 and S2). Similarly, the structural maintenance of chromosome (Smc) 5 and Smc6 subunits of the Smc5/6 complex, which is a known Sumo target involved in the DNA damage response, exhibited increased sumoylation in all stresses with the exception of osmotic stress, where, conversely, both Smc5 and Smc6 showed a significant decrease in sumoylation (Tables S1 and S2).

To gain further insight into the cellular processes and biological pathways that are targeted by the different SSRs, we performed Gene Ontology (GO) analysis of the proteomic data sets. Comparative GO (biological process) analysis of differentially sumoylated substrates revealed that proteins that were differentially sumoylated upon heat shock, hyperosmotic stress, and nitrogen starvation were mainly involved in chromatin organization, whereas DNA alkylation and oxidative stress resulted in differential sumoylation of the translation machinery (Fig. 1*F*). Surprisingly, there was substantial overlap between several stresses, notably nitrogen starvation, heat stress, and hyperosmotic stress (Fig. 1*F*). Thus, although the differentially sumoylated substrates varied between different stresses, they broadly fell into the same biological process with transcription being the sole biological process that appeared in all five stresses. These data indicate that the SSR affects similar cellular processes yet targets specific sets of proteins according to the type of cellular injury.

We also performed comparative GO (cellular component) analysis to identify protein complexes that are differentially targeted by the various stresses. As expected, RNAPIII was the only common complex to all five stresses (Fig. 1*G*). Hyperosmotic stress, nitrogen starvation, and heat stress exhibited a certain degree of overlap in chromatin-associated complexes, whereas DNA alkylation and oxidative stress overlapped under the "cytosolic ribosome" term.

Taken together, our data suggest that each of the cellular stresses we have tested triggers its own unique SSR that involves a particular set of proteins (discussed in further detail below), whereas RNA polymerase complexes and their respective transcription machineries, and the RNAPIII complex in particular, are the only SSTs common to each SSR.



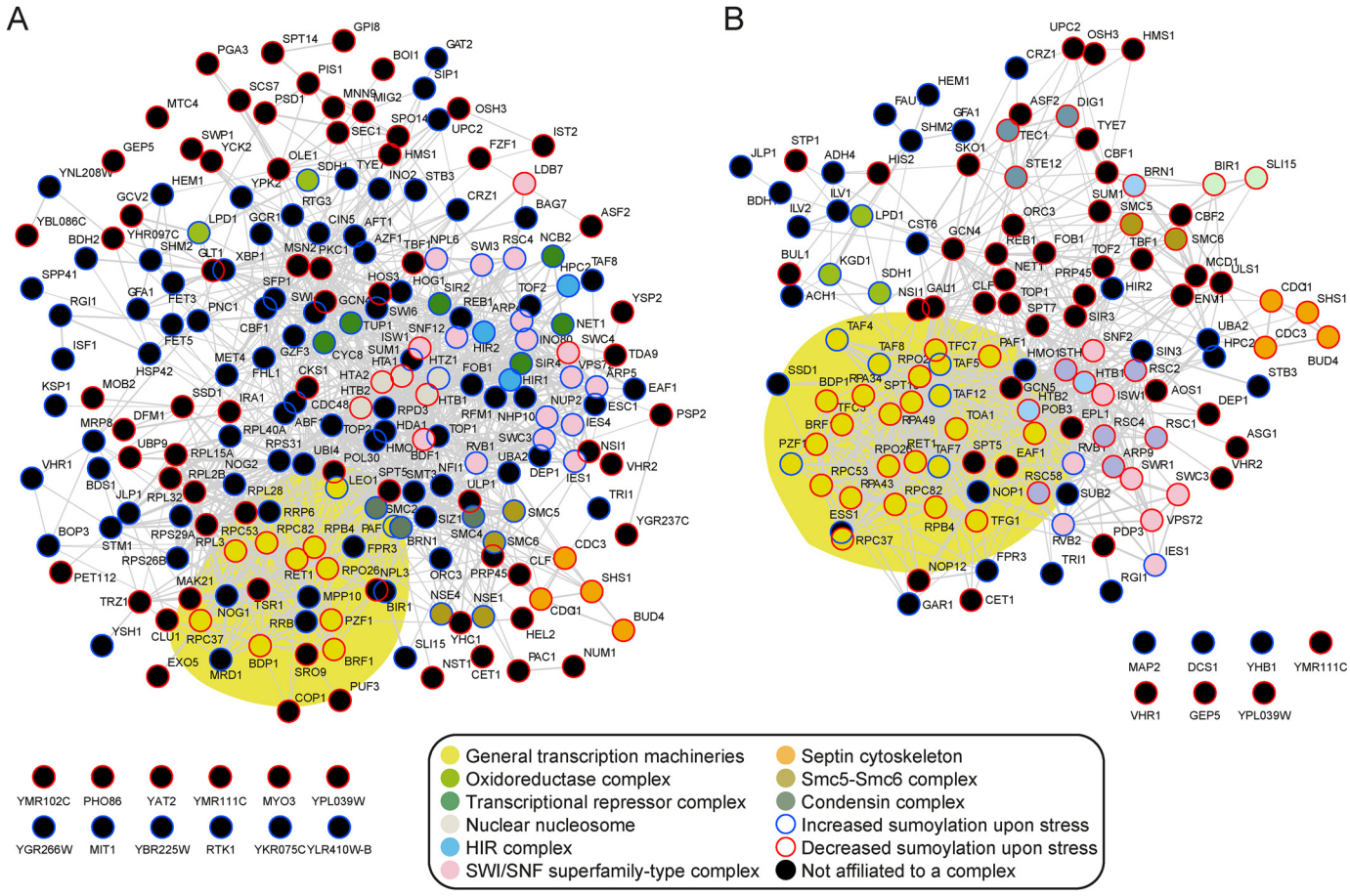


Figure 2. Identification of sumo-modified complexes: heat stress and osmotic stress. Networks generated for differentially sumoylated substrates following heat stress (*A*) and osmotic stress (*B*). Nodes represent proteins, and edges represent protein–protein associations. Desumoylated proteins are circled in *red*, whereas hypersumoylated proteins are circled in *blue*. Enriched complexes are depicted with different colors (see legend). *Black nodes* represent proteins not associated with enriched complexes. Networks were generated using the STRING database (55).

Heat stress-regulated sumoylome

Next, we wished to gain further insight into the various stress-specific SSRs. We identified 211 proteins that were reproducibly differentially sumoylated upon exposure to elevated temperature (Table S1). We identified several regulators of the septin cytoskeleton (Fig. 2*A*), including the septins Cdc3, Cdc11, and Shs1, which have previously been shown to be Sumo-modified proteins (26) and which all became hyposumoylated following heat stress (Table S2). We also identified 13 components of the SWI/SNF superfamily-type complex, which became hypersumoylated upon heat stress and four that became hyposumoylated. This chromatin remodeling complex has previously been shown to be Sumo-modified upon exposure to elevated temperatures in the plant *Arabidopsis thaliana* (27), suggesting evolutionary conservation of this stress response. Other proteins that became hypersumoylated are the Smc2 and Smc4 subunits of the condensin complex and four components of the aforementioned eight-subunit Smc5/6 complex. Similarly, three subunits of the four-subunit histone regulation complex, Hir1, Hir2, and Hpc2 also appeared more sumoylated. As mentioned above, exposure to heat resulted in desumoylation of all three transcription machineries (Fig. 2*A*): the RNAPIII subunits Ret1, Rpc37, Rpc82, and Rpc53; the TFIIIB subunits Brf1 and Bdp1; the TFIIIA Pzf1; and the RNAPII subunit Rpb4. Finally, Rpo26 (common to all three polymerases) was also desumoylated after heat stress (Fig. S2 and Table S1).

Figure 1. Characterization of Sumo proteomes in five stress conditions by MS. *A*, experimental workflow for Ni–NTA enrichment of sumoylated proteins in diverse stress conditions. Exponentially growing cells expressing *HIS_6-FLAG-SMT3* were exposed to different cellular stressors as indicated on the scheme. The cells were lysed under denaturing conditions, and sumoylated proteins were enriched with Ni–NTA affinity matrix. Sumo substrates in each condition were identified by MS. *B* and *C*, heat maps showing specific signatures of differentially sumoylated proteins under different stress conditions. All identified Sumo targets are shown in *B*, whereas *C* shows only transcription machineries subunits. Relative changes in the sumoylation status of identified targets after each stress are displayed, where *green* denotes an increase in sumoylation, and *red* denotes a decrease. Values from two biological replicates per stress are depicted. The *asterisk* to the *right* of each heat map highlights proteins displaying a SSR that is consistent across all five stresses. *D* and *E*, Circos plot representation of overlaps between the different stresses for proteins enriched in Sumo pulldowns. The outer arcs (*multiple colors*) on each plot represent a given stress, whereas the inner arcs (*blue*) represent proteins depleted (*D*) or enriched (*E*) from Sumo pulldowns in the given stress. *Lines* connect the same proteins shared by multiple stresses. *F* and *G*, heat map of differentially enriched biological processes (*F*) or cellular components (*G*) based on GO identifiers generated using Metascape (52). The GO terms were assigned from the list of proteins identified in Sumo pulldowns in stressed *versus* unstressed samples. The respective −log10 (*p* values) are visualized with a color scale ranging from 0 for no representation to +20 for overrepresentation. *HS*, heat stress; *Os*, osmotic stress; *Ox*, oxidative stress; *DA*, DNA alkylation; *-N*, nitrogen starvation.

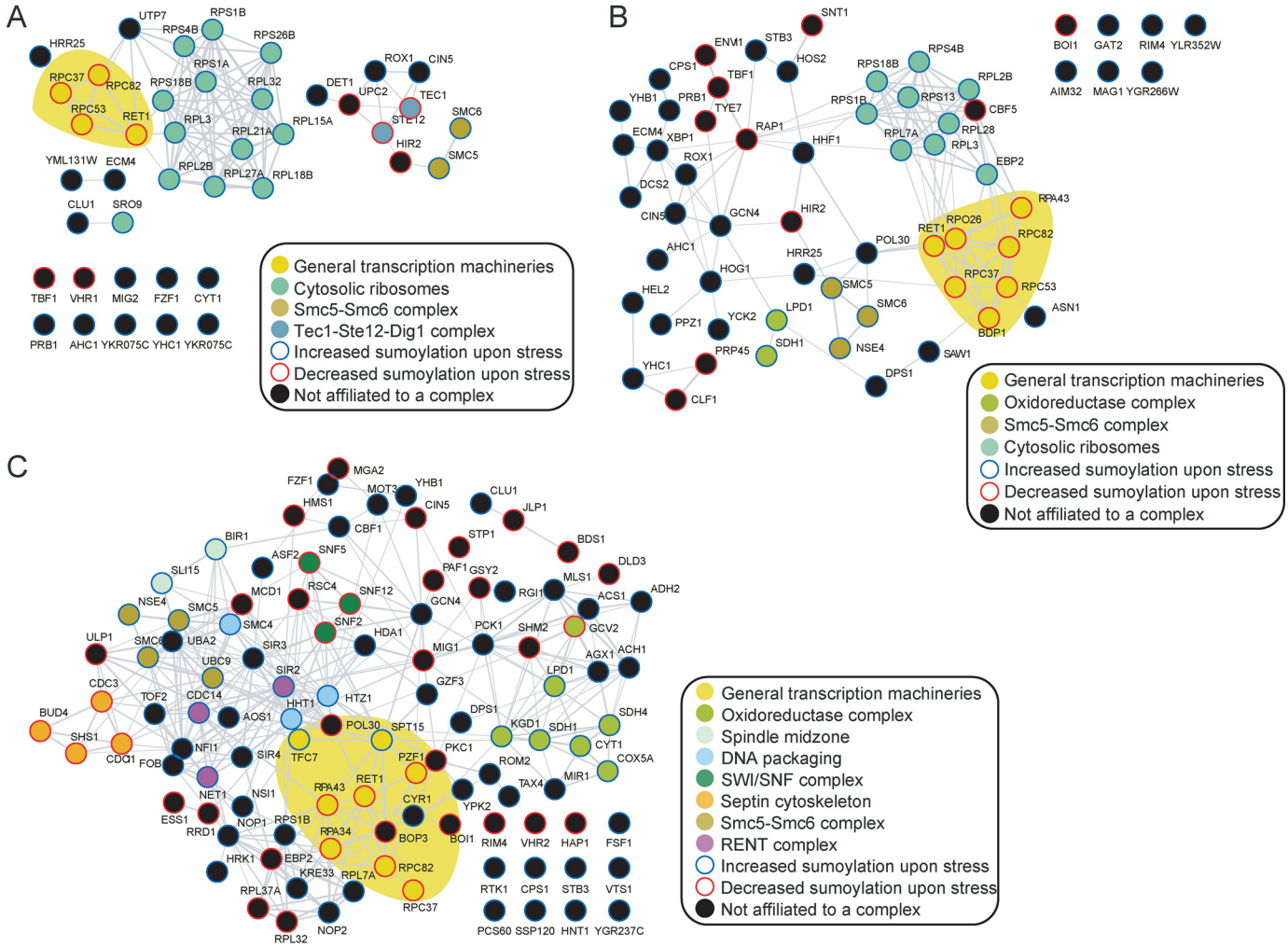


Figure 3. Identification of sumo-modified complexes: oxidative stress, DNA alkylation, and nitrogen starvation. Networks were generated for differentially sumoylated substrates following oxidative stress (*A*), DNA alkylation (*B*), and nitrogen starvation (*C*). Nodes represent proteins and edges represent protein–protein associations. Desumoylated proteins are circled in *red*, whereas more sumoylated proteins are circled in *blue*. Enriched complexes are depicted with different colors (see legend). *Black nodes* represent proteins not associated with enriched complexes. Networks were generated using the STRING database (55).

Hyperosmotic stress-regulated sumoylome

We identified 128 differentially sumoylated substrates after hyperosmotic stress. We found that 87 of 128 substrates were desumoylated (Table S1). These desumoylated proteins participate in various cellular processes, and the vast majority of differentially sumoylated proteins comprised nuclear proteins (Table S2 and Fig. 2*B*). A prominent group of differentially sumoylated proteins included transcription factors previously shown to participate in general stress responses, including Crz1, Asg1, and Yhb1 (28–30), as well as the osmotic stress-regulated transcription factor Sko1 (31). In addition to these DNA sequence-specific transcription factors, we found that several subunits of chromatin silencing and remodeling complexes are Sumo targets, such as the RSC (remodeling the structure of chromatin)–type complex, and two SWI/SNF superfamily-type complexes, Ino80 and Swr1 (Fig. 2*B* and Tables S1 and S2). Furthermore, multiple subunits of RNAPI, RNAPII, and RNAPIII were desumoylated upon hyperosmotic stress, as well as the basal transcription factors TFIIA and TFIIF, and subunits of the Mediator complex, whereas subunits of the TFIID (Taf4, Taf5, Taf7, Taf8, and Taf12) showed increased sumoylation. In agreement with our findings, a previous study also found that hyperosmotic stress affects sumoylation of multiple components of the chromatin remodeling SWI/SNF complex, as well as subunits of TFIID (25). Finally, we found that a major target of the hyperosmotic SSR was the RNAPIII machinery, including subunits of the RNAPIII basal transcription factors TFIIIB and TFIIIC, all of which were desumoylated (Fig. 2*B*, Fig. S2, and Table S2).

Oxidative stress-regulated sumoylome

Upon exposure to H_2O_2, 40 proteins were found differentially sumoylated, which is the smallest number of all the stresses we tested (Table S1). Ribosome components were most frequently observed to be differentially sumoylated, including 12 ribosomal proteins and one ribosome-associated protein (Sro9), which underwent increased sumoylation (Fig. 3*A* and Table S2). The few proteins that became less sumoylated after oxidative stress were the RNAPIII subunits Rpc37, Ret1, Rpc53, and Rpc82, as well as two subunits of the Tec1–Ste12–Dig1

translational repressor complex involved in filamentous growth (Figs. 1*C* and 3*A* and Table S1).

DNA alkylation stress-regulated sumoylome

DNA alkylation stress resulted in differential sumoylation of 60 Sumo substrates, including proteins involved in diverse DNA repair pathways that were previously reported to be Sumo substrates upon exposure to DNA damage (Table S1). These include Mag1, which is a 3-methyl-adenine DNA glycosylase involved in the base excision repair pathway, and Saw1, which is involved in DNA repair by recruiting the Rad1–Rad10 complex. We also identified the aforementioned Smc5/6 complex (Table S2 and Fig. 3*B*). In addition, multiple components of the translational machinery were differentially sumoylated after treatment with alkylating agents, including eight ribosomal proteins that were more sumoylated and one component of the 66S preribosomal particle, Ebp2, which was desumoylated (Table S1 and S2). Similar to the other stresses, RNAPIII was among the most represented complex, with five subunits being desumoylated (Ret1, Rpc82, Rpc37, Rpc53 and the TFIIIB subunit Bdp1). In addition, one subunit of RNAPI (Rpa43) and one subunit shared by all three polymerases (Rpo26) were desumoylated in response to treatment with alkylation agents (Fig. 3*B*, Fig. S2, and Table S1).

Nitrogen starvation stress-regulated sumoylome

We identified 105 proteins that were differentially sumoylated after nitrogen stress, 65 of which showed increased sumoylation (Table S1). The septin ring organization complex became desumoylated, which was also observed after heat and hyperosmotic stresses (Fig. 3*C*). Interestingly, our data suggest that some Sumo targets display a sumoylation behavior during nitrogen starvation that is opposite to that of hyperosmotic stress. For instance, hyperosmotic stress resulted in desumoylation of the Sir2-containing RENT complex subunits, the Smc5/6 complex, and the chromosomal passenger proteins Bir1 and Sli15, whereas nitrogen starvation stress resulted in a significant increase in their sumoylation levels (Figs. 2*B* and 3*C* and Table S1). Proteins that became desumoylated mainly include RNAPI subunits Rpa43 and Rpa34; RNAPIII subunits Ret1, Rpc82, and Rpc37; and the RNAPII-associated transcription factor Paf1 (Fig. S2). Two proteins that are particularly relevant for the response to nitrogen starvation are the transcription factors Gcn4 and Stp1, which are involved in the transcription of amino acid biosynthetic genes and amino acid permease genes, respectively (32). Gcn4 is a known Sumo target (33) and showed increased Sumo modification after nitrogen starvation, whereas Stp1 became desumoylated (Fig. 3*C* and Table S1). Interestingly, a unique feature of the nitrogen starvation stress response was an apparent increase in sumoylation of multiple subunits of the mitochondrial respiratory chain, with the notable exception of Gcv2 (glycine decarboxylase), which was desumoylated (Tables S1 and S2).

Desumoylation of RNAPIII constitutes the core of the SSR

Among the most consistently regulated SSTs were subunits of RNAPIII and its general transcription factors (Fig. 1, *B–D*, and Table S1 and S2). We validated these MS data by immunoblotting. Sumo-conjugated proteins were affinity-purified from exponentially growing or stressed yeast cultures under denaturing conditions, and SDS-PAGE immunoblotting was performed with antibodies recognizing HA-tagged Rpc82 (Fig. 4*A*) or Myc-tagged Ret1 (Fig. 4*B*). Sumoylation of Rpc82 was significantly reduced following exposure to heat stress, nitrogen starvation, hyperosmotic stress, and DNA damage, whereas desumoylation of Rpc82 in response to oxidative stress appeared to be prone to experimental variation for reasons we do not currently understand (Fig. 4*A*). Sumoylation of Ret1 was also strongly reduced after exposure to all stressors (Fig. 4*B*).

Physiological relevance of stress-induced desumoylation of Rpc82 and Ret1

We previously reported that TORC1 inhibition and nitrogen starvation result in desumoylation of Ret1 and Rpc82, which decreases their presence at class III genes (RNAPIII-transcribed genes) (23). We then asked whether oxidative stress, hyperosmotic stress, heat stress, and DNA damage provoke a similar response as nitrogen starvation. We analyzed the association of Rpc82 and Ret1 to class III genes by ChIP-qPCR following exposure to stress. We found that Rpc82 occupancy was significantly reduced at $tDNA^{Leu}$ in response to heat stress, hyperosmotic stress, oxidative stress, alkylation stress, and nitrogen starvation stress (Fig. 4*C*; see Fig. 4*F* for primer locations). These forms of stress also reduced Rpc82 levels at *SCR1*, which is another noncoding RNA transcribed by RNAPIII, although the reduction in Rpc82 levels at this gene did not appear to be significant under oxidative stress (Fig. 4, *C* and *F*). Ret1 occupancy also appeared significantly decreased at $tDNA^{Leu}$ after exposure to stress, with the exception of oxidative stress, which only caused a slight, nonsignificant reduction in Ret1 levels (Fig. 4, *D* and *F*). Furthermore, Ret1 levels at *SCR1* were only significantly reduced after hyperosmotic stress and nitrogen starvation, whereas heat stress, oxidative stress, and alkylating stress had little or no effect. We also assessed the recruitment of Brf1, a subunit of the RNAPIII transcription factor TFIIIB, to these loci. Brf1 occupancy was decreased at $tDNA^{Leu}$ in response to all stresses, whereas Brf1 levels at *SCR1* were only reduced after hyperosmotic stress and nitrogen starvation (Fig. 4, *E* and *F*).

We previously reported that Rpc82 sumoylation, but not Ret1 SUMOylation, was necessary to promote RNAPIII assembly and transcriptional activity at class III genes (23). tRNA molecules are transcribed as precursors (pre-tRNAs) that undergo sequential post-transcriptional modifications to generate mature tRNAs. These steps include removal of 3′ and 5′ ends, addition of 3′ and 5′ tails, intron removal for intron-containing tRNAs and a number of nucleoside modifications. Some of these modifications may impair analysis of RNA levels by RT-qPCR, because they block efficient conversion into cDNA (34, 35). Given that mature tRNAs are very stable, it is important to study the immature form to determine the effect of a given stimulus on *tDNA* transcription. Therefore, to assess whether *tDNA* transcription was affected by stress, we performed Northern blotting using probes specific for the precursors and mature forms of $tRNA^{Leu}$ and $tRNA^{Trp}$. Both $tRNA^{Leu}$

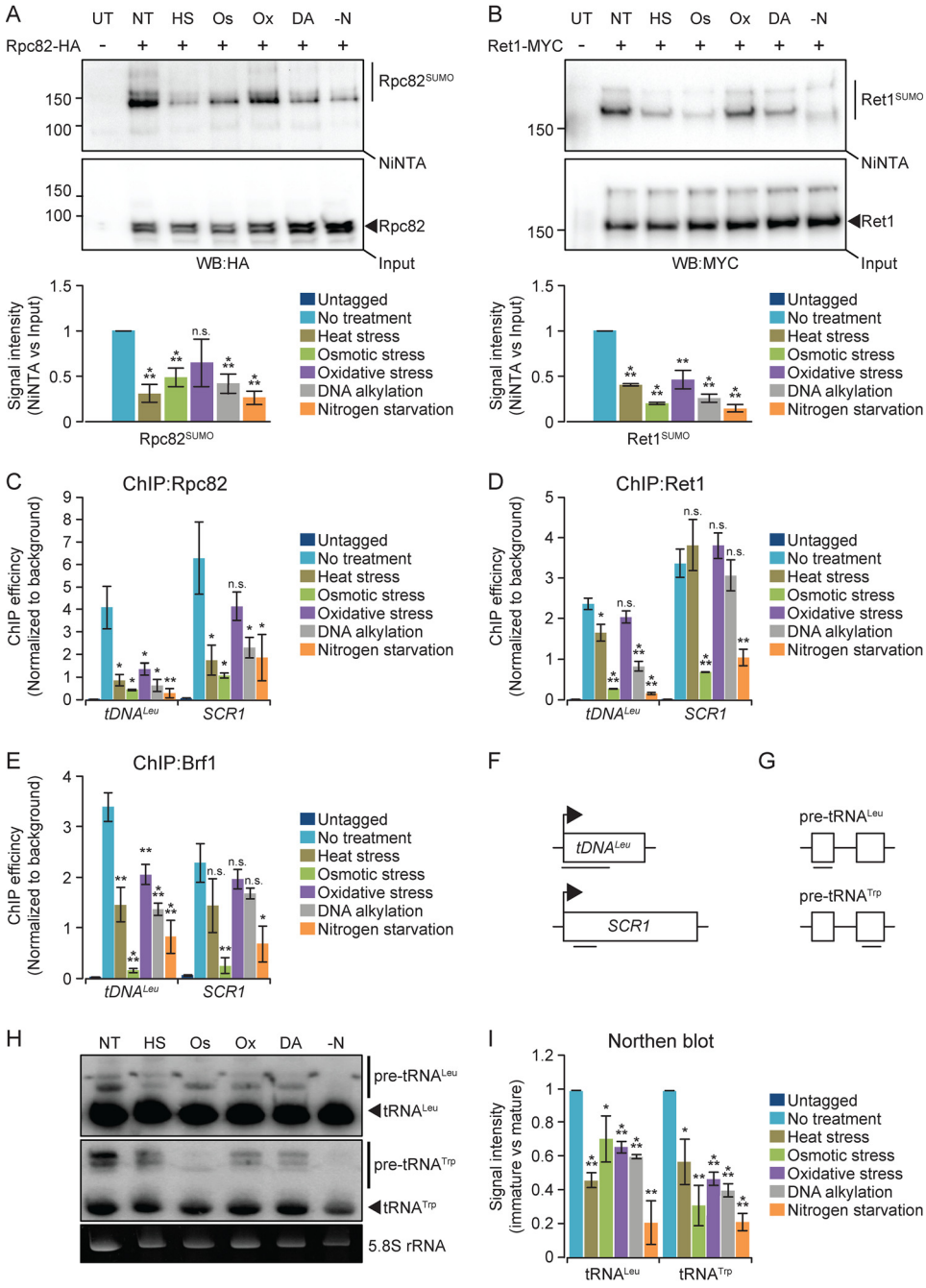


Figure 4. Desumoylation of Ret1 and Rpc82 upon stress rewire their binding to class III genes and affect RNAPIII transcriptional activity. *A* and *B*, validation of MS data. Sumo pulldowns were performed under denaturing conditions using cell lysates of *6HF-SMT3* cells grown in the indicated conditions. The levels of copurifying Rpc82-HA and Ret1-MYC were analyzed by Western blotting (*WB*) using anti-HA (*A*) and anti-MYC (*B*) antibodies, respectively. The *lower graphs* display the quantification of Rpc82-HA (*A*) and Ret1-MYC (*B*) signals. *Error bars* indicate the deviation from the average of three different experiments. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. *n.s.*, nonsignificant (Student's *t* test); *UT*, untagged; *NT*, no treatment; *HS*, heat stress; *Os*, osmotic stress; *Ox*, oxidative stress; *DA*, DNA alkylation; *-N*, nitrogen starvation. *C–E*, stress reduces the recruitment of RNAPIII holoenzyme and general transcription factor TFIIIB at class III genes. The occupancy of RNAPIII and TFIIIB subunits were analyzed by ChIP-qPCR. Yeasts were stressed as indicated in Fig. 1, and the relative levels of Rpc82-HA (*C*), Ret1-MYC (*D*), and Brf1-GFP (*E*) at *tDNA*^{Leu} and *SCR1* were determined by ChIP-qPCR using anti-HA, -MYC, and -GFP antibodies, respectively. *F*, schematic representation of the regions where primers used for ChIP anneal. *G*, schematic representation or Norther blotting probes. *H*, precursor tRNAs (pre-tRNA) do not accumulate under stress. Pre-tRNA levels were analyzed by Northern blotting. Northern blots are representative of three independent biological replicates of experiments. WT cells were grown at 30 °C to exponential phase and then stressed as specified in Fig. 1. Total RNA was isolated and analyzed by Northern hybridization with oligonucleotide probes specific to precursor and mature tRNA$^{Leu(CAA)}$ (*top panel*) and tRNA$^{Trp(CCA)}$ (*middle panel*). Loading control is provided by ethidium bromide–stained 5.8S rRNA (*bottom panel*). *I*, ratios of precursor over mature tRNAs. *Errors bars*, S.E. of three different experiments. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

and tRNA^{Trp} are synthesized from intron-containing genes (Fig. 4*G*), and mature tRNAs migrate faster than the unprocessed intron-containing forms. We observed that mature tRNA^{Leu} and mature tRNA^{Trp} levels were unaffected in response to cell stress (Fig. 4, *H* and *I*). In contrast, the relative abundance of precursor tRNA^{Leu} and tRNA^{Trp} was significantly decreased following exposure to stress (Fig. 4, *H* and *I*). Taken together, these results suggest that stress generally trig-

gers desumoylation of RNAPIII subunits Ret1 and Rpc82, which affects their occupancy at *tDNA* genes, resulting in reduced tRNA synthesis.

Discussion

Sumo has previously been implicated in adaptation to cellular stress, and cell stress can induce increased global sumoylation, which is referred to as the Sumo stress response (reviewed in Ref. 24). Despite the preexisting literature on stress-induced changes in the sumoylation levels of several Sumo substrates (36–38), it remains unclear whether the SSR constitutes a broad pathway consisting of a common set of Sumo targets (regardless of the type of stress) or whether each type of stress triggers its own specific SSR involving a unique core set of proteins.

In this study, we addressed this question by exploring the SSR induced by five different forms of stress, *i.e.* heat stress, hyperosmotic stress, oxidative stress, DNA alkylation stress, and nitrogen starvation stress. We isolated sumoylated proteins and performed a comparative analysis of targets obtained for each stress using MS. Interestingly, each form of stress resulted in differential sumoylation of largely nonoverlapping sets of Sumo substrates. The only Sumo targets shared by each SSRs were the RNAPIII subunits Rpc37, Ret1, and Rpc82, which invariably became desumoylated upon cellular injury. We conclude that each stress triggers its own unique SSR, which in some cases partially overlaps with SSRs of other forms of stress. A notable exception is desumoylation of RNAPIII components, which appears to be a key effect shared by all SSRs.

Different stresses trigger stress-specific SSRs

We observed limited overlap between the SSR patterns induced by the five different stressors that we have tested. However, we found that SSTs belong to a relatively small number of 26 mainly nuclear protein complexes involved in transcription, chromatin organization, centromeric function, translation, and energy metabolism. Of these 26 complexes, 19, 15, 13, 8, and 5 complexes were differentially sumoylated upon osmotic stress, nitrogen starvation, heat stress, DNA alkylation, and oxidative stress, respectively. These data suggest that each SSR mobilizes the sumoylation pathway in a stress-specific manner (Table S2).

The nature of these 26 complexes is remarkably consistent with Sumo targets identified in previous studies (33). It will be interesting to determine the physiological effect of Sumo on these complexes under normal growth conditions *versus* stress conditions. Given that Sumo is thought to act as a molecular glue that stabilizes protein–protein interactions (21), it is tempting to hypothesize that the SSR regulates the activity of these protein complexes by controlling their stability/assembly. Indeed, differential sumoylation triggered by DNA damage and nitrogen starvation affects the stability of DNA repair complexes and transcription factor complexes (20, 22, 23).

We have identified SSTs that belong to the general transcription machinery, including the three RNA polymerases and their general transcription factors (Table S1 and S2); for instance, sumoylation of TFIIIA, TFIIIB, TFIIIC, RNAPIII, and RNAPI, all of which are involved in synthesis of factors involved in mRNA translation, is particularly affected in response to multiple forms of stress, emphasizing the importance for the cell to regulate its translational capacity under conditions that may compromise cellular integrity. A large number of SSTs belong to complexes involved in chromatin organization and regulation (Table S2). The Nua4, Ino80, SWR1, and RSC-type complexes were all differentially sumoylated in several, yet nonoverlapping stresses. This suggests that in response to each stress Sumo may regulate a specific pathway leading to chromatin remodeling and potential stress-specific differential gene regulation.

In response to all stresses other than oxidative stress, Sumo is differentially linked to cytoplasmic protein complexes that belong to the mitochondrial respiratory chain (Table S2). This is consistent with the rewiring of cellular energy metabolism in response to stress. Nevertheless, the effect of Sumo on energy production by mitochondria during SSR remains to be clarified.

A large part of the cell's energy is devoted to the translation process, and decreasing translational capacity helps cells survive stressful conditions (39). Several ribosomal proteins and ribosome-associated factors were differentially sumoylated (mainly hypersumoylated) in all stresses but oxidative stress. Whether sumoylation regulates ribosomal assembly or activity during various SSRs remains to be determined.

Sumoylation is a dynamic process, and different stressors induce different kinetics of SSR activation and inactivation (25). It is possible that stress-specific SSRs share a greater number of common SSTs than the three that we identified in the present study (*i.e.* Rpc82, Rpc37, and Ret1; see below), depending on the time the cell is exposed to a given stressor. One limitation of our study is that we analyzed single time points, and it will be interesting to perform time-course analyses with multiple stresses to determine the degree of overlap between stress-dependent SSRs at high temporal resolution.

Desumoylation of RNAPIII constitutes the core of the SSR

PTMs of the RNAPIII and subsequent alteration of tRNA synthesis is still an understudied field (40). In normally growing cells, sumoylated RNAPIII and TORC1 maintain high translational capacity by sustaining strong levels of tRNA synthesis and ribosomal protein gene transcription (22, 23). Under these conditions the active conformation of the RNAPIII holoenzyme is supported by the sumoylation of its subunit Rpc82, which promotes *tDNA* transcription (23). However, nitrogen starvation or treatment with the TORC1 inhibitor rapamycin triggers the desumoylation of several RNAPIII subunits, including Rpc82, Ret1, and Rpc53. This results in reduced Rpc82 levels at RNAPIII-transcribed genes, such as *tDNAs* and *SCR1*, and subsequent inhibition of tRNA synthesis.

In the present study we reiterated these findings, and more strikingly, we found that Rpc82, Ret1, and Rpc37 sumoylation was reduced upon all stresses that we have tested. We also found that Rpc53 was desumoylated upon all stresses except for one nitrogen starvation biological replicate. In this replicate, although Rpc53 sumoylation was reduced, it did not reach the significance cutoff (Fig. 1 and Fig. S2). However, we previously detected Rpc53 desumoylation upon nitrogen starvation or rapamycin treatment using label-free MS and stable isotope labeling of amino acids in cell culture, and these findings were

confirmed by Western blotting (23). We therefore believe that despite minor experimental variation, Rpc53 is a fourth subunit of RNAPIII belonging to the core of the SSR. Now, the question is: why are these four subunits invariably desumoylated upon stress?

Interestingly Ret1–Rpc82 on one side and Rpc37–Rpc53 on the other side physically interact and form two subcomplexes of the RNAPIII holoenzyme, which interact with TFIIIB and TFIIIC, respectively (41, 42). Given the preeminent role of Sumo in regulating protein–protein interactions, it is plausible that the stability of these two subcomplexes within the holoenzyme, or their interaction with TFIII-B or -C, is altered by desumoylation during the SSR. This could result in the destabilization of the RNAPIII transcriptional complex and decreased tRNA synthesis. This hypothesis is supported by the lower levels of Rpc82 and Ret1 detected at *tDNA* genes in all stresses, which correlated with a decrease in tRNA synthesis (Fig. 4). These data indicate that cells carefully regulate Rpc82, Ret1, Rpc37, and Rpc53 sumoylation to control tRNA synthesis under optimal growth conditions and during cell stress.

Contrary to Rpc82, we observed that recruitment of Ret1 and Brf1 (a subunit of TFIIIB) was impaired mainly at tRNA genes, whereas it was only moderately affected at *SCR1* in three of the five stresses that were tested (Fig. 4). This suggests that stress predominantly regulates RNAPIII assembly at tRNA genes rather than at other genes transcribed by the RNAPIII such as *SCR1* (Fig. 4). This could be due to the different promoter and gene structure of *SCR1* compared with tRNA genes and may involve additional regulatory pathways during SSR.

Another interesting question is the relevance of our findings to mammalian cells. Indeed, previous high-throughput MS studies found that the human RNAPIII subunits RPC3, RPC4, and RPC5 (yeast Rpc82, Rpc53, and Rpc37, respectively) are Sumo substrates in normally growing HeLa cells (43). Interestingly, RPC3 and RPC4 are differentially sumoylated in response to heat shock, and RPC4 and RPC5 are desumoylated upon MMS treatment (43, 44). Of note, in these studies the human RNAPI subunit RPA34 appeared desumoylated in response to heat stress and MMS, which we have also found upon starvation and osmotic stress in yeast. Strong sumo signals were also found at tRNA genes in K562 cells using ChIP-seq (37, 45, 46). These Sumo signals decreased at tRNA genes upon heat shock, suggesting that, as we previously found in yeast (23), desumoylation of the RNAPIII complex also occurs during the stress response in mammals. These data indicate that differential Sumo modification of basal transcription factors may constitute an evolutionarily conserved regulatory hub altering tRNA (and rRNA) synthesis in response to stress.

As mentioned above, previous reports showed that the TORC1 pathway and the sumoylation pathway cooperate to trigger a decrease in translational capacity that allows cells to survive during nutrient stress (22, 23, 39, 47, 48). In the present study we extend the role of the sumoylation pathway beyond cellular response to starvation, by showing that inhibition of tRNA synthesis via desumoylation of Rpc82, Ret1, and Rpc37 (and possibly Rpc53) is triggered by several other stressors. We propose that although each stress triggers a stress-specific SSR, desumoylation of RNAPIII and subsequent inhibition of tRNA synthesis form the general mechanism of adaptive response to stress that constitutes the core of each SSR.

Experimental procedures

Yeast strains and growth conditions

S. cerevisiae strains used in this study are derivatives of either the S288c strains RDKY3615 (49) or BY4741 and are listed in Table S3. Yeast genetic manipulations were performed using standard gene-replacement methods or intercrossing. Yeast strains were grown at 30 °C to mid-logarithmic phase in complete synthetic medium (CSM) (2% glucose, 0.67% yeast nitrogen base without amino acids, 0.08% complete dropout mixture). Heat stress was performed at 42 °C in preheated CSM. Hyperosmotic stress was performed in CSM in which glucose was substituted with 1.5 M sorbitol. Oxidative stress and DNA damage were induced with addition of, respectively, 0.75 mM hydrogen peroxide or 0.1% methyl methanesulfonate. Strains were grown in minimal medium (2% glucose, 0.17% yeast nitrogen base without amino acids without ammonium sulfate) to induce nitrogen starvation. For hyperosmotic and oxidative stresses and DNA damage samples, the cells were harvested after 30 min of treatment. Heat stress and nitrogen starvation samples were harvested after 10 min or 3 h of treatment, respectively.

Whole cell lysates for Western blotting

The cells (10 ml) were collected by centrifugation, washed with cold water, and flash-frozen in liquid nitrogen. Pellets were resuspended in 250 μl of lysis solution (1.85 M NaOH, 7.4% β-mercaptoethanol (v/v)). After 10 min on ice, 250 μl of 55% TCA was added, and the samples were incubated 10 min on ice. Proteins were pelleted by centrifugation (10 min at 4 °C at 10,000 rpm). The remaining pellets were washed 30 min at −20 °C with 90% acetone. Pellets were then resuspended in SDS-PAGE sample buffer and incubated at 95 °C for 10 min prior to SDS-PAGE (Fig. S1).

Sumo pulldown under denaturing conditions for MS analysis

Sumo pulldowns were performed as previously described (22) in two independent biological repeats. Briefly, for each growth condition, 1 liter of cells expressing His_6-FLAG-Smt3 were harvested, washed in cold water, and flash-frozen in liquid nitrogen. Lysates were prepared by alkaline lysis (1.85 M NaOH) in presence of β-mercaptoethanol (7.5% (v/v)) and TCA protein precipitation. Proteins were pelleted by centrifugation, washed with cold water, and resuspended in buffer A (6 M guanidine HCl, 100 mM NaH_2PO_4, 10 mM Tris-HCl, 0.05% Tween; adjusted to pH 8.0). The suspension was homogenized at room temperature for 1 h, and insoluble material was removed by centrifugation. The supernatants were supplemented with 10 mM imidazole and incubated overnight at 4 °C in presence of Ni–NTA–agarose beads (Qiagen). The beads were washed in buffer A and buffer C (8 M urea, 100 mM, 10 mM Tris-HCl, 0.05% Tween; adjusted to pH 6.3), and proteins bound to the Ni–NTA–agarose were eluted in buffer C supplemented with 250 mM imidazol. Proteins were precipitated with 55% TCA, and the pellets were washed with −20 °C acetone before proceeding to proteolytic digestion.

Sumo pulldown under denaturing conditions for Western blotting

The cells (20 ml) were collected by centrifugation, washed in ice-cold water, and flash-frozen. The pellets were resuspended in lysis buffer (8 M urea, 50 mM Tris, pH 8.0, 0.05% SDS) supplemented with 10 mM N-Ethylmaleimide and protease inhibitor cocktail (Roche), and the cells were lysed by mechanical disruption with glass beads. Cells debris was discarded by centrifugation, and 5% of the supernatant was saved as input material. The remaining supernatant was incubated with 50 μl of Ni–NTA–agarose beads and incubated overnight at 4 °C. The beads were washed three times for 5 min with wash buffer (8 M urea, 50 mM Tris, pH 8.0, 200 mM NaCl, 0.05% SDS) supplemented with 5 mM imidazol. Proteins bound to the resin were eluted by the addition of loading buffer (8 M urea, 10 mM MOPS, 10 mM EDTA, 1% SDS, 0.01% bromphenol blue, pH 6.8). Samples were incubated 10 min at 65 °C prior to SDS-PAGE.

Mass spectrometry and data analyses

Protein pellets were solubilized in 50 mM NH_4HCO_3 containing 2% rapigest surfactant (Waters), reduced using DTT (5 mM, 45 min, 56 °C), and digested overnight at 37 °C with trypsin (Promega). Rapigest was removed by acidification (0.5–1% TFA), and peptide samples were desalted using c18 StageTips prior to MS analysis.

LC-MS/MS analyses were performed using an Easy nLC1000 LC system (Thermo Electron) connected to a quadrupole Orbitrap (QExactive Plus) mass spectrometer (Thermo Electron) and involved a nanoelectrospray ion source (EasySpray; Thermo Electron). An EasySpray analytical column (C18, 2-μm beads, 100 Å, 75-μm inner diameter; Thermo) was used for peptide separation, with a flow rate of 0.3 μl/min, and solvent gradient of 2% to 30% (v/v) acetonitrile in 0.1% (v/v) formic acid for 120 min, after which columns were washed using 90% (v/v) acetonitrile in 0.1% (v/v) formic acid for 20 min. All LC-MS/MS analyses involved data-dependent acquisition, where selected peptides were fragmented using high-energy collision dissociation. Ions selected for MS/MS were dynamically excluded for 30 s.

Protein identification and label-free quantification was performed using the MaxQuant software (50). Statistical analyses were performed in the Perseus software (51) using log2-transformed LFQ intensities. For analysis of desumoylation events, the proteins that were not significantly enriched in the SUMO pulldown in mid-logarithmic phase compared with nontagged SUMO controls were removed. For analysis of increased sumoylation events, proteins that were not significantly enriched in SUMO pulldowns under a stress condition compared with nontagged SUMO controls were removed. Comparisons were then performed between each SUMO pulldown under a stress condition and the SUMO pulldown in mid-logarithmic phase. The samples were compared using a Student's *t* test method (permutation-based false discovery rate correction (250 randomizations); false discovery rate cutoff = 0.05; S0 = 0.1). Proteins were considered significantly reduced or increased under a specific stress condition if they passed the Student's *t* test significance threshold across two biological repeat experiments. For display purposes, samples were also compared using Volcano plots as performed in Perseus. Network enrichment analysis of each list of significant proteins was performed using Metascape (52). Processed data for all proteins enriched in at least one stress condition *versus* the mid-logarithmic control are displayed in Table S1. All raw MS data and MaxQuant search parameter details are available via the PRIDE database (see details below). Experiment labels for PRIDE data are as follows: control/untagged (S1 or 398); no treatment/mid-logarithmic phase (S2 or 484); nitrogen starved (S3 or S.D.); oxidative stress (S4 or H_2O_2); osmotic stress (S5 or sorbitol); DNA damage (S6 or MMS); heat shock (S7 and S8 in biological repeat 2). Biological repeat 1 includes an extra experiment (S7 or 46c) that was not used for final analyses.

ChIP

ChIP assays were performed as previously described (53). Briefly, 50 ml of mid-log phase cells were fixed with 1% (v/v) formaldehyde for 30 min, and formaldehyde was quenched by adding glycine to a final concentration of 125 mM. The cells were centrifuged, and pellets were washed with cold TBS then lysed in lysis buffer (50 mM Hepes-KOH, pH 7.5, 140 mM NaCl, 1 mM EDTA, 1% (v/v) Triton X-100, 0.1% (w/v) sodium deoxycholate, and protease inhibitor mixture). Samples were sonicated twice using a diagenode Bioruptor Twin, for 15 min with alternating cycles of 30-s pulses. The resulting samples were centrifuged, and the supernatant was collected. ChIP was performed overnight at 4 °C on 100 μg of chromatin. The beads were washed with the lysis buffer and then washed with the lysis buffer supplemented with 500 mM NaCl. The beads were further washed with wash buffer (10 mM Tris, pH 8.0, 250 mM LiCl, 0.5% (v/v) Nonidet P-40, 0.5% (w/v) sodium deoxycholate, and 1 mM EDTA) and with a buffer containing 10 mM Tris and 1 mM EDTA, pH 8.0, prior elution in 50 mM Tris, pH 8.0, 10 mM EDTA, 1% (v/v) SDS at 65 °C. The cross-link was reversed through at 65 °C for 6 h. RNA and proteins were degraded with RNase and proteinase K treatments, respectively, and DNA was purified using QIAquick PCR purification kit (Qiagen). DNA fragments were analyzed by qPCR with an AB StepOnePlus machine (Applied Biosystem) using primers amplifying *tDNA*^{Leu} (forward, GTCTAAGGCGCCTGATTCAAGA, and reverse, GTTCACTGCGGTCAAGAT) or *SCR1* (forward, GAATTCTGGCCGAGGAACAAATCC, and reverse, CAGCTCTGCCCAGGACAAATTTAC).

Northern blotting

The cells (10 ml) were harvested by centrifugation, washed in ice-cold water, and frozen in liquid nitrogen. Total RNA was extracted using mirVANA miRNA isolation kit (Thermo Fisher Scientific) following the manufacturer's instructions. After heat denaturation at 55 °C for 5 min, the samples were separated by 12% PAGE in 1× taurine at 200 V for 80 min. tRNAs were transferred to a nylon membrane (Hybond XL, GE Healthcare) by electroblotting in 1× taurine at room temperature and 200 mA. RNAs were UV-cross-linked to membranes (120 mJ cm^{-2} in a CL-1000 UV-Cross-linker, UVP) and prehybridization, hybridization, and washing steps were performed. tRNAs were detected by hybridization of [^{32}P]-5′-end-labeled oligonucleo-

tides probes complementary to specific sequences of their non-intron regions. Immature forms and other post-transcriptional modifications migrate more slowly than the mature forms. Hybridized probes were removed from the filters by boiling in 0.1% SDS. The sequences of the probes are CAGGAATTGAA-CCTGCAACCCTTC for $tRNA^{Trp(CCA)}$ and GGTTGCTAAG-AGATTCGAACTC for $tRNA^{Leu(CAA)}$. Hybridization signals were detected using a Amersham™ Typhoon™ biomolecular imager and analyzed with the ImageQuant TL software.

Data deposition

The MS proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE (54) partner repository with the data set identifier PXD013884.

Author contributions—A. N. P. and J. R. data curation; A. N. P. software; A. N. P., P. C., and J. M. E. formal analysis; A. N. P. validation; A. N. P., J. R., and M. C. H. investigation; A. N. P. and J. R. visualization; A. N. P., J. R., P. C., and J. M. E. writing-original draft; A. N. P., J. R., M. C. H., P. C., and J. M. E. writing-review and editing; P. C. and J. M. E. resources; P. C. and J. M. E. supervision; P. C. and J. M. E. funding acquisition; P. C. and J. M. E. project administration; P. C. and J. M. E. conceptualization.

Acknowledgments—We thank Tuula Nyman and Gustavo De Souza from the Proteomics Core Facility of the University of Oslo for assistance with experimental approaches, data analysis and data representation. We also thank Ragnhild Eskeland and Jørgen Wesche for their critical reading of the manuscript.

References

1. Wilkinson, K. A., and Henley, J. M. (2010) Mechanisms, regulation and consequences of protein SUMOylation. *Biochem. J.* **428,** 133–145 CrossRef Medline
2. Giaever, G., Chu, A. M., Ni, L., Connelly, C., Riles, L., Véronneau, S., Dow, S., Lucau-Danila, A., Anderson, K., André, B., Arkin, A. P., Astromoff, A., El-Bakkoury, M., Bangham, R., Benito, R., *et al.* (2002) Functional profiling of the *Saccharomyces cerevisiae* genome. *Nature* **418,** 387–391 CrossRef Medline
3. Li, S. J., and Hochstrasser, M. (1999) A new protease required for cell-cycle progression in yeast. *Nature* **398,** 246–251 CrossRef Medline
4. 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 CrossRef Medline
5. Desterro, J. M., Thomson, J., and Hay, R. T. (1997) Ubch9 conjugates SUMO but not ubiquitin. *FEBS Lett.* **417,** 297–300 CrossRef Medline
6. Johnson, E. S., and Blobel, G. (1997) Ubc9p is the conjugating enzyme for the ubiquitin-like protein Smt3p. *J. Biol. Chem.* **272,** 26799–26802 CrossRef Medline
7. Gareau, J. R., and Lima, C. D. (2010) The SUMO pathway: emerging mechanisms that shape specificity, conjugation and recognition. *Nat. Rev. Mol. Cell Biol.* **11,** 861–871 CrossRef Medline
8. Takahashi, Y., Toh-e, A., and Kikuchi, Y. (2001) A novel factor required for the SUMO1/Smt3 conjugation of yeast septins. *Gene* **275,** 223–231 CrossRef Medline
9. Smet-Nocca, C., Wieruszeski, J. M., Léger, H., Eilebrecht, S., and Benecke, A. (2011) SUMO-1 regulates the conformational dynamics of thymine–DNA glycosylase regulatory domain and competes with its DNA binding activity. *BMC Biochem.* **12,** 4 CrossRef Medline
10. Ulrich, H. D. (2005) SUMO modification: wrestling with protein conformation. *Curr. Biol.* **15,** R257–R259 CrossRef Medline
11. Kolesar, P., Altmannova, V., Silva, S., Lisby, M., and Krejci, L. (2016) Pro-recombination role of Srs2 protein requires SUMO (small ubiquitin-like modifier) but is independent of PCNA (proliferating cell nuclear antigen) interaction. *J. Biol. Chem.* **291,** 7594–7607 CrossRef Medline
12. Li, Y. J., Stark, J. M., Chen, D. J., Ann, D. K., and Chen, Y. (2010) Role of SUMO:SIM-mediated protein–protein interaction in non-homologous end joining. *Oncogene* **29,** 3509–3518 CrossRef Medline
13. Rojas-Fernandez, A., Plechanovová, A., Hattersley, N., Jaffray, E., Tatham, M. H., and Hay, R. T. (2014) SUMO chain-induced dimerization activates RNF4. *Mol. Cell* **53,** 880–892 CrossRef Medline
14. Wu, C. S., Ouyang, J., Mori, E., Nguyen, H. D., Maréchal, A., Hallet, A., Chen, D. J., and Zou, L. (2014) SUMOylation of ATRIP potentiates DNA damage signaling by boosting multiple protein interactions in the ATR pathway. *Genes Dev.* **28,** 1472–1484 CrossRef Medline
15. Churikov, D., Charifi, F., Eckert-Boulet, N., Silva, S., Simon, M. N., Lisby, M., and Géli, V. (2016) SUMO-dependent relocalization of eroded telomeres to nuclear pore complexes controls telomere recombination. *Cell Reports* **15,** 1242–1253 CrossRef Medline
16. Escobar-Ramirez, A., Vercoutter-Edouart, A. S., Mortuaire, M., Huvent, I., Hardivillé, S., Hoedt, E., Lefebvre, T., and Pierce, A. (2015) Modification by SUMOylation controls both the transcriptional activity and the stability of delta-lactoferrin. *PLoS One* **10,** e0129965 CrossRef Medline
17. Van Rechem, C., Boulay, G., Pinte, S., Stankovic-Valentin, N., Guérardel, C., and Leprince, D. (2010) Differential regulation of HIC1 target genes by CtBP and NuRD, via an acetylation/SUMOylation switch, in quiescent versus proliferating cells. *Mol. Cell. Biol.* **30,** 4045–4059 CrossRef Medline
18. Desterro, J. M., Keegan, L. P., Jaffray, E., Hay, R. T., O'Connell, M. A., and Carmo-Fonseca, M. (2005) SUMO-1 modification alters ADAR1 editing activity. *Mol. Biol. Cell* **16,** 5115–5126 CrossRef Medline
19. El McHichi, B., Regad, T., Maroui, M. A., Rodriguez, M. S., Aminev, A., Gerbaud, S., Escriou, N., Dianoux, L., and Chelbi-Alix, M. K. (2010) SUMOylation promotes PML degradation during encephalomyocarditis virus infection. *J. Virol.* **84,** 11634–11645 CrossRef Medline
20. Psakhye, I., and Jentsch, S. (2012) Protein group modification and synergy in the SUMO pathway as exemplified in DNA repair. *Cell* **151,** 807–820 CrossRef Medline
21. Chymkowitch, P., Nguéa, P. A., and Enserink, J. M. (2015) SUMO-regulated transcription: challenging the dogma. *Bioessays* **37,** 1095–1105 CrossRef Medline
22. Chymkowitch, P., Nguéa, A. P., Aanes, H., Koehler, C. J., Thiede, B., Lorenz, S., Meza-Zepeda, L. A., Klungland, A., and Enserink, J. M. (2015) Sumoylation of Rap1 mediates the recruitment of TFIID to promote transcription of ribosomal protein genes. *Genome Res.* **25,** 897–906 CrossRef Medline
23. Chymkowitch, P., Nguéa, P. A., Aanes, H., Robertson, J., Klungland, A., and Enserink, J. M. (2017) TORC1-dependent sumoylation of Rpc82 promotes RNA polymerase III assembly and activity. *Proc. Natl. Acad. Sci. U.S.A.* **114,** 1039–1044 CrossRef Medline
24. Enserink, J. M. (2015) Sumo and the cellular stress response. *Cell Div.* **10,** 4 CrossRef Medline
25. Lewicki, M. C., Srikumar, T., Johnson, E., and Raught, B. (2015) The *S. cerevisiae* SUMO stress response is a conjugation-deconjugation cycle that targets the transcription machinery. *J. Proteomics* **118,** 39–48 CrossRef Medline
26. Johnson, E. S., and Blobel, G. (1999) Cell cycle–regulated attachment of the ubiquitin-related protein SUMO to the yeast septins. *J. Cell Biol.* **147,** 981–994 CrossRef Medline
27. Miller, M. J., and Vierstra, R. D. (2011) Mass spectrometric identification of SUMO substrates provides insights into heat stress-induced SUMOylation in plants. *Plant Signal. Behav.* **6,** 130–133 CrossRef Medline
28. Akache, B., and Turcotte, B. (2002) New regulators of drug sensitivity in the family of yeast zinc cluster proteins. *J. Biol. Chem.* **277,** 21254–21260 CrossRef Medline
29. Mendizabal, I., Rios, G., Mulet, J. M., Serrano, R., and de Larrinoa, I. F. (1998) Yeast putative transcription factors involved in salt tolerance. *FEBS Lett.* **425,** 323–328 CrossRef Medline
30. Zhao, X. J., Raitt, D., Burke, P. V., Clewell A. S, Kwast, K. E., and Poyton, R. O. (1996) Function and expression of flavohemoglobin in *Saccharomyces cerevisiae*: evidence for a role in the oxidative stress response. *J. Biol. Chem.* **271,** 25131–25138 CrossRef Medline

31. Sri Theivakadadcham, V. S., Bergey, B. G., and Rosonina, E. (2019) Sumoylation of DNA-bound transcription factor Sko1 prevents its association with nontarget promoters. *PLoS Genet.* **15,** e1007991 CrossRef Medline
32. Hinnebusch, A. G., and Natarajan, K. (2002) Gcn4p, a master regulator of gene expression, is controlled at multiple levels by diverse signals of starvation and stress. *Eukaryot. Cell* **1,** 22–32 CrossRef Medline
33. Esteras, M., Liu, I. C., Snijders, A. P., Jarmuz, A., and Aragon, L. (2017) Identification of SUMO conjugation sites in the budding yeast proteome. *Microb. Cell* **4,** 331–341 CrossRef Medline
34. Motorin, Y., Muller, S., Behm-Ansmant, I., and Branlant, C. (2007) Identification of modified residues in RNAs by reverse transcription-based methods. *Methods Enzymol.* **425,** 21–53 CrossRef Medline
35. Wilusz, J. E. (2015) Removing roadblocks to deep sequencing of modified RNAs. *Nat. Methods* **12,** 821–822 CrossRef Medline
36. Manza, L. L., Codreanu, S. G., Stamer, S. L., Smith, D. L., Wells, K. S., Roberts, R. L., and Liebler, D. C. (2004) Global shifts in protein sumoylation in response to electrophile and oxidative stress. *Chem. Res. Toxicol.* **17,** 1706–1715 CrossRef Medline
37. Niskanen, E. A., Malinen, M., Sutinen, P., Toropainen, S., Paakinaho, V., Vihervaara, A., Joutsen, J., Kaikkonen, M. U., Sistonen, L., and Palvimo, J. J. (2015) Global SUMOylation on active chromatin is an acute heat stress response restricting transcription. *Genome Biol.* **16,** 153 CrossRef Medline
38. Xiao, Z., Chang, J. G., Hendriks, I. A., Sigurethsson, J. O., Olsen, J. V., and Vertegaal, A. C. (2015) System-wide analysis of SUMOylation dynamics in response to replication stress reveals novel small ubiquitin-like modified target proteins and acceptor lysines relevant for genome stability. *Mol. Cell. Proteomics* **14,** 1419–1434 CrossRef Medline
39. Loewith, R., and Hall, M. N. (2011) Target of rapamycin (TOR) in nutrient signaling and growth control. *Genetics* **189,** 1177–1201 CrossRef Medline
40. Chymkowitch, P., and Enserink, J. M. (2018) Regulation of tRNA synthesis by posttranslational modifications of RNA polymerase III subunits. *Biochim. Biophys. Acta Gene Regul. Mech.* **1861,** 310–319 CrossRef Medline
41. Khoo, S. K., Wu, C. C., Lin, Y. C., and Chen, H. T. (2018) The TFIIE-related Rpc82 subunit of RNA polymerase III interacts with the TFIIB-related transcription factor Brf1 and the polymerase cleft for transcription initiation. *Nucleic Acids Res.* **46,** 1157–1166 CrossRef Medline
42. Wu, C. C., Lin, Y. C., and Chen, H. T. (2011) The TFIIF-like Rpc37/53 dimer lies at the center of a protein network to connect TFIIIC, Bdp1, and the RNA polymerase III active center. *Mol. Cell. Biol.* **31,** 2715–2728 CrossRef Medline
43. Hendriks, I. A., D'Souza, R. C., Chang, J. G., Mann, M., and Vertegaal, A. C. (2015) System-wide identification of wild-type SUMO-2 conjugation sites. *Nat. Commun.* **6,** 7289 CrossRef Medline
44. Hendriks, I. A., Treffers, L. W., Verlaan-de Vries, M., Olsen, J. V., and Vertegaal, A. C. (2015) SUMO-2 orchestrates chromatin modifiers in response to DNA damage. *Cell Reports* **10,** 1778–1791 CrossRef Medline
45. Cossec, J. C., Theurillat, I., Chica, C., Búa Aguín, S., Gaume, X., Andrieux, A., Iturbide, A., Jouvion, G., Li, H., Bossis, G., Seeler, J. S., Torres-Padilla, M. E., and Dejean, A. (2018) SUMO safeguards somatic and pluripotent cell identities by enforcing distinct chromatin states. *Cell Stem Cell* **23,** 742–757.e8 CrossRef Medline
46. Neyret-Kahn, H., Benhamed, M., Ye, T., Le Gras, S., Cossec, J. C., Lapaquette, P., Bischof, O., Ouspenskaia, M., Dasso, M., Seeler, J., Davidson, I., and Dejean, A. (2013) Sumoylation at chromatin governs coordinated repression of a transcriptional program essential for cell growth and proliferation. *Genome Res.* **23,** 1563–1579 CrossRef Medline
47. Cieśla, M., and Boguta, M. (2008) Regulation of RNA polymerase III transcription by Maf1 protein. *Acta Biochim. Pol.* **55,** 215–225 Medline
48. Willis, I. M., and Moir, R. D. (2007) Integration of nutritional and stress signaling pathways by Maf1. *Trends Biochem. Sci.* **32,** 51–53 CrossRef Medline
49. Chen, C., and Kolodner, R. D. (1999) Gross chromosomal rearrangements in *Saccharomyces cerevisiae* replication and recombination defective mutants. *Nat. Genet.* **23,** 81–85 CrossRef Medline
50. Cox, J., and Mann, M. (2008) MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. *Nat. Biotechnol.* **26,** 1367–1372 CrossRef Medline
51. Tyanova, S., Temu, T., Sinitcyn, P., Carlson, A., Hein, M. Y., Geiger, T., Mann, M., and Cox, J. (2016) The Perseus computational platform for comprehensive analysis of (prote)omics data. *Nat. Methods* **13,** 731–740 CrossRef Medline
52. Zhou, Y., Zhou, B., Pache, L., Chang, M., Khodabakhshi, A. H., Tanaseichuk, O., Benner, C., and Chanda, S. K. (2019) Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nat. Commun.* **10,** 1523 CrossRef Medline
53. Kruitwagen, T., Chymkowitch, P., Denoth-Lippuner, A., Enserink, J., and Barral, Y. (2018) Centromeres license the mitotic condensation of yeast chromosome arms. *Cell* **175,** 780–795.e15 CrossRef Medline
54. Perez-Riverol, Y., Csordas, A., Bai, J., Bernal-Llinares, M., Hewapathirana, S., Kundu, D. J., Inuganti, A., Griss, J., Mayer, G., Eisenacher, M., Pérez, E., Uszkoreit, J., Pfeuffer, J., Sachsenberg, T., Yilmaz, S., *et al.* (2019) The PRIDE database and related tools and resources in 2019: improving support for quantification data. *Nucleic Acids Res.* **47,** D442–D450 CrossRef Medline
55. Jensen, L. J., Kuhn, M., Stark, M., Chaffron, S., Creevey, C., Muller, J., Doerks, T., Julien, P., Roth, A., Simonovic, M., Bork, P., and von Mering, C. (2009) STRING 8: a global view on proteins and their functional interactions in 630 organisms. *Nucleic Acids Res.* **37,** D412–D416 CrossRef Medline

Desumoylation of RNA polymerase III lies at the core of the Sumo stress response in yeast

Aurélie Nguéa P, Joseph Robertson, Maria Carmen Herrera, Pierre Chymkowitch and Jorrit M. Enserink

J. Biol. Chem. 2019, 294:18784-18795.
doi: 10.1074/jbc.RA119.009721 originally published online November 1, 2019