Yeast Ataxin-7 links histone deubiquitination with gene gating and mRNA export

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Targeting of a gene to the nuclear pore complexes (NPCs), or gene gating, can affect its transcriptional state1–9. However, the mechanism underlying gene gating is poorly understood. Here, we have identified SAGA-associated Sgf73 (ref. 10), the yeast orthologue of human Ataxin-7 (ref. 11), as a regulator of histone H2B ubiquitin levels, a modification linked to both transcription initiation and elongation12,13. Sgf73 is a key component of a minimal histone-deubiquitinating complex. Activation of the H2B deubiquitinating protease, Ubp8, is cooperative and requires complex formation with the amino-terminal zinc-finger-containing domain of Sgf73 and Sgf11–Sus1. Through a separate domain, Sgf73 mediates recruitment of the TREX-2 mRNA export factors Sac3 and Tep1 to SAGA and their stable interaction with Sus1–Cdc31. This latter step is crucial to target TREX-2 to the NPC. Loss of Sgf73 from SAGA abrogates gene gating of GAL1 and causes a GAL1 mRNA export defect. Thus, Sgf73 provides a molecular scaffold to integrate the regulation of H2B ubiquitin levels, tethering of a gene to the NPC and export of mRNA.

Proximal gene positioning of NPC was proposed to be coupled to transcription initiation and mRNA export on the basis of the observation that SAGA, a multifunctional co-activator of RNA polymerase II [AU: OK?] (PolII), interacts with the NPC-associated TREX-2 mRNA export complex (Sac3–Tep1–Cdc31–Sus1; refs 14–17). Accordingly, GAL1 gene gating depends on Sus1, a subunit of both SAGA and TREX-2, but the precise function of Sus1 in both complexes has remained unclear9. Within SAGA, Sus1 associates with the H2B deubiquitinating enzyme Ubp8 and Sgf11, a protein of unknown function18–21. H2B monoubiquitination at Lys 123 (H2Bub1), catalysed by Rad6/Bre1, is linked to histone H3 methylation13,22–24, and both the addition and subsequent removal of ubiquitin at the promoter and coding region of a gene are required for optimal transcription15,25,26. Apart from mediating the assembly of a pre-initiation complex at the core promoter, a SAGA-related complex was shown to enter the GAL1 coding region, where Ubp8 promotes transcription elongation25,27–29. However, the regulation of Ubp8 activity is ill-defined.

To gain insights into a potential connection between H2B deubiquitination, gene gating and mRNA export, we studied the role of Sus1 in SAGA by searching for regulatory factors in the structural vicinity of the Ubp8–Sgf11–Sus1 module. Notably, Sgf11, a binding partner of Sus1 in SAGA, contains a predicted zinc-finger domain with similarity to a region in the N-terminus of Sgf73 (Supplementary Information, Figs S1a, b). Sgf73 and its human orthologue Ataxin-7 are subunits of the SAGA and TFFC–STAGA co-activators, respectively10,20,31. Besides the N-terminal domain (ZnF1, Supplementary Information, Figs S1a, b), Sgf73 contains a second conserved zinc-finger (ZnF2) with a different spacing, which in Ataxin-7 is required for binding to TFFC–STAGA11.

Affinity-purification of TAP-tagged Sgf73 showed a typical SAGA complex profile (Fig. 1a; preparations also contain SLIK–SALSA variants of SAGA). When Sgf73–TAP purifications were carried out with increasing concentrations of salt, a portion of SAGA subunits, including Tra1, Spt7 and Gcn5, dissociated but Ubp8, Sgf11 and Sus1 were specifically retained (Fig. 1a). This finding suggests that within SAGA, Sgf73 has contact with the Ubp8–Sgf11–Sus1 heterotrimer (subsequently termed Ubp8 module).

Next, we carried out SAGA purifications using Ada2 (a bona fide SAGA subunit). Deletion of Sgf73 caused a specific loss of Ubp8 and Sus1 but did not cause major changes of the SAGA subunit composition, as assessed by Coomassie-staining (Fig. 1b; however, other SAGA-associated proteins, for example substoichiometric or co-migrating bands, could be affected). In contrast, Sgf73 remained associated with SAGA when UBP8 was deleted (Fig. 1b), although this deletion does cause dissociation of Sgf11–Sus1 from SAGA20. These data indicate that Sgf73 anchors the Ubp8 module to SAGA. Consistently, the global level of H2Bub1 at Lys 123 was increased in an sgf73 deletion mutant (Fig. 1c) to a similar magnitude as that of a strain disrupted for UBP8 (Fig. 1c). Thus, Sgf73 functions to target or activate Ubp8 for histone deubiquitination in vivo.

Given that Sgf11 and the Sgf73 ZnF1 domain are conserved in the primary sequence, we tested whether the Ubp8 module binds to the N-terminal region of Sgf73. Truncation of the first 104 residues of Sgf73, which included the N terminus and the adjacent ZnF1 domain,
Figure 1 Sgf73 anchors Ubp8 to SAGA. (a) Sgf73-TAP was affinity-purified and treated with 0.1, 0.5 and 1 M MgCl₂, before TEV-elution and subsequent purification using the CBP-tag. Eluates were analysed by SDS–PAGE and Coomassie staining. Filled circles show the position of Sgf73. The indicated co-purifying proteins were identified by mass spectrometry. Asterisks indicate common contaminants of the TAP method (from top to bottom: Ssa2, Ssb1, GA-3P-DH and Rps0a). Molecular weight protein standards are indicated. (b) Affinity-purification of Ada2–TAP expressing Sus1–13myc from wild-type (WT), ubp8Δ and sgf73Δ strains. Filled circles show the position of Ada2. Eluates were analysed by SDS–PAGE and Coomassie staining (upper panel) and western blotting (lower panel) using anti-Myc antibodies. Assigned subunits were determined by mass spectrometry. Note that Ubp8, Sus1 and Sgf11 are substoichiometric in Ada2 preparations. TAP, eluate after TAP-purification; WCE, whole cell extract. (c) Ubiquitination of H2B (H2B–Ub) is increased in sgf73Δ cells. Anti-Flag immunoprecipitates derived from wild-type cells expressing untagged H2B (lane 1), Flag–H2B–K123R mutated in the ubiquitination site (lane 2), intact Flag–H2B (lane 3) and sgf73Δ cells (lane 5). Recovered proteins were analysed by SDS–PAGE and western blotting using an anti-Flag antibody to detect unmodified Flag–H2B and ubiquitinated Flag–H2B.

caused the loss of Sus1 and Ubp8 from SAGA, when purified using Ada2 (Fig. 2a). On the other hand, purification of the corresponding Sgf73 fragment (Sgf73 1–104–TAP) recovered Ubp8, Sgf11 and Sus1 but no other SAGA subunits (Fig. 2b, lane 3). Removal of the last zinc-coordinating residue (Cys 98) of the ZnF1 domain still supported Ubp8 module binding (Fig. 2b, lane 4), whereas truncation of an additional four amino acids caused a marked decrease in the yield of the purification (Fig. 2b, lane 5; see Supplementary Information, Fig. S1c for expression levels). Thus, a region comprising the first 96 N-terminal residues of Sgf73 is necessary and sufficient for anchoring the Ubp8 module to SAGA.

Previously, Ubp8 was reported to be enzymatically inactive when not associated with SAGA. To assess the contribution of Sgf73 to Ubp8 activity, we used an assay involving Ub [AU: abbreviation used later, OK?]. Ub–AMC (ubiquitin C-terminal 7-amido-4-methylcoumarin), a model substrate for deubiquitinating enzymes, various minimal deubiquitinating complexes consisting of the Sgf73 N terminus and the attached Ubp8 module showed robust Ub–AMC-hydrolysing activity (Fig. 2c). The SAGA-liberated Ubp8-containing complexes were slightly more active enzymatically than the SAGA holoenzyme (Fig. 2c).

We then examined whether these minimal Ubp8-containing complexes would be able to deubiquitinate the natural H2Bub1 substrate. All minimal deubiquitinating complexes could hydrolyse H2Bub1; however, the ZnF1-mutant complex (Sgf73 1–96) cleaved ubiquitin from H2B less efficiently, compared with a minimal complex containing an intact ZnF1 domain (for example, Sgf73 1–104) or the SAGA holoenzyme used as a positive control (Fig. 2d). Notably, the ZnF1 mutant (Sgf73 1–96) complex was not impaired in Ub–AMC hydrolysis (Fig. 2c). These results suggest that the Sgf73 ZnF1 domain has a non-catalytic role and potentially aids in recognizing the structurally more complex H2Bub1 substrate.

We next asked how Ubp8 activation is mechanistically accomplished. Recombinant Sgf73 N-terminal domain (1–96), Sus1, Sgf11 and Ubp8 were used to probe the requirements for Ubp8 activation (see Supplementary Information, Fig. S1d for input). Notably, Ubp8 alone was devoid of Ub–AMC hydrolysing activity (Fig. 2e; Ubp8 alone also failed to hydrolyse H2Bub1, data not shown). We then assayed the Ubp8 interacting factors in pairwise combinations for their ability to stimulate Ubp8 and found that it could be stimulated [AU: OK?] only when Sgf73 1–96, Sus1 and Sgf11 were present in the reaction (Fig. 2e).

To analyse whether these four proteins interact physically with each other [AU: OK?] under the conditions of the assay, we tested for in vitro complex formation. Ubp8 or an Sgf11–Sus1 dimer cannot bind significantly to the Sgf73 N terminus on their own, but a tetrameric complex is reconstituted when all four proteins are present (Fig. 2f). Thus, Ubp8 activation correlates with the assembly of a minimal deubiquitinating complex.
Figure 2 An N-terminal domain of Sgf73 regulates Ubp8 activity. (a) Affinity-purifications of Ada2–TAP from strains with full-length 3HA–Sgf73 or the indicated N-terminal truncation, both co-expressing Myc-tagged Sus1. Eluates were analysed by SDS–PAGE and Coomassie staining (upper panel) and western blotting (lower panel) using anti-Myc antibodies. Filled circles denote Ada2. Ubp8, full-length and truncated Sgf73 were identified by mass spectrometry. (b) Affinity-purification of full-length (WT) and indicated N-terminal Sgf73–TAP constructs. Eluates were analysed by SDS–PAGE and Coomassie staining. Filled circles show the position of Sgf73 constructs. The indicated co-purifying proteins were identified by mass spectrometry. Asterisks indicate contaminants (from top to bottom: Sse1, Ssa2, Ssb1). (c) Ub–AMC hydrolysis assay using the indicated Sgf73–TAP constructs or ubiquitin–AMC alone. Coomassie staining shows equalized amounts of Ubp8 in the various complexes. Numbers correspond to the lanes [AU: OK? If not, please clarify] in b. (d) In vitro deubiquitination assay. Hyperubiquitinated Flag–H2B purified from ubp8Δ cells was incubated with the indicated complexes followed by SDS–PAGE and western blotting using an anti-Flag antibody. As a control, the substrate was incubated with buffer alone. The assay was performed in parallel with c. The asterisk denotes cross-reaction of the antibody. (e) Ub–AMC hydrolysis assay performed with equimolar amounts of recombinant subunits. Ubp8 alone had near-baseline levels of hydrolytic activity. Ub–AMC hydrolytic activity was blocked when the protein mixture was reacted with a twofold excess of Ub–VS (ubiquitin vinyl sulfone, an irreversible DUB inhibitor) before the assay. (f) Reconstitution assay with equimolar amounts of recombinant subunits. Indicated subunits were preincubated together, followed by GST affinity-purification. Eluates were analysed by SDS–PAGE (12% gel, MES buffer) and Coomassie staining.
Figure 3 Sgf73 affects the integrity of the TREX-2 mRNA export complex. (a) Affinity-purification of Sus1–TAP from wild-type (WT), ubp8Δ and sgf73Δ strains. Eluates were analysed by SDS–PAGE and Coomassie staining and the indicated bands were identified by mass spectrometry. Asterisks indicate contaminants of the TAP method (from top to bottom: Crn1, Ssa2, Ssb1 and Act1). (b) Schematic drawing of the assembly status of SAGA and TREX-2 in wild-type and mutant conditions. Sus1 is depicted in red to indicate the complexes purified by Sus1–TAP from wild-type and mutant strains, as shown in a. Sac3 is marked in the sgf73Δ mutant to indicate its instability during purification. (c, d) Affinity-purification of Sus1–TAP in strains expressing full-length Sgf73 or indicated C-terminal Myc-tagged (c) and N-terminal HA-tagged (d) truncation constructs. Eluates were analysed by SDS–PAGE and Coomassie staining. The labelled co-purifying proteins were identified by mass spectrometry. Filled circles show the position of Sgf73 constructs; open circles indicate degradation products of Sgf73. Arrows mark Thp1. Asterisks indicate contaminants of the TAP method (from top to bottom: Enolase, Act1, Rpi and TEV protease; note that Enolase, lane 4, runs slightly below Thp1, lane 3). (e) Schematic drawing of functional domains within Sgf73 (not drawn to scale). (f) Native gel electrophoresis of whole-cell lysates derived from the indicated Sus1–TAP (left panel) or Thp1–TAP strains (right panel) followed by western blotting with antibodies against the ProtA-epitope (a part of the TAP-tag). Lower panel shows loading controls analysed under denaturing conditions (SDS–PAGE). The open circle indicates the position of the Sgf73ΔC (287–657)–Ubp8–Sgf11–Sus1 subcomplex, the closed diamond the position of a TREX-2 intermediate. (g) Ada2–TAP was affinity-purified from wild-type (WT), ubp8Δ and sgf73 truncated (sgf73ΔC287–657) strains, which co-expressed both Sac3–Myc and Thp1–Myc. Co-purification of Sac3 and Thp1 was detected by western blotting using an anti-Myc antibody.
As Sgf73 mediates Sus1 binding to SAGA, and Sus1 is also a subunit of TREX-2, we asked whether Sgf73 would influence TREX-2 function. Within TREX-2, both Sus1 and Cdc31 bind to a carboxy-terminal motif of Sac3, termed CID (Cdc31-interacting domain), which is required for correct NPC-targeting of the complex. Sus1–TAP affinity-purifications were used to simultaneously monitor its ability to associate with SAGA and TREX-2. Previously, we had shown that deletion of UBP8 or SGF11 from cells has no influence on the incorporation of Sus1 into the TREX-2 complex, whereas the interaction with SAGA is abolished (Fig. 3a, lane 2). When Sus1–TAP was purified from an sgf73Δ strain, no SAGA subunits (for example, Tra1, Spt7, Ubp8), except Sgf11, were recovered (Fig. 3a, lane 3). Remarkably, the TREX-2 core components Sac3 and Thp1 were lost, whereas Cdc31 was still retained (Fig. 3a, lane 3).

To determine the assembly status of Thp1 and Sac3 in strains where SGF73 had been disrupted, we subjected the Sus1–TAP-depleted lysate to Thp1–FLAG immunoprecipitation. Thp1 and degradation products of associated Sac3 could be recovered, but Cdc31 and Sus1 were missing, supporting the notion that the absence of Sgf73 causes fragmentation of TREX-2 and an instability of Sac3 during biochemical isolation (Supplementary Information, Fig. S2a). These findings suggest that Sgf73 is required for stable interaction between Sus1–Cdc31 and an intact Sac3–Thp1 heterodimer (Fig. 3b). Importantly, the disintegration of TREX-2, which is observed when SGF73 is deleted, cannot be an indirect result of uncoupling Sus1 from SAGA because deletion of UBP8 did not affect the TREX-2 assembly state.

To determine which functional element of Sgf73 maintains an intact TREX-2 complex, Sgf73 was progressively truncated from the C or N terminus. When Sus1–TAP was purified from a strain lacking the 255 C-terminal residues (Sgf73ΔC 403–657), Sus1 was still efficiently associated with TREX-2 and SAGA (Fig. 3c, lane 3). Deletion of an additional 20 residues from Sgf73 (Sgf73ΔC 383–657) revealed a concomitant loss of Sac3–Thp1 and SAGA from Sus1–TAP, indicative of a close overlap

**Figure 4** Sgf73 and Sus1 affect targeting of Sac3 and Thp1 to the NPC. (a, b) Subcellular localization of Sac3–GFP and Thp1–GFP in the indicated wild-type, ubp8Δ, sgf73Δ and sus1Δ strains are shown. Fluorescence microscopic and Nomarski photographs of representative cells are shown (scale bar, 2 µm). (c) Phenotypic analysis of sgf73Δ cells in liquid medium containing either 2% glucose or 2% galactose and the semi-log graph is depicted (d) Growth of wild-type and the indicated mutant strains on rich medium prepared with glucose (YPD) or galactose (YPG). Cell density was normalized, tenfold serial dilutions were prepared and then plated. Plates were incubated for 2 days at 30 °C.
between the regions responsible for TREX-2 integrity and SAGA anchoring. Sus1–TAP still retained Cdc31 (Fig. 3c, lanes 4–6) and remained bound to Sgf73∆C 383–657 together with Sgf11 and Ubp8.

On the other hand, removal of the first 104 residues from Sgf73 abolished the interaction between Ubp8 and Sus1–TAP, but binding to the TREX-2 complex was still observed (Fig. 3d, lane 2; see also ref. 15). However, Sus1–TAP was uncoupled from these large complexes in the sgf73∆C 287–657 mutant and shifted into a faster-migrating species that may correspond to the Sgf73∆C (287–657)–Ubp8–Sgf11–Sus1 subcomplex (Fig. 3f; see also Fig. 3c, lane 6). Small Sus1-containing species (for example, Sus1–Cdc31) and free Sus1 were not detected in this gel system.

To monitor the influence of Sgf73 on TREX-2 integrity with respect to Thp1, the migration of Thp1–TAP was followed by native gel electrophoresis. In wild-type cells, Thp1–TAP was mainly present in a large assembly that corresponds to TREX-2 (Fig. 3f). In comparison, Thp1–TAP was shifted into a faster-migrating band in sgf73∆ cells, consistent with the observed disintegration of TREX-2 in this SAGA mutant (Fig. 3a, f; Supplementary Information, Fig. S2a). Thus, native gel analysis revealed an Sgf73-dependent uncoupling of Sus1 not only from SAGA but also TREX-2, which correlates with a disruption of TREX-2 integrity.

To determine whether Sgf73 exerts its influence on TREX-2 by transiently recruiting TREX-2 subunits to SAGA, Sac3 and Thp1 were tested...
for SAGA association. When SAGA was purified from wild-type cells using Ad2–TAP, co-enrichment of Sac3–Myc and Thp1–Myc was observed (Fig. 3g; Supplementary Information, Fig. S2d). However, Sac3–Myc and Thp1–Myc were no longer associated with SAGA when Ad2–TAP was purified from a strain expressing the C-terminal-truncated Sgf73 (Sgf73AC 287–657) that dissociates from SAGA and impairs TREX-2 integrity. In contrast, recruitment of Sac3–Myc and Thp1–Myc to SAGA was not affected in a strain in which Ub8p was deleted (Fig. 3g). Thus, Sgf73 is required to recruit Sac3 and Thp1 to SAGA either directly or in conjunction with other SAGA subunits.

We then asked whether Sgf73 affects the subcellular TREX-2 localization. In contrast to wild-type yeast where the TREX-2 core subunits Sac3–GFP and Thp1–GFP localize predominantly to the nuclear periphery, Sac3–GFP and Thp1–GFP partly mislocalized to the cytoplasm in the sgf73Δ strain (Fig. 4a, b). However, deletion of Ub8p did not induce a cytoplasmic mislocalization of Sac3–GFP and Thp1–GFP (Fig. 4a, b). As Sgf73 promotes the association of Sus1–Cdc31 with Sac3, we tested whether Sus1 has a role in targeting Sac3 to the NPC. Indeed, Sac3–GFP and Thp1–GFP became mislocalized in sus1Δ cells (Fig. 4a, b). We attribute this defect to a TREX-2-related function of Sus1, as uncoupling of Sus1 from SAGA in ubp8Δ cells did not elicit such an effect. These results indicate that Sgf73 and Sus1 are required for the efficient targeting of Sac3 and Thp1 to the NPC.

To estimate the physiological importance of Sgf73-dependent functions, we examined the effects of sgf73Δ deletion on cell growth. sgf73Δ cells have a slight growth retardation in glucose-containing (YPD) medium, but a pronounced growth defect in galactose-containing (YPG) medium (Gal-phenotype; Fig. 4c). We then tested whether the components required for histone deubiquitination and mRNA export interact genetically. As with sgf73Δ cells, growth of sac3Δ and thp1Δ strains was slower in galactose-containing medium (Fig. 4d). However, the combination of either sac3Δ or thp1Δ with the sgf73Δ deletion synergistically enhanced growth inhibition, independently of the carbon source (Fig. 4d). Moreover, the sgf73Δ;sus1Δ double mutant showed a synthetic growth defect, which was particularly pronounced on galactose-containing plates (Fig. 4d). In contrast, sgf73Δ is epistatic to ubp8Δ on both carbon sources (Fig. 4d), consistent with the strict requirement of Sgf73 for Ub8p function. This suggests that the function of Sgf73 is dependent on TREX-2 factors.

The TREX-2 subunits Sac3 and Sus1 are both required for galactose-dependent peripheral repositioning of the GAL1 gene locus3. Thus, we hypothesized that Sgf73 could be important for GAL1 gene gating. As shown in Fig. 5a, expression of sgf73AC 287–657 (a mutant of SGF73) inhibited GAL1 movement to the nuclear periphery [AU: rewording OK? If not, please clarify]. Moreover, we noticed an activation-dependent repulsion of the GAL1 locus from the periphery but the significance of this finding is not understood to date. Notably, the SGF73 mutant is defective both in TREX-2 integrity and H2B deubiquitination (Fig. 3c, lane 6). Global H2Bub1 levels are increased in this mutant, although to a lesser extent than in ubp8Δ cells. This indicates that the SAGA-liberated deubiquitinating complex (Sgf73AC287–657–Ubp8–Sgf11–Sus1) is enzymatically active without being targeted correctly to chromatin in vivo (Supplementary Information, Fig. S3a). Impaired GAL1 repositioning was also observed for deletion of UBP8, a mutation that is known to increase H2Bub1 levels at the GAL1 promoter and ORF25 (Fig. 5a). The GAL1 gating defect is related to Ubp8 enzymatic activity, as it is also observed in a catalytically inactive ubp8C146S mutant (Supplementary Information, Fig. S3b). In contrast, SAGA histone acetyltransferase activity (mediated by Gcn5) is dispensable for GAL1 repositioning1, suggesting a differential in vivo requirement for histone deubiquitination and acetylation in the gene gating pathway.

As Sgf73 is required for TREX-2 integrity, we tested whether it is necessary for GAL1 mRNA export. GAL1 mRNA forms a single nuclear dot in close proximity to the GAL1 chromosomal locus and it was proposed that GAL1 mRNA is channelled to the NPC via the mRNA dot. We conducted FISH experiments with Cy3-labelled GAL1-specific RNA probes and quantified fluorescence intensity in the nuclear mRNA dot. Analysis of the gene-gating-deficient Sgf73 [AU: Sgf73, as above!] mutant (sgf73AC 287–657) revealed a significant reduction in cytoplasmic GAL1 mRNA signal and a concomitant increase in nuclear dot fluorescence intensity, compared with wild-type cells (Fig. 5b; Supplementary Information, Fig. S3c). Nuclear dot mRNA accumulation was also observed in ubp8Δ cells and cells harbouring the ubp8C146S active-site mutant, although to a lesser extent (Fig. 5b; Supplementary Information, Fig. S3c). Notably, total GAL1 mRNA levels were not significantly altered when measured at the same time point of galactose induction (Supplementary Information, Fig. S3d). Thus, we conclude that the SAGA subunits Sgf73 and Ub8p are required for efficient GAL1 mRNA export.

In summary, Sgf73 is crucial for the regulation of H2B deubiquitination and through its bifunctional protein design, creates a link to the export of specific mRNA transcripts. Ub8p activity, stringently controlled by Sgf73, is required for gene gating and H2B is a likely substrate candidate. On the other hand, Sgf73 recruits the TREX-2 mRNA export complex to SAGA and could contribute to gene gating by triggering the NPC-repositioning of a gene or by subsequently tethering it to the nuclear periphery. We speculate that Sgf73 alters the CID domain of Sac3 to allow for a stable interaction between Sus1–Cdc31 and Sac3–Thp1, a prerequisite for efficient NPC-targeting of TREX-2. Seen in a wider context, Sgf73 may integrate two functions that are both necessary for efficient gene expression: the acquisition of a Sac3 NPC-targeting signal and the establishment of a correct H2Bub1 chromatin code. This mechanism may involve communication between the central ZnF2 domain (responsible for SAGA binding and TREX-2 integrity) and the Sgf73 N-terminus, and could either result in allosteric regulation or de novo recruitment of the Ubp8 module. Our findings may be relevant for understanding the pathogenesis of spinocerebellar ataxia type 7, a neurodegenerative disease caused by polyglutamine-expanded versions of Ataxin-7, the human orthologue of Sgf73 (ref. 33).

**METHODS**

**Yeast strains and plasmid constructions.** Genomic integrations of N- and C-terminal tags (TAP, HA, Myc and FLAG) and gene deletions were performed using standard methods [AU: please provide a brief description]. The ubp8C146S mutant was created by site-directed mutagenesis and confirmed by sequencing. All strains and plasmids used in this study are listed in Supplementary Table S1.

**Affinity purifications.** TAP-tagged proteins were affinity-purified according to published methods31. Proteins were detected by SDS–PAGE and colloidal Coomassie staining. Stepwise salt disruption of Sgf73–TAP purified SAGA complex was performed essentially as described previously32. Standard methods were used to purify 6His-tagged or GST-tagged proteins from *Escherichia coli*.

H2B ubiquitination/deubiquitination assays and complex reconstitution. Global H2B ubiquitination was examined as described previously30. Ub–AMC
hydrolysis assays were conducted essentially as described previously\(^\text{22}\). For reconstitution of Ubp8 activity, recombinant proteins were preincubated at 16°C for 30 min before performing the Ub–AMC assay. Measurements were made using a Gemini XPS spectrofluorometer. To purify the reconstituted deubiquitinating complex, recombinant proteins were mixed, GST-immunoprecipitated and, after washing and elution, detected by SDS–PAGE and Coomassie staining. In *in vitro* H2B deubiquitination assays, a hyperubiquitinated Flag-tagged H2B substrate was purified from a *ubp8Δ* strain. H2Bub1 bound to Flag M2 agarose beads was incubated with different TAP-purified complexes at 30°C for 45 min in DUB buffer (50 mM Tris pH 7.5, 50 mM NaCl, 1 mM dithiothreitol). For detection of the Flag epitope, peroxidase-coupled anti-Flag M2 antibody ([AU: please provide dilution] Sigma) was used.

**Native gel analysis and immunoblotting** Whole-cell extracts were treated with an endonuclease ([AU: please provide concentration] Benzonase; Sigma) for 30 min at 16°C before loading onto a nativePAGE 4–16% Bis-Tris gel (Invitrogen). Electrophoresis was performed for 1 hr at 150 V, followed by 80 min at 250 V. The gel was soaked for 5 min in SDS–PAGE running buffer (25 mM Tris base, 2.8 mM SDS, 192 mM glycine), followed by semi-dry blotting onto a PVDF membrane (Millipore) and immunodetection with an anti-ProteinA antibody.

**Fluorescence microscopy** Fluorescence microscopy was performed using an Axioimager Z1 (Carl Zeiss) with a ×100 NA 1.4 Plan-Apo-Chromat oil immersion lens (Carl Zeiss) and DICIII, HEEGF or HECy3 filter set, respectively. Pictures were acquired with an AxioCamMRm camera (Carl Zeiss) and software AxioVision 4.3 (Carl Zeiss) at resolution 1388 × 1040 (Binning 1 × 1, gain factor 1).

**GAL1 mRNA FISH** was performed using six Cy3 internally labelled 50-mer oligonucleotide probes. Cells were grown to mid-log phase in raffinose-containing (Millipore) and immunodetection with an anti-ProteinA antibody ([AU: please provide dilution and source].

**COMPETING FINANCIAL INTERESTS** The authors declare no competing financial interests.

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