Histone H2B Ubiquitination Promotes the Function of the Anaphase-Promoting Complex/Cyclosome in Schizosaccharomyces pombe

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ABSTRACT

Ubiquitination and deubiquitination of proteins are reciprocal events involved in many cellular processes, including the cell cycle. During mitosis, the metaphase to anaphase transition is regulated by the ubiquitin ligase activity of the anaphase-promoting complex/cyclosome (APC/C). Although the E3 ubiquitin ligase function of the APC/C has been well characterized, it is not clear whether deubiquitinating enzymes (DUBs) play a role in reversing APC/C substrate ubiquitination. Here we performed a genetic screen to determine what DUB, if any, antagonizes the function of the APC/C in the fission yeast Schizosaccharomyces pombe. We found that deletion of ubp8, encoding the Spt-Ada-Gcn5-Acetyl transferase (SAGA) complex associated DUB, suppressed temperature-sensitive phenotypes of APC/C mutants cut9-665, lid1-6, cut4-533, and slp1-362. Our analysis revealed that Ubp8 antagonizes APC/C function in a mechanism independent of the spindle assembly checkpoint and proteasome activity. Notably, suppression of APC/C mutants was linked to loss of Ubp8 catalytic activity and required histone H2B ubiquitination. On the basis of these data, we conclude that Ubp8 antagonizes APC/C function indirectly by modulating H2B ubiquitination status.

KEYWORDS
anaphase promoting complex/cyclosome (APC/C) deubiquitinating enzymes (DUBs) SAGA complex histone H2B ubiquitination

The purpose of cell division is to accurately replicate the genetic material of a dividing cell and evenly distribute it between mother and daughter cells. Precise degradation of critical cell-cycle regulators by the ubiquitin proteasome system (UPS) ensures unidirectionality of cell-cycle progression (reviewed in Mocciaro and Rape 2012; Wickliffe et al. 2009).

Targeting of cellular proteins for degradation by the UPS involves the covalent attachment of ubiquitin to substrate proteins, creating a degradation signal that targets substrates to the 26S proteasome. Ubiquitin is attached to substrates through a tightly coordinated enzyme cascade including E1-activating, E2-conjugating, and E3-ligating enzymes (reviewed in Komander and Rape 2012; Teixeira and Reed 2013; Tomko and Hochstrasser 2013).

In Schizosaccharomyces pombe and other eukaryotic organisms, the metaphase to anaphase transition is controlled by the anaphase-promoting complex/cyclosome (APC/C) E3 ubiquitin ligase. The APC/C carries out its mitotic function by promoting the degradation of securin and cyclin B through the UPS (reviewed in McLean et al. 2011; Primorac and Musacchio 2013; Teixeira and Reed 2013). To prevent precocious sister chromatid separation, the APC/C is inhibited by the spindle assembly checkpoint (SAC) (reviewed in Jia et al. 2013; Musacchio 2011), which is only silenced when all kinetochores achieve bipolar attachment to spindle poles. SAC inactivation promotes APC/C activation, leading to chromosome segregation and mitotic exit (reviewed in Jia et al. 2013; McLean et al. 2011; Musacchio 2011).

Ubiquitin is removed from proteins by deubiquitinating enzymes (DUBs) (reviewed in Komander et al. 2009; Reyes-Turcu et al. 2009). DUBs are cysteine or metalloproteases that are classified based on their catalytic domain structure. The 5 DUB families include ubiquitin C-terminal hydrolases, ubiquitin-specific proteases (USPs), Machado-Joseph disease proteases, JAB1/MPN/Mov34 metalloenzymes, and ovarian tumor proteases (OTU) (Nijman et al. 2005). DUBs have diverse roles in regulating the ubiquitin cycle. They are responsible for processing ubiquitin precursors into their conjugation competent form, cleaving ubiquitin from target proteins, trimming of ubiquitin chains, and replenishing the free ubiquitin pool (Komander et al. 2009; Nijman et al. 2005; Reyes-Turcu et al. 2009). In mammalian cells, the DUB USP44 reverses APC/C mediated ubiquitination of the...
APC/C activator Cdc20 to maintain the SAC (Stegmeier et al. 2007). Although it seems plausible that reversal of APC/C ubiquitination is a conserved mechanism, there is no known USP44 homolog in non-mammals.

In this work, we used a genetic approach to determine whether a conserved DUB exists that antagonizes the function of the APC/C in S. pombe, a model organism with many mechanisms of conserved cell cycle control. In contrast to mammals, which encode approximately 80 DUB genes (reviewed in Komander et al. 2009; Reyes-Turcu et al. 2009), the S. pombe genome encodes only 20 catalytically active DUBs belonging to four of the five DUB superfamilies (ubiquitin C-terminal hydrolase, USP, OTU, and JAB1/MPN/Mov34 metalloenzymes) (Kouranti et al. 2010). All S. pombe DUBs except for the proteasomal DUB Rpn11 are nonessential for viability (Iwaki et al. 2007; Kim et al. 2010; Shimannuki et al. 1995; Stone et al. 2004; Zhou et al. 2003) making our genetic screen straightforward. Here, we provide evidence that a single DUB, Ubp8, antagonizes the APC/C in a mechanism independent of the SAC. Genetic analysis revealed that Ubp8’s ability to antagonize APC/C function depends on its catalytic activity and the ubiquitination status of histone H2B. Accordingly, our work reveals a new interaction between chromatin signatures and cell cycle progression, mediated by a DUB.

**MATERIALS AND METHODS**

**Yeast strains, media, and genetic methods**

S. pombe strains used in this study (Supporting Information, Table S1) were grown in yeast extract (YE) medium (Moreno et al. 1991). Crosses were performed in glutamate medium and strains were constructed by tetrad analysis. YE G418 (100 mg/L; Sigma-Aldrich, St. Louis, MO) was used for selecting KanR cells. For serial dilution spot tests, cells were cultured in liquid YE at 25°C, three serial 10-fold dilutions starting at 4 × 10^6 cells/mL were made, 4 μL of each dilution was spotted on YE plates and cells were grown at the indicated temperatures for 3–4 d. Overexpression of pREP1-His-biotin-his (HBH)-tagged ubiquitin was achieved by growth in the absence of thiamine for 18–22 hr, whereas repression was achieved by growth in the presence of 5 μM of thiamine. htb1-Flag and htb1-K119-Flag strains were a gift from Dr. Jason Tanny (McGill University).

**Molecular biology methods**

*ubp8* was tagged after the stop codon of its endogenous open reading frame (ORF) with sequences encoding the Kanamycin resistance gene fragment (digested from pIRT2-Flank vector (Life Technologies) and then subcloned into a pRT2 vector (Hindley et al. 1987). *ubp8-C154S H387A* was created by mutating a pRT2-plasmid containing *ubp8*+ using a QuikChange site-directed mutagenesis kit (Agilent Technologies). For *ubp8* gene replacements, a haploid *ubp8::ura4* strain was transformed with a linear *ubp8* gene fragment (digested from pRT2- *ubp8-C154S H387A* plasmid) using standard lithium acetate transformations. Integrants were selected based on resistance to 5-FOA and validated by colony PCR using primers homologous to endogenous sequences that flank the genomic clone within pRT2 in combination with those within the ORF. All constructs were sequenced to ensure their accuracy.

**S. pombe protein methods**

Cell pellets were frozen in a dry ice/ethanol bath and lysed by bead disruption in NP-40 lysis buffer under denaturing sodium dodecyl sulfate (SDS) lysis conditions as previously described (Gould et al. 1991), except with the addition of a complete protease inhibitor mixture (Calbiochem). Cell pellets for Htb1-FLAG immunoblots were lysed by bead disruption using a FastPrep cell homogenizer (MP Biomedicals). Proteins were immunoprecipitated with IgG sepharose beads (GE Healthcare) as described previously (Kouranti et al. 2010). Proteins were separated on a 4–12% Bis-Tris gel (Life Technologies), transferred to Immobilon-P PVDF (Millipore) membrane, and immunoblotted with anti-FLAG (Sigma-Aldrich), anti-GFP (Roche), IgG primary and fluorescent mouse, and rabbit secondary antibodies (LI-COR Biosciences) according to the manufacturer’s instructions. H2B ubiquitination was quantified relative to total H2B protein using Odyssey software (LI-COR Biosciences).

**In vivo ubiquitinome purifications**

HBH-tagged ubiquitin was overexpressed in *wildtype* and *ubp8Δ* strains utilizing the thiamine repressible *mmt1* promoter in pREP1 (Kouranti et al. 2010; Maundrell 1993). Ubiquitinated proteins were purified using two-step affinity purifications performed under denaturing conditions as described (Tagwerker et al. 2006). In summary, cell pellets were lysed by bead disruption in buffer 1 (8 M urea, 300 mM NaCl, 50 mM NaPO4, 0.5% NP40, and 4 mM Imidazole, pH 8) and incubated with Ni2+-NTA agarose beads (QIAGEN) for 3–4 hr at room temperature. After incubation, beads were washed 4 times with buffer 3 (8 M urea, 300 mM NaCl, 50 mM NaPO4, 0.5% NP40, and 20 mM Imidazole, pH 6.3) and eluted in buffer 4 (8 M urea, 200 mM NaCl, 50 mM NaPO4, 0.5% NP40 and 2% SDS, 100 mM Tris and 10 mM EDTA, pH 4.3). The pH of the eluate was adjusted to 8 and streptavidin ultra-link resin (Pierce) was added and incubated overnight at room temperature. After the overnight incubation, streptavidin beads were washed 4 times with buffer 6 (8 M urea, 200 mM NaCl, 2% SDS and 100 mM Tris, pH 8) and once with buffer 7 (8 M urea, 200 mM NaCl and 100 mM Tris, pH 8). Purifications were performed in duplicate and purified proteins were subjected to mass spectrometric (MS) analysis.

**Mass spectrometry methods**

Purified ubiquitin-HBH on streptavidin beads was washed three times with Tris-urea buffer (100 mM Tris, pH 8.5, 8M urea). Proteins were reduced with 3mM TCEP (Tris(2-carboxyethyl)phosphine hydrochloride), alkylated with 10mM iodoacetamide, and digested with trypsin (0.4 μg of Trypsin Gold, Promega). Two-dimensional liquid chromatography–mass spectrometry 2D-LC-MS/MS analysis was performed in the following manner. Peptides were loaded onto 26-cm columns with a bomb pressure cell and then separated and analyzed by three-phase multidimensional protein identification technology on a Velos LTQ mass spectrometer (Thermo Scientific, West Palm Beach, FL) coupled to a nanoHPLC (NANOAcquity; Waters Corporation). The NANOAcquity autosampler was used for the 12 salt elution steps, each with 2 μL of ammonium acetate. Each injection was followed by elution of peptides with a 0–40% acetonitrile gradient (60 min) except the first and last injections, in which a 0–90% acetonitrile gradient was used. One full precursor MS scan (400–2000 mass-to-charge ratio) and five tandem MS scans of the most abundant ions detected in the precursor MS scan under dynamic exclusion was performed. Ions with a neutral loss of 98 Da (singly charged), 49 Da (doubly charged), or 32.7 Da (triply charged) from the parent ions during MS2 were
subjected to MS$^3$ fragmentation. MS data analysis was done as previously described (Chen et al. 2013) with the following changes. A newer version of Scaffold (Scaffold v4.2.0) was used and the filtering criteria were changed to: minimum of 90.0% peptide identification probability, minimum of 99.0% protein identification probability, and minimum of 2 unique peptides.

RESULTS

Ubp8 antagonizes the function of the APC/C in S. pombe

We surmised that if a DUB antagonizes APC/C function in S. pombe, the deletion of that DUB should suppress a mutant defective in APC/C activity. Thus, we crossed each of the 19 nonessential DUB deletion mutants and a temperature-sensitive mutant of rpm11 (the essential DUB) to cut9-665, an APC/C mutant (Kouranti et al. 2010; Penney et al. 1998; Samejima and Yanagida 1994). Only three mutations affected growth of cut9-665 (Figure 1, A and B). In a serial dilution growth test, only upb8Δ suppressed cut9-665 at its semipermissive temperature (32°C) (Figure 1A). cut9-665 showed a negative genetic interaction with upb14Δ and pad1-1 (a temperature-sensitive allele of the proteasomal DUB rpm11) (Figure 1, A and B). These DUB mutants decrease the cellular ubiquitin pool most likely contributing to the exacerbated APC/C temperature-sensitive phenotype (Penney et al. 1998; Verma et al. 2002) (see discussion).

lid1+ and cut4+ encode other components of the APC/C whereas slp1+ encodes the APC/C coactivator termed Cdc20p in other organisms (Yu 2007). Temperature-sensitive lid1-6, cut4-533, cut9-665, and slp1-362 mutants all display a “cut” phenotype at restrictive temperatures where chromosome segregation and spindle elongation fail to occur; therefore, subsequent cytokinesis bisects the nucleus or results in segregation of DNA to only one daughter cell (Berry et al. 1999; Matsumoto 1997; Yamashita et al. 1996). To determine whether suppression of cut9-665 by upb8Δ was indicative of general suppression of hypomorphic APC/C function, we tested other APC/C mutants and found that upb8Δ suppressed lid1-6, cut4-533, and slp1-362 (Figure 1C, data not shown). On the basis of these data, we conclude that Ubp8 antagonizes APC/C function.

Suppression of APC/C temperature-sensitive mutants is not dependent on the SAC or enhanced proteasome function

The SAC is a well-characterized APC/C inhibitor (Jia et al. 2013; Musacchio 2011). Therefore, we investigated whether suppression of cut9-665 by upb8Δ was achieved by diminishing SAC activity. Deletion of key SAC components mph1Δ, mad2Δ, and mad3Δ (He et al. 2013) suppressed the temperature-sensitive growth of cut9-665. Strains were grown at 25°C to an OD$_{595}$ = 0.2. Serial dilutions (10-fold) of the indicated single and double mutant strains were spotted on yeast extract plates and incubated at the indicated temperatures. (B) List of the S. pombe deubiquitinating enzymes that showed genetic interactions with cut9-665 and their homologs. (C) upb8Δ suppresses the temperature-sensitive phenotype of APC/C mutants cut4-533 and lid1-6. Strains were grown and spotted as described in (A).
ubp8Δ suppressed APC/C mutations as expected but also enhanced the suppression of the APC/C by ubp8Δ additively (Figure 2). Interestingly, the ability of SAC components to suppress APC/C mutants was rather allele-specific (Figure 2). In any case, these data indicate that Ubp8 antagonizes the APC/C through a SAC-independent mechanism(s).

The APC/C is responsible for ubiquitinating critical cell-cycle regulatory proteins, targeting them for their subsequent degradation by the 26S proteasome (reviewed in McLean et al. 2011; Primorac and Musacchio 2013; Teixeira and Reed 2013). Thus, it was possible that ubp8Δ lowered the threshold for APC/C function by enhancing proteasome-mediated degradation. To test this idea, we combined ubp8Δ with a mutation in the proteasome subunit Mts3, mts3-1. This mutant is defective in proteasome-mediated proteolysis and in the metaphase to anaphase transition (Gordon et al. 1993; Gordon et al. 1996; Seeger et al. 1996). ubp8Δ did not suppress the temperature-sensitive growth of mts3-1 (Figure S1), indicating that suppression of APC/C mutants is likely not mediated by enhanced proteasome function.

Figure 2 ubp8Δ suppression of cut9-665 is independent of the spindle assembly checkpoint. ubp8Δ suppresses the temperature-sensitive phenotype of cut9-665 in both mph1+ and mph1Δ strains. ubp8Δ suppression of cut9-665 is enhanced in mad2Δ and mad3Δ strains. Serial dilutions (10-fold) of the indicated single, double and triple mutant strains were spotted on yeast extract plates and incubated at the indicated temperatures.

Figure 3 Suppression of (APC/C) temperature-sensitive mutants is dependent on the catalytic activity of Ubp8. (A) Anti-FLAG western blots of whole-cell extracts prepared from untagged and the indicated FLAG-tagged strains. H2Bub corresponds to the slower migrating ubiquitinated form of H2B. Graph indicates fold change of ubiquitinated histone H2B in the indicated strain backgrounds. (B) ubp8-C154S H387A suppressed the temperature sensitive phenotype of lid1-6, and cut9-665 mutants. Serial dilutions (10-fold) of the indicated single and double mutant strains were spotted on yeast extract plates and incubated at the indicated temperatures.
Suppression of APC/C temperature-sensitive mutants is dependent on the activity of the SAGA DUB module

We next tested whether suppression of APC/C temperature-sensitive mutants was due to loss of Ubp8 catalytic activity or loss of the entire protein. Ubp8 is a papain-like cysteine protease that uses an Asn-His-Cys triad for catalytic function. We altered the sequence at the endogenous ubp8 locus to produce solely Ubp8-C154S H387A, which based on sequence homology is predicted to be a catalytically inactive mutant (Ingvarsdottir et al. 2005). To ensure that Ubp8-C154S H387A lacked activity, we assayed the levels of ubiquitinated histone H2B, a known substrate of Ubp8 in multiple organisms (Daniel et al. 2004; Henry et al. 2003). We observed increased levels of ubiquitinated histone H2B in both ubp8Δ and ubp8-C154S H387A cells (Figure 3A). Like ubp8Δ, ubp8-C154S H387A suppressed the temperature sensitive phenotype of multiple APC/C mutants although it suppressed lid1-6 the best (Figure 3B), indicating that antagonization of the APC/C by Ubp8 depends on its catalytic function.

Ubp8 is part of the evolutionarily conserved DUB module of the SAGA transcriptional complex. In S. pombe, the SAGA DUB module consists of Sgf11, Sgf73, and Sus1, in addition to Ubp8 (Helmlinger et al. 2008). In Saccharomyces cerevisiae, all four analogous DUB module components are required for ubiquitin protease activity in vitro and in vivo (Lee et al. 2009; Weake et al. 2008). As expected based on these data, deletion of each S. pombe SAGA DUB module component (ubp8Δ, sgf11Δ, sgf73Δ, or sus1Δ) led to increased levels of ubiquitinated H2B in vivo (Figure 4A). Furthermore, each deletion suppressed the temperature-sensitive phenotype of APC/C mutants (Figure 4B). These results indicate that suppression of APC/C mutants is achieved by loss of the DUB activity associated with the SAGA complex.

Suppression of APC/C temperature-sensitive mutants is specific to the SAGA DUB module

The SAGA complex is organized into distinct subcomplexes, each with discrete regulatory activities (reviewed in Koutelou et al. 2010; Samara and Wolberger 2011). In S. pombe, these modules include the DUB, histone acetyltransferase, TATA-binding protein, structural, Tra1, and Sgf29 (reviewed in Helmlinger 2012) (Figure 5A). The main function of the DUB module is to deubiquitinate histone H2B to regulate gene expression. The histone acetyltransferase module is responsible for acetylation of histone H2B and H3, whereas the TATA-binding protein module regulates preinitiation complex assembly and transcriptional activation. Tra1 has an important role in the recruitment of transcriptional coactivator complexes to specific promoters, while Sgf29 plays a role in the binding of methylated histones (Helmlinger 2012). Components of the structural module TBP-associated factors (TAFs) are responsible for maintaining SAGA architectural integrity and are involved in regulating gene expression through their association with the TFIID general transcription factor complex (Grant et al. 1998). In accordance with their role in multiple complexes, TAF components are encoded by essential genes in S. pombe (Helmlinger et al. 2011).

![Figure 4](image-url) Suppression of APC/C temperature-sensitive mutants is dependent on the SAGA DUB module. (A) Anti-FLAG western blots of whole-cell extracts prepared from untagged and the indicated FLAG-tagged strains. H2B ub corresponds to the slower migrating ubiquitinated form of H2B. (B) Individual deletions of SAGA DUB module components (sgf11Δ, sgf73Δ, or sus1Δ) suppress the temperature-sensitive phenotype of APC/C mutants. Serial dilutions (10-fold) of the indicated single and double mutant strains were spotted on yeast extract plates and incubated at the indicated temperatures.
To determine whether SAGA subunits outside the DUB module affected the cells’ requirement for APC/C function, we tested whether deletion mutants of non-DUB SAGA components could suppress APC/C mutants. gcn5Δ, sgf29Δ, ada2Δ, ngg1Δ, and tra1Δ, did not suppress the temperature-sensitive phenotype of lid1-6 (Figure 5B). Further, spt8Δ had a negative growth phenotype in combination with lid1-6 (Figure 5B). These data indicate that the activity of the DUB module of the SAGA complex is responsible for antagonizing APC/C function.

**Suppression of APC/C temperature-sensitive mutants is dependent on H2B ubiquitination**

To better understand how Ubp8 inhibits APC/C function, we identified putative substrates of the SAGA DUB module by semiquantitative comparison of all ubiquitinated proteins (the ubiquitinome) in wild-type cells relative to that of ubp8Δ. We overexpressed a HBH-Ub fusion in wildtype and ubp8Δ strains, performed purifications under fully denaturing conditions, and identified all ubiquitinated proteins using 2D-LC-MS/MS. We compared the abundance of each ubiquitinated protein in ubp8Δ relative to that in the wild type to identify potential Ubp8 substrates (i.e., ubiquitinated proteins enriched in the ubp8Δ strain). Histone H2B, an important substrate of Ubp8 (reviewed in Koutelou et al. 2010; Samara and Wolberger 2011), was the only protein identified that was more than two-fold more abundant in ubp8Δ than in the wildtype (Figure S2).

With this knowledge in mind, we investigated whether the ability of ubp8Δ to suppress APC/C mutants required histone H2B ubiquitination. BRE1-like Brl1 and small histone ubiquitination factor Shf1, components of the HULC ubiquitin ligase complex, are required in *S. pombe* for H2B ubiquitination (Tanny et al. 2007; Zofall and Grewal

**Figure 5** Suppression of APC/C temperature-sensitive mutants is specific to the SAGA DUB module. (A) Schematic organization of *S. pombe* SAGA complex. Structural subunits are colored lavender; Tra1 (coactivator binding and promoter recruitment), peach; histone acetyltransferase (HAT), pink; TATA-box binding protein module (TBP), green; DUB module, red; and Sgf29 (methylated histone binding), cream. Underlined subunits indicate mutants used in this study. (B) Single deletions of SAGA complex components and the effect on the temperature sensitive phenotype of lid1-6. Serial dilutions (10-fold) of the indicated single- and double-mutant strains were spotted on yeast extract and incubated at the indicated temperatures.
Deletions of brl1 and shf1 (brl1Δ and shf1Δ) abolished ubiquitination of histone H2B as did a lysine to arginine mutant of histone H2B in which the ubiquitin acceptor site is lost (htb1-K119R) (Figure 6A). ubp8Δ no longer suppressed APC/C temperature mutants when combined with mutants in which H2B ubiquitination was abolished (Figure 6B). Furthermore, H2B ubiquitination mutants exhibited a negative growth phenotype when combined with cut9-665 (Figure 6B). Together these results indicate that elevated levels of histone H2B ubiquitination reduce the requirement for APC/C function in S. pombe.

**DISCUSSION**

In this work we found that of all DUB single deletions, only one, ubp8Δ, reduced the requirement for APC/C function in S. pombe. Our data support a model wherein Ubp8 antagonizes APC/C indirectly via modulating the ubiquitination status of H2B.

**Multiple DUBs influence APC/C function**

In our genetic approach to identify DUB(s) that antagonize APC/C function, we found that ubp14Δ and pad1-1, mutations in the conserved DUBs Ubp14 and Rpn11, respectively, showed negative genetic interactions with APC/C mutants, indicating that these DUBs are important for APC/C function. Ubp14 cleaves free ubiquitin chains to liberate monomeric ubiquitin, thereby replenishing the ubiquitin pool (Amerik et al. 1997). Deletion of ubp14 leads to the accumulation of free ubiquitin chains that compete with other cellular proteins for proteasome binding, thereby interfering with normal proteasome function and presumably the degradation of APC/C substrates (Amerik et al. 1997). Similarly, Rpn11, an essential proteasomal DUB, cleaves the proximal ubiquitin molecule in a ubiquitin chain when polyubiquitinated substrates bind the proteasome (Verma et al. 2002). This proximal cleavage releases the ubiquitin chain from the proteasome and allows target proteins to be properly degraded. pad1-1 (rpn11-ts) mutants have compromised ubiquitin-dependent proteasome function and arrest at metaphase at the restrictive temperature (Penney et al. 1998; Verma et al. 2002). Therefore, through decreased proteasome function in ubp14Δ and pad1-1 (rpn11-ts) mutants, we predict that the degradation of APC/C substrates is compromised, further exacerbating the cut9-665 temperature sensitive phenotype.

**Suppression of APC/C mutants depends on SAGA DUB module activity**

Only mutations of the DUB module of the SAGA complex suppressed mutations in APC/C components. The yeast SAGA DUB module is...
a highly conserved complex in which the proteins Sgf11, Sgf73, Sus1, and Ubp8 are orthologous to the human proteins ATXN7L3, ATXN7, ENY2, and USP22, respectively (Lang et al. 2011; Zhao et al. 2008). In *S. cerevisiae*, each subunit of the Ubp8-Sgf11-Sus1-Sgf73 complex makes extensive contact with all other subunits and all four components are required for DUB activity in *in vitro* and *in vivo* (Lee et al. 2009; Weake et al. 2008). In contrast, in *Drosophila melanogaster* and in human cells, the presence of Ataxin-7 (Sgf73) is not necessary for DUB activity, and, instead, loss of Ataxin-7 results in increased deubiquitination and reduced levels of H2B ubiquitination (Mohan et al. 2014). This is presumably through the release of an active DUB module population no longer regulated by other components of the SAGA complex. These results reveal the complexity of regulation of H2B ubiquitination and present an interesting backdrop for understanding the role of the DUB module in regulating APC/C function in higher eukaryotes.

**Increased histone H2B ubiquitination reduces the threshold for APC/C function**

Histone H2B ubiquitination regulates many cellular processes, including transcriptional activation and silencing, maintenance of chromatin structure, and DNA repair (Weake and Workman 2008), and we have now linked this modification to a function in APC/C regulation in *S. pombe*. The exact mechanism by which H2B ubiquitination promotes APC/C function remains unclear. Because of its known role in transcriptional regulation, it is possible that increased H2B ubiquitination in *ubp8Δ* modulates the levels of APC/C components, inhibitors or activators. However, there is no evidence in support of this explanation from the results of extensive transcriptome analyses (Helmlinger et al. 2011). For example, the transcript level of the *S. pombe* APC/C coactivator *slp1*, which is known to be cell cycle regulated (Anderson et al. 2002), is not altered in deletions of DUB module components nor are the transcripts for any core APC/C component. Transcript levels of members of the PKA pathway, an APC/C inhibitor, are also unaltered in DUB module mutants (Helmlinger et al. 2011).

A second possibility for the reduced requirement of APC/C function when H2B ubiquitination levels are raised in *ubp8Δ* cells could be related to the effects of H2B ubiquitination on centromere structure and function. H2B mutations that affect its structure and ubiquitination lead to centromeric defects (Maruyama et al. 2006). Indeed, ubiquitination of H2B is required to maintain active centromeric chromatin and therefore enhances proper kinetochore formation (Sadeghi et al. 2014). Improved kinetochore formation could promote spindle microtubule-kinetochore attachment thereby reducing SAC activity, which could then lead to suppression of APC/C mutants. Although this is an attractive possibility, we did not obtain evidence supporting this mechanism. Importantly, we found that *ubp8Δ* suppressed APC/C mutants independently of the SAC. Furthermore, *ubp8Δ* was unable to suppress mutants of the essential kinetochore protein Nufl2 (nufl2-1, nufl2-2, and nufl2-3) (Nabatani et al. 2001) (Figure S3). However, more complicated mechanisms of influencing the requirement for APC/C function through an effect of H2B ubiquitination on centromeric chromatin structure cannot be ruled out.

A third possibility is that H2B ubiquitination regulates a downstream signaling event that modulates APC/C activity. For example, H2B ubiquitination is required for methylation of the Dam1 kinetochore component (Latham et al. 2011) and has a role in regulation of DNA replication in *S. cerevisiae* (Trujillo and Osley 2012). Thus, it is possible that Ubp8 regulates signaling events beyond chromatin that antagonize APC/C function. Further work is needed to dissect the molecular mechanism by which H2B ubiquitination impacts APC/C function.

Because of its essential roles in chromosome segregation and mitotic progression, the APC/C has become a therapeutic target for the treatment of multiple neoplastic diseases (Bassermann et al. 2014). Interestingly, the Ubp8 homolog USP22 has been identified as a member of an I1-gene “death from cancer” signature that acts as a predictor of tumor aggressiveness, treatment resistance, and metastatic probability in cancer patients (Atanassov and Dent 2011; Glinsky 2006; Liu et al. 2010). Therefore, advancing our understanding of how the SAGA DUB module controls cell division may provide insights into the role of USP22 in tumor progression.

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