Anaphase-promoting Complex/Cyclosome-mediated Proteolysis of Ams2 in the G1 Phase Ensures the Coupling of Histone Gene Expression to DNA Replication in Fission Yeast

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Background: GATA-type transcription factor Ams2 required for core histone transcription is degraded by the SCF during G1 and M phases.

Results: Ams2 is also a substrate for the APC/C ubiquitin system, and its deregulation alters progression through S phase.

Conclusion: Ams2 is regulated by both the APC/C and SCF ubiquitin ligases.

Significance: Discovering ubiquitylation and proteolysis events by the APC/C is crucial for understanding cell division cycle control.

Histone transcription and deposition are tightly regulated with the DNA replication cycle to maintain genetic integrity. Ams2 is a GATA-containing transcription factor responsible for core histone gene expression and for CENP-A loading at centromeres in fission yeast. Ams2 levels are cell cycle-regulated, and after the S phase Ams2 is degraded by the SCFprofiubiquitin ligase; however, the regulation of Ams2 in G1 or meiosis is poorly understood. Here we show that another ubiquitin ligase, the anaphase-promoting complex/cyclosome (APC/C) targets Ams2 for destruction in G1. Ubiquitylation and destruction of Ams2 is dependent upon a coactivator Cdh1/Ste9 and the KEN box in the C terminus of Ams2. We also find that stabilization of Ams2 sensitizes cells to the anti-microtubule drug thiabendazole and the histone deacetylase inhibitor tricostatin A when a histone deacetylase gene is deleted, suggesting that histone acetylation together with Ams2 stability ensures the coupling of mitosis to DNA replication. Furthermore, in meiosis, the failure of the APC/C-mediated destruction of Ams2 is deleterious, and pre-meiotic DNA replication is barely completed. These data suggest that Ams2 destruction via both the APC/C and the SCF ubiquitin ligases underlies the coordination of histone expression and DNA replication.

The ubiquitin system is an ATP-dependent tagging process, by which ubiquitin is attached to acceptor lysine residue(s) on a substrate via an enzymatic cascade consisting of a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2), and a ubiquitin ligase (E3) (1, 2). Depending upon the number of ubiquitins or type of ubiquitin chain formed, the signal may differ from destruction of the substrate by the 26 S proteasome to a change in substrate function or localization. Two highly conserved cell cycle-regulated E3 ubiquitin ligases are the anaphase-promoting complex/cyclosome (APC/C)2 and the SCF (Skp1-Cullin 1-F-box) protein complexes (3, 4). APC/C E3 catalytic activity is tightly regulated during the cell cycle and is active from metaphase to the end of G1. This is mainly achieved by phosphorylation of APC/C subunits and binding to one of two cell cycle-specific coactivator proteins, Cdc20/Slp1 and Cdh1/Ste9. In contrast, SCF E3 catalytic activity can be present throughout the cell cycle, and the timing of substrate destruction is regulated by the phosphorylation status of those substrates. Therefore, the two E3 ligases can be active at different times in the cell cycle and thus complement each other.

The APC/C controls cell cycle transition by catalyzing the ubiquitylation of cell cycle regulators. Securin and cyclin B are two major substrates whose destruction is required for sister chromatid separation and mitotic exit, respectively (5–7). In addition, the APC/C controls other cell cycle events such as spindle dynamics, spindle checkpoint, cytokinesis, and DNA replication. However, it is believed that more APC/C substrates are still awaiting discovery, and thus systematic genome-wide screening for APC/C substrates is required. In addition to its well studied role in the mitotic cycle, the APC/C is essential for meiosis, a specialized form of cell division that gives rise to haploid gametes from diploid germ cells (8). Meiosis occurs from one round of DNA replication followed by two consecutive rounds of chromosome segregation, known as meiosis I (MI) and meiosis II (MII) (9). Progression through meiosis in fission yeast has been shown to require Mes1, which acts as a competitive inhibitor of the APC/C in MI, ensuring sufficient levels of cyclin B/Cdk1 remain to initiate MII (10, 11). In addition, the APC/C regulates proteins that function in a variety of meiotic processes. The destruction of a transcriptional repres...
sor Ume6 by the APC/C-Cdc20 is required for meiotic gene transcription, as well as meiotic progression (12). We have recently shown that destruction of Rhp54 (Rad54 homolog in fission yeast) in G1, prior to entry into meiosis is required for faithful meiotic recombination and thus the generation of genetic variation (13).

In eukaryotic cells, histone gene expression is regulated in a cell cycle–dependent manner. Histone proteins are essential for nucleosome formation. Nucleosomes consist of ~150 base pairs of DNA wrapped around a histone octamer containing two sets of each of the histone H2A/H2B and H3/H4 dimers (14). New nucleosomes are assembled as DNA replication occurs, and thus histone expression and DNA replication need to be coupled, so histone proteins are regulated in a delicate balance transcriptionally, post-transcriptionally, and posttranslationally. Ams2 in fission yeast was originally identified as a multicopy suppressor of the temperature-sensitive cnp1-1 mutant, defective in the centromere–specific histone H3 variant CENP-A (15). Ams2 promotes the loading of CENP-A and centromere nucleosome formation; thus the loss or overproduction of Ams2 interferes with the core centromere structure (15, 16). Ams2 is a member of the GATA-type transcription factor family (15) and regulates the transcription of all core histone genes during the S phase, and expression levels oscillate periodically during the cell cycle, peaking at the S phase when core histones are expressed. Transcriptional activation of ams2+ from the G1 to S phases is partly involved in this spike expression. In addition, the destruction of Ams2 in the post-S phase by the SCF (Skp1-Cullin-F box) E3 ligase containing Po3 as an substrate. We demonstrate that Ams2 is ubiquitylated by APC/C. Schizosaccharomyces pombe have set out a genome-wide search of APC/C substrates in order to understand.

In budding yeast, acetylation of histone H3 at lysine 56 (H3-K56Ac) has been shown to occur during the replication fidelity (17), the regulation of histone gene expression is crucial. Furthermore, histone modification, such as acetylation, plays a role in nucleosome assembly as well as DNA damage response control. In budding yeast, acetylation of histone H3 at lysine 56 (H3-K56Ac) has been shown to occur during the S phase and can be stimulated when DNA is damaged (18–21); however, how the acetylation of histones and expression of core histones are coordinated to preserve genome integrity remains to be understood.

Using a cell-free APC/C-dependent destruction assay, we have set out a genome-wide search of APC/C substrates in Schizosaccharomyces pombe and identified Ams2 as a new substrate. We demonstrate that Ams2 is ubiquitylated by APC/C-Cdh1, but not APC/C-Cdc20. We also show that Ams2 is degraded in vivo, and its destruction in G1 is a prerequisite for timely progression during DNA replication, in particular in meiosis. Ams2 is regulated by both SCF and APC/C ubiquitin ligases, underscoring a role of Ams2 proteolysis in the maintenance of histone homeostasis and genome integrity, in conjunction with the control of histone acetylation.

**EXPERIMENTAL PROCEDURES**

**Xenopus Egg Extracts and Destruction Assays**—Xenopus cytostatic factor–arrested egg extracts (CSF extracts) were prepared as described previously (22). A cell-free Cdh1-APC/C-dependent destruction assay was performed as previously described (13).

**Yeast General Methods**—Methods of handling *S. pombe* were described previously (23). Thiamine (2 μM) was added to the medium to repress the nmt1 promoter. The strains used in this study are shown in supplemental Table S1.

**Plasmid Construction and Mutagenesis**—The coding region of ams2+ was amplified from an *S. pombe* cDNA library and subcloned using the Invitrogen gateway system. Ams2 constructs with mutations were generated by PCR-based mutagenesis. All of the constructs were confirmed by DNA sequencing (Cogenics and University College London in-house).

**Synchronous Cultures**—To induce synchronous meiosis, homozygous diploid (h−/h−) cells containing the pat1-114 mutation were grown in Edinburgh minimal medium 2 (EMM2) to mid-log phase (Asyn), washed into EMM2-nitrogen at 25 °C for 15 h (G1), and shifted to 34 °C to inactivate Pat1 kinase and induce meiosis. Cells were collected every 20 min and analyzed by microscopy and immunoblotting.

**Flow Cytometry**—CyAn ADP high performance flow cytometry was used to analyze samples, and FlowJo software and the Watson Pragmatic were used to analyze the percentage of cells in the S and G1 phases.

**RNA Analysis**—RNA samples were prepared, and Northern Blotting was carried out as previously described (24). The probes were prepared as described in Ref. 25. The one-step Mesa Green qRT-PCR MasterMix for SYBR assay (Eurogentec, Southampton, U.K.) was used for quantitative RT-PCR experiments. The data were analyzed using MJ Opticon monitor analysis software 3.0.

**Ubiquitylation Assay**—Ubiquitylation assays were essentially performed as described (26). Xenopus APC/C was immunoprecipitated from 15 μl of interphase extract using anti-Apc3 mAb (AF3.1) immobilized on Dynabeads protein A (Invitrogen). Reactions were performed at 23 °C in 10 μl of buffer (20 mM Tris-HCl, pH 7.5, 100 mM KCl, 2.5 mM MgCl2, 2 mM ATP, 0.3 mM DTT) containing 0.05 mg/ml E1, 0.025 mg/ml UbcX, 0.75 mg/ml ubiquitin, 1 μM ubiquitin-aldehyde, 150 μM MG132, 0.01 mg/ml purified His-Cdh1 protein, and 1 μl of 35S-labeled substrates. The reactions were stopped at the indicated time points with SDS sample buffer and resolved by SDS-PAGE followed by autoradiography.

**Antibodies**—Antibodies were used as follows: anti-Pk (AbD Serotec, 1:200), anti-Myc 4A6 (Millipore 05–724, 1:300), anti-Cdc2 (mAb Y100, 1:2,000), anti-Cdc13 (RbAb HY1, 1:1,000), anti-Cig2 (mAb 3A11, 1:1,000), anti-Cut2 (RbAb HY19, 1:100), anti-Cdc2 pTpY (mAb CP3.2, a gift from Dr. J. Gannon, 1:10), and anti-histone H3 (Abcam ab1791, 1:500).

**RESULTS**

**Ams2 Is a New APC/C Substrate**—In a genome-wide screen for a KENXP motif (where X is any amino acid) containing proteins in the fission yeast *S. pombe* (13), Ams2 was shown to be a substrate of the APC/C in a cell-free APC/C–dependent destruction assay reconstituted in Xenopus egg extract. Ams2 was radiolabeled by coupled transcription and translation in the presence of [35S]methionine and added into a Cdh1-driven destruction assay. We found that Ams2 destruction was...
dependent upon the addition of recombinant *Xenopus* Cdh1 (Fig. 1B), but not Cdc20 (supplemental Fig. S1). In addition, Ams2 destruction was blocked by the presence of the N-terminal 70 amino acids of cyclin B, a known competitive inhibitor of the APC/C (27), as well as the 26 S proteasome inhibitor, MG132. Sequence inspection of Ams2 revealed two KEN boxes within its C terminus region, KEN 542 and KEN 575 (Fig. 1A). A single KEN box mutation did not stabilize Ams2, whereas when both of these KEN boxes were mutated (dKm), Ams2 became stable (Fig. 1C), suggesting that recognition of either KEN box by Xenopus APC/C-Cdh1 is sufficient to target Ams2 for destruction. We also confirmed that *S. pombe* Cdh1, Ste9, was able to bind Ams2 in a KEN box-dependent manner (Fig. 1D). Finally, to determine whether the APC/C-Cdh1 complex was directly capable of ubiquitylating Ams2, we employed an *in vitro* APC/C ubiquitin ligase assay. In this assay, APC/C purified from interphase egg extracts and recombinant *Xenopus* Cdh1 were used together with 35S-labeled substrate, Ams2. Wild type Ams2 was ubiquitylated only when Cdh1 was added, but mutation of both KEN boxes (dKm) abolished its ubiquitylation (Fig. 1E). These results indicate that Ams2 is an APC/C-Cdh1/Ste9 substrate.

**Ams2 Is Regulated in a Cell Cycle-dependent Manner**—Next, we investigated the stability of Ams2 in *S. pombe* by measuring
the half-life of Ams2 (WT and dKm). Pk-tagged versions of Ams2 were expressed from an inducible nmt1 promoter in cells blocked in G1 or G2 using a temperature-sensitive allele of cdc10 or cdc25, respectively, and then cycloheximide and thiamine were added at time 0. Samples were taken at the indicated times and analyzed by immunoblotting.

### FIGURE 2. Ams2 is destroyed in G1 in a KEN box-dependent manner in vivo.

A and B, Ams2 stability in G1 and G2 phase was measured. Ams2 was expressed from the nmt1 (rep81) promoter in cdc10-129 (HY96) or cdc25-22 (HY8) cells that had been incubated at the restrictive temperature to arrest cells at G1 or G2, respectively. Then cycloheximide (a protein synthesis inhibitor) and thiamine (a repressor of the nmt1 promoter) were added at time 0. Samples were taken at the indicated times and analyzed by immunoblotting using indicated antibodies. As control, endogenous Cdc13 and Cdc2 were used. The graph represents relative levels of Ams2 and Ams2-dKm. C, cells expressing Myc-Ams2 (HY908) and Myc-Ams2-dKm (HY909) from the endogenous ams2+ promoter were grown in EMM2 (asynchronous, lane A), washed with EMM2-N and then cultured for 15 h at 25 °C. Upon addition of NH4Cl2 (nitrogen source), the cells were released from the G1 block into the S phase or G2, respectively. Then cycloheximide (a protein synthesis inhibitor) and thiamine (a repressor of the nmt1 promoter) were added at time 0. Samples were taken at the indicated times and analyzed by immunoblotting.

Ams2 and HDAC Act in Concert to Control Cell Cycle Progression—In most eukaryotes, newly synthesized histones during S phase are acetylated and deposited into nucleosomes. We hypothesized that histone gene expression by Ams2 might be coupled with histone acetylation. To explore this model, we created double mutant strains containing ams2-dKm with HDACs and investigated whether the presence of Ams2 in G1 had an impact on their cellular sensitivity toward genotoxic stress or anti-mitotic agents. We studied HDACs from the “classical” family, which has two classes: class I Hda1/Hos2 and Clr6 and class II Clr3, as well as the class III SIR2 family of NAD+-dependent HDACs: Sir2, Hst2, and Hst4 (29). Clr6 is an essential gene; thus a temperature-sensitive strain clr6-1 was used (30). The growth and sensitivity of these double mutants to a variety of drugs were investigated (Fig. 3 and supplemental Fig. S4). Stabilization of Ams2 in a clr6Δ, hos2Δ, sir2Δ, hst2Δ, or clr6-1 background did not show any clear synergistic effects, whereas stabilization of Ams2 in a hst4Δ background (hst4Δams2-dKm) significantly elevated sensitivity to thiamethazole (a microtubule inhibitor) and tricostatin A (an inhibitory HDACi).
Because the KEN box mutation stabilizes Ams2 in G1, we next investigated what effect \textit{ams2-dkm} might have upon entry into and progression through meiosis. To this end, we constructed strains where either Myc-tagged wild type \textit{ams2} or \textit{ams2-dkm} had been integrated into the \textit{ams2} locus and expressed from the native promoter. Meiosis was zygotically induced in these cells, and meiotic progression was monitored by counting the number of nuclei. Meiosis was synchronously induced by thermal inactivation of the Pat1 kinase in prestarved homozygous (\textit{h\textsuperscript{−}/h\textsuperscript{−}}) diploid cells. As shown in Fig. 4A, the presence of Ams2 (\textit{ams2-dkm}) in G1 significantly delayed meiotic progression, meiosis I not starting until 4.5 h after Pat1 inactivation. In contrast, WT (\textit{ams2\textsuperscript{+}}) entered meiosis I around 4 h, completing it by 5 h. As cells progressed through the pre-meiotic S phase, WT Ams2 appeared and became phosphorylated. Ams2 was then destroyed prior to MI as judged by nuclei counting and was absent from the rest of the time course (Fig. 4B). The destruction of Ams2 around 3 h (after pre-meiotic S phase) is likely to be via the SCF as seen in mitosis. On the other hand, Ams2-dKm was present in G1 cells as a single form (Fig. 4C, and \textit{ams2-dkm} cells were incubated with decreasing cell numbers onto rich medium containing 12.5 \mu g/ml thiabendazole (TBZ) or 12.5 \mu g/ml trichostatin A (TSA), and incubated at 30 °C for 4 days. B and C, WT (HY908), \textit{ams2-dkm} (HY909), \textit{hst4A} (HY1032), and \textit{hst4Aams2-dkm} (HY1051) cells were incubated in the presence of 50 \mu g/ml CBZ and “cut” phenotype together with cells with multiple septa were counted at the indicated time points. Error bars, S.E. from three independent experiments.

FIGURE 3. Genetic interaction between \textit{ams2-dkm} and histone deacetylases. A, Ams2 shows a genetic interaction with Hst4. Asynchronous wild type (HY908), \textit{ams2-dkm} (HY909), \textit{hst4A} (HY1032), and \textit{hst4Aams2-dkm} (HY1051) cells were spotted with decreasing cell numbers onto rich medium containing 12.5 \mu g/ml thiabendazole (TBZ) or 12.5 \mu g/ml trichostatin A (TSA), and incubated at 30 °C for 4 days. B and C, WT (HY908), \textit{ams2-dkm} (HY909), \textit{hst4A} (HY1032), and \textit{hst4Aams2-dkm} (HY1051) cells were incubated in the presence of 50 \mu g/ml CBZ and “cut” phenotype together with cells with multiple septa were counted at the indicated time points. Error bars, S.E. from three independent experiments.

It is also possible that this is due to Ams2-dKm-dependent pre-
mitotic progression is severely delayed if Ams2 is present in G1
before meiosis is initiated.

Transcriptional Control of Histone Genes Is Deregulated by
\textit{ams2-dkm} in Meiosis—Because Ams2 has been shown to be a
transcriptional activator of core histones during mitosis (25), we
analyzed the mRNA levels of the core histones (H2A, H3, and
H4) during meiotic progression (Fig. 5A), as well as \textit{ams2\textsuperscript{+}}
and \textit{mei4\textsuperscript{+}} (a key regulator of several middle phase meiotic
genes). In WT, the \textit{ams2\textsuperscript{+}} transcript appeared before those of
the core histones and then disappeared as the cells enter into
meiosis. The core histones had a narrow window of transcription
appearing around 1.5 h and vanishing by 3 h, coinciding with
DNA replication (Fig. 5, A and B). In contrast, in \textit{ams2-dkm}
mutant, the \textit{ams2\textsuperscript{+}} transcripts and those of the core histones
appeared earlier than WT and persisted until 6 h (during
the whole experiment). Although levels were reduced at later
time points as cells attempted to cross the MI boundary, overall
levels of histone mRNAs were significantly enhanced in the
\textit{ams2-dkm} cells compared with WT. Because the S phase index
was evident for over 5 h in \textit{ams2-dkm} cells, it is likely that
\textit{ams2-dkm} cells are impaired for S phase entry and/or progres-
sion and \textit{ams2-dkm} transcription stays on, which in turn keeps
core histone transcription switched on. Expression of the mei-
osis-specific transcription factor, mei4/H11001 was also monitored. Mei4 is not required for pre-meiotic S phase but is essential for MI and sporulation (32), which is achieved by regulating cdc25/H11001 expression (33). In WT cells, a peak of mei4/H11001 expression was seen around 5 h (at the end of MI), whereas in ams2-dKm cells mei4/H11001 expression never peaked even after 6 h, which is consistent with the failure of the mutant to complete MI (Fig. 5A). To investigate whether this S phase delay is dependent on the activation of checkpoint proteins Cds1 or Chk1, we have made cdc1Δams2-dKm and chklΔams2-dKm homozygous (h+/h−) diploid cells, However, neither cdc1Δ nor chklΔ mutation could rescue the ams2-dKm phenotype in meiosis, indicating that this delay is not due to Cds1 or Chk1 (see supplemental Fig. S6). Please note that elevated levels of the histone transcripts did not affect the global level of histone proteins (Fig. 4B). This result is consistent with previously published data in an ams2 shut off strain where no ams2 or histone transcripts are observed (25).

**Feedback Control of Ams2 Expression—**Ams2-dKm is more stable than wild type Ams2 in G1 (Fig. 2A); however, we noticed that Ams2-dKm levels are less abundant than wild type Ams2 in the presence of HU, a competitive inhibitor of ribonucleotide reductase that depletes the deoxyribonucleotide pool and stalls DNA replication (Fig. 6A). The addition of HU into ams2-dkm cells released from G1 arrest also gave a similar result (supplemental Fig. S7). This unexpected result suggests the existence of a cellular mechanism by which Ams2 protein levels are monitored in G1 and then down-regulated, presumably to avoid...
uncoordinated histone expression with DNA replication. First, we examined whether this is due to SCF<sup>pof3</sup>, which is responsible for Ams2 destruction after the S phase and in the G<sub>2</sub> phase. However, deletion of <i>pof3</i> (<i>pof3Δ</i>) did not rescue the low abundance of Ams2-dKm (Fig. 6B), so <i>pof3</i> is unlikely to be involved in this regulation. Next, we examined whether this is due to repression of transcriptional activation. Ams2 is a Mlu I cell cycle box-binding factor (MBF)-regulated G<sub>1</sub>/S transcript. It has been shown that Nrm1 is an inhibitor of MBF, and in the presence of HU, Cds1 phosphorylates and inactivates Nrm1 to maintain MBF-dependent transcripts (34). We observed an increase of RNA levels of both <i>ams2</i> and <i>ams2-dKm</i> after HU addition (supplemental Fig. S8), so it is unlikely that low abundance of Ams2-dKm is because of transcriptional repression. In agreement with this, deletion of Nrm1 repressor (<i>nrm1Δ</i>) was unable to rescue low levels of Ams2-dKm in the presence of HU (Fig. 6C). In addition, we investigated whether checkpoint proteins Cds1 or Chk1 might be involved in this negative feedback. As expected, Ams2 is poorly expressed if <i>cds1</i> is deleted (<i>cds1Δ</i>), because Nrm1 stably inhibits MBF. Intriguingly, the absence of Chk1 mostly rescued the phenotype and showed early accumulation of Ams2-dKm (Fig. 6D). Expression of <i>ams2-dKm</i> mRNA in <i>chk1Δams2-dKm</i> was very similar to that in <i>ams2-dKm</i> (supplemental Fig. S8), so deletion of <i>chk1</i> (<i>chk1Δ</i>) does not regulate <i>ams2-dKm</i> transcription in the presence of HU. Thus, Chk1 presumably regulates Ams2-dKm levels via proteolysis or translation, but we do not know the precise mechanism(s) at the moment. We examined whether this feedback mechanism would reflect the sensitivity of cells to S phase DNA damage. Because <i>hst4Δ</i> and <i>hst4Δams2-dKm</i> were both sensitive to higher doses of S phase DNA-damaging agents such as CPT, HU, or MMS (supplemental Fig. S4), lower doses of CPT and HU were used (Fig. 6E). Although <i>hst4Δ</i> cells were still sensitive to low doses of these agents, <i>ams2-dKm</i> partly rescued the drug sensitivity of <i>hst4Δ</i>.

**DISCUSSION**

Histone expression and deposition has to be coordinated with DNA replication. The results presented here demonstrate how Ams2, a GATA-containing transcription factor responsible for core histone gene expression, is controlled during G<sub>1</sub> in fission yeast. Using a cell-free system reconstituted in <i>Xenopus</i> egg extracts, we searched for new APC/C substrates in fission yeast and identified Ams2 as a target of Cdh1/Ste9-APC/C. Ams2 has two KEN boxes, and when both were mutated, it was not longer ubiquitylated or degraded in G<sub>1</sub>. To see the physiological effect of Ams2 destruction in G<sub>1</sub>, cells expressing Ams2-dKm under a native promoter (<i>ams2-dKm</i>) were carefully investigated rather than using overexpression based experiments. We showed a collaborative role for both the APC/C and SCF ubiquitin ligases in regulating Ams2. In addition, our data highlight the important role of Ams2 in histone homeostasis in both the mitotic and meiotic cell cycles. The absence of Ams2 in G<sub>1</sub> apparently plays a role in the transcription repression of the core histone genes in this phase of the cell cycle.

The regulation of acetylation of lysine 56 of histone H3 (H3K56-Ac) is important for the deposition of newly synthesized histones into nucleosomes, as well as for the maintenance of DNA damage responses (18–20, 35, 36). H3K56-Ac is carried out by Rtt109 in fungi (37, 38), whereas Hst4 is responsible for...
for deacetylation during G₂/M (39, 40). The genetic interaction observed between *ams2-dKm* and *hst4Δ* (Fig. 3) may suggest a relationship between the G₁ stability of Ams2 and hyperacetylation of histone H3. Histone acetylation alongside histone expression seems to be another important element coupling DNA replication and mitosis (41–44). Ams2 might directly or indirectly regulate the status of H3K56-Ac using histone acetyltransferases. Because we have been unable to detect an interaction between Ams2 and histone H3, it is possible that the Ams2 effect we are seeing involves histone chaperones Caf1 and Rtt106, which promote histone H3 acetylation on lysine 56 (20). It should be noted that members of the GATA transcription factor family are known to be substrates of acetylation themselves; both human GATA-1 and GATA-4 are acetylated by p300 stimulating GATA-dependent transcription (45, 46).

When cells are treated by genotoxic stress such as HU, it appears that Ams2 levels are significantly reduced if Ams2 is present in G₁, suggesting the existence of a feedback system that might monitor Ams2 and/or histone levels in G₁. In this paper, we have observed, in *pof3Δ* cells where Ams2 can only be destroyed in G₁, a normal accumulation of Ams2 in the presence of HU. Less Ams2 could result in a slower synthesis/incorporation of histone H3 into nucleosomes and thus rescue from DNA-damaging agents such as CPT, HU, and MMS in *hst4Δams2-dKm* might be observed (Fig. 6E), compared with *hst4Δ* where restoring Ams2 levels could result in more or faster incorporation of H3, which compromises genomic integrity (16). It is also possible that double KEN box mutations (*ams2-dkm*) might result in a loss or gain of function of Ams2, and thus phenotypes of *hst4Δ* such as DNA damage sensitivity are indirectly rescued in *hst4Δams2-dKm*. It seems that Chk1 is involved in this feedback control, although the mechanism remains unclear. In budding yeast, transcriptional repression via Chk1 following DNA damage has already been elucidated. Under normal conditions, Chk1 acts through histone H3 phosphorylation to recruit GCN5 to the promoters of relevant genes such as Cdk1 and cyclin B. Upon DNA damage, however, Chk1 dissociates from chromatin reducing transcription levels. The transcription of some 200 genes and the transcriptional elongation/3′ processing machinery have been found to be effected by Chk1 (47, 48).

We surmise that through the destruction of Ams2 in G₁, the APC/C plays a role in histone regulation in meiosis by repressing the early expression of histone transcripts, as seen for Ume6 (Figs. 4 and 5) (12). The pre-meiotic S phase is prolonged in *ams2-dkm* cells. However, this delay is not due to a histone surveillance mechanism or DNA checkpoint Cds1/Rad53 or Chk1 (supplemental Fig. S6) (49, 50). Because failure of Ams2 destruction in G₁ has more evident effects on meiotic progress than mitosis, it is tempting to speculate that additional layers of histone regulation might be present in mitosis, which are absent in meiosis. Indeed, *S. pombe* histone transcription is regulated by the HIRA-like protein Hip1, which represses transcription outside of the S phase (24). In *ams2-dKm*, histone H2A, H3, and H4 transcripts appear early in G₁, during a period when Hip1 silences histone transcription, suggesting that it is the elevated Ams2 levels that override the repression system and that this is important as cells progress into meiosis.

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