Genome Integrity: A HAT Needs a Chaperone

Histone acetylation helps to maintain integrity of the yeast replisome in response to genotoxic agents. Four groups have now identified protein Rtt109p as the catalytic subunit of a novel histone H3 acetylase complex that harbors a histone chaperone subunit essential for significant activity.

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Each day a cell faces a constant battle with DNA lesions which, if left unrepaired, can lead to a high mutation rate, genomic instability and even cell death. The response to genotoxic damage is crucial during S phase of the cell cycle, where DNA damage can cause catastrophic collapse of the replication fork. Such defects in replisome function commonly lead to formation of DNA double-strand breaks which can initiate chromosome translocations or other rearrangement events that often lead to cancer. Consequently, cells have evolved complex machineries that stabilize stalled replication forks, enhance DNA repair, and facilitate re-start of replication at the fork [1].

During S phase, the template for eukaryotic DNA replication is a folded chromatin fiber which is at least 30 nanometers thick in all eukaryotes. These chromatin fibers are composed of long, linear arrays of nucleosomes which each contain approximately 147 base pairs of DNA wrapped around a histone octamer composed of two H2A–H2B dimers and a H3/H4 tetramer. As a replication fork proceeds along the chromosome, nucleosomes in front of the fork are disrupted, and parental H3/H4 tetramers are randomly transferred to both sister chromatids closely behind the fork [2]. Newly synthesized histones are then rapidly deposited by the fork-associated CAF1 and Asf1p histone chaperones to "fill in the gaps" [3,4]. These newly synthesized histones contain several acetylated lysine residues on their flexible amino termini, such as residues lysine 5 and lysine 12 on histone H4 (H4 K5Ac and H4 K12Ac) [4]. Although these amino-terminal modifications are conserved throughout eukaryotes, they are not essential for cell function, at least in fungi [5].

Recently, the acetylated residue H3 K56Ac has emerged as an additional modification of newly synthesized histones that is deposited into chromatin in the wake of the replication fork [6]. H3 K56Ac is an abundant histone mark in budding yeast, *Drosophila* and *Tetrahymena*, and low levels are detected in cultured human cells [6,7]. In budding yeast, H3 K56Ac is bound to the nucleosome assembly factor CAF1 [8], and detection of H3 K56Ac in cell extracts requires the Asf1p chaperone [9,10]. Strikingly, both *asf1* mutants and yeast that contain substitutions at H3 K56, such as H3 K56R, show extreme sensitivity to a variety of genotoxic agents, such as hydroxyurea, methyl methane sulfonate, bleomycin, and camptothecin [6]. Furthermore, stalling of the replication fork in the absence of H3 K56Ac leads to collapse of the replisome [3,11] and aberrant fork accumulation of DNA polymerase [3].

Given that replication forks are known to stall at particular genomic locations even in the absence of genotoxic lesions, the instability of stalled forks provides a simple explanation for why *asf1* mutants show constitutive activation of the DNA damage checkpoint, enhanced levels of spontaneous DNA double-strand breaks, increased global chromosome rearrangements, and increased levels of sister-chromatid recombination [12,13]. Taken together, these observations indicate that H3 K56Ac is required to maintain genomic integrity, most likely by stabilizing stalled replication forks and/or by providing a favorable environment for repair or prevention of DNA double strand breaks that occur during replication.

Surprisingly, even though the yeast genome sequence has been known for years, the identity of the histone acetyltransferase (HAT) that catalyzes acetylation of H3 on residue K56 has remained elusive. Indeed, a comprehensive analysis of each of the HAT enzymes predicted from the yeast genome sequence failed to identify a gene product essential for H3 K56Ac [14]. Four recent papers [11,15–17] have now reported the identification of the *RTT109* gene product as the founding member of a novel class of acetyltransferases that generate H3 K56. In two cases, identification of Rtt109p involved the laborious task of western blot screening 4,700 protein extracts prepared from each of the viable yeast gene deletion strains with antibodies specific for H3 K56Ac [11,17,18].

In a more classical approach, Driscoll *et al.* [16] extended previous genetic studies that hinted at a role for Rtt109p in the DNA damage response and performed a detailed phenotypic analysis of an *rtt109* mutant. They found that the phenotypes of an *rtt109* mutant looked a lot like an *asf1* mutant — sensitivity to genotoxic agents that induce DSBs during replication, increased spontaneous double-strand breaks, enhanced global chromosome rearrangements, accumulation of cells in the G2 phase of the cell cycle, and increased recombination between tandem repeats. Indeed, double mutant analyses from several groups show that *RTT109*, *ASF1* and H4 K56Ac function within the same genetic pathway for maintaining genome stability [11,15–17]. Likewise, large scale genetic interaction studies led Collins *et al.* to Rtt109p [15].

But does *RTT109* encode the catalytic subunit of a H3 K56 HAT? Surprisingly, the sequence of Rtt109p does not show any obvious similarity to known acetyltransferases, and it even lacks the conserved residues that

comprise known acetyl-CoA binding pockets. Rtt109p is well conserved among distantly related fungi, such as fission yeast, but homologs in more complex eukaryotes remain to be identified [11]. Despite the lack of similarity to known HATs, recombinant Rtt109p is sufficient to weakly acetylate both itself and histone H3 *in vitro*, and the acetylated H3 is recognized by antibody to H3 K56Ac [11,15–17]. However, recombinant Rtt109p generates H3 K56Ac groups too slowly and too inefficiently for the rapid demands of S phase in yeast.

How does Rtt109p solve this problem? What is remarkable is that Rtt109p associates within cells with one of two different histone chaperones, Vps75p [11,15,17,19] or Asf1p [11,16]; of these two, the Asf1p interaction plays a key role in genome stability [11]. Interactions with either Vps75p [11,15,17] or Asf1p [11,15,16] lead to a Rtt109p–histone chaperone HAT complex that robustly and specifically acetylates H3 K56, with kinetic parameters very similar to canonical HATs [11]. Although a detailed catalytic mechanism for acetylation of H3 K56Ac is not yet known, it appears that it will share at least some similarities with other HAT reactions. For instance, HATs such as Gcn5p or Esa1p require an acidic residue during the catalytic cycle to deprotonate the lysine prior to acetylation. In the case of Rtt109p, several aspartate (D) residues are conserved among fungal homologs, for example D89, D287 and D288, and substitution of these residues for either alanine or asparagine eliminates Rtt109p HAT activity *in vitro* and *in vivo* [11,15,17]. Rtt109p does not bind strongly to histones [11], and therefore it appears that the histone chaperone subunit must present the histone substrate for subsequent acetylation. Whether the histone chaperone enhances only the binding affinity for histone substrate or contributes to catalysis is not yet clear and awaits further mechanistic studies.

Of course, a central question remains: how does Rtt109p-dependent histone acetylation maintain integrity of the replisome? One type of model is based on the location of H3 K56 within the nucleosome. H3 K56 is located near where the DNA enters and exits the nucleosome, and it makes a water-mediated contact with nucleosomal DNA about ten bases into the nucleosome [6]. Acetylation of H3 K56 eliminates this histone–DNA contact and is likely to weaken the interaction of DNA at the nucleosome periphery. This weakened interaction may enhance the mobility of H3 K56Ac nucleosomes or disrupt the folding of nucleosomal arrays into 30 nanometer-like fibers (Figure 1). Such alterations in chromatin structure may facilitate regression ('backing up') of stalled replication forks.

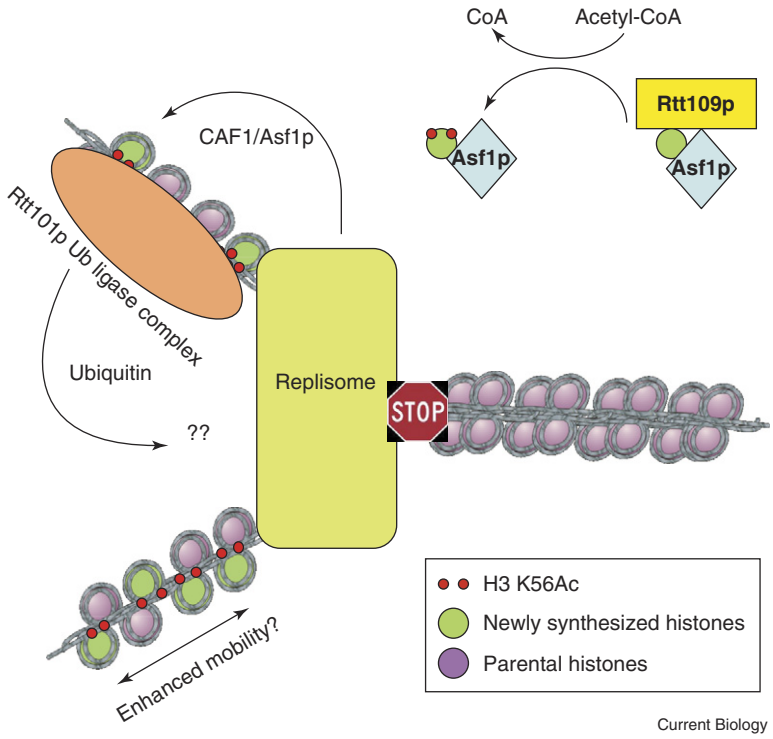

Figure 1. Schematic of a stalled eukaryotic replisome.

Stop sign denotes a genotoxic lesion that blocks replication fork progression. The model illustrated suggests that H3 K56Ac present on newly assembled nucleosomes may enhance nucleosome mobility or promote recruitment of the Rtt101p ubiquitin ligase complex. The substrate for Rtt101p is not known. (See text for details.)

Alternatively, H3 K56Ac might function like many other histone marks — by directly binding repair or fork stabilization protein(s) and recruiting them to a stalled replication fork (Figure 1). This recruitment model provides a possible explanation for why the presence of constitutive H3 K56Ac, as in a *hst3 hst4* deacetylase mutant, also leads to genome instability phenotypes [6], because genome-wide levels of the histone mark may titrate a limiting repair/replisome factor.

A good candidate for such a putative H3 K56Ac interacting partner is a subunit of the Rtt101p–Mms2p–Mms22p cullin ubiquitin ligase complex [19]. Genetic studies indicate that Rtt109p functions in the same genetic pathway as this ubiquitin ligase [15], and the Rtt101p cullin subunit, like the Rtt109p HAT, is required to prevent the collapse of stalled forks [20]. This raises the intriguing possibility that H3 K56Ac promotes the Rtt101p-dependent ubiquitination of components(s) of the replication machinery (Figure 1). This modification may stabilize stalled forks or enhance removal of the blocking lesion. Given how chromatin dynamics plays a central role in gene transcription and DNA repair, it should come as no surprise that histone modifications will play

center stage in orchestrating multiple events during chromosomal replication.

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Chromosome Congression: The Kinesin-8-Step Path to Alignment

During mitosis, chromosomes must become aligned at the equator of the mitotic spindle before segregation. Recent work suggests that a kinesin-8 motor uses a unique combination of activities to regulate this process.

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To segregate their chromosomes properly during cell division, eukaryotic cells employ a system of proteins to capture, bi-orient and position chromosomes at the equator of the microtubule-based mitotic spindle. Chromosomes make relatively stable attachments to spindle microtubule plus-ends through specialized protein complexes called kinetochores. Throughout the process of chromosome alignment, referred to as 'congression', kinetochore microtubules elongate and shorten while maintaining attachment to the chromosomes. Detailed observations of vertebrate chromosome movements and micromanipulation studies have established that assembly (lengthening) and disassembly (shortening) of the 20–25 microtubules that bind each vertebrate kinetochore may contribute the bulk of the forces required for chromosome congression during early mitosis, as well as chromosome segregation during anaphase [1]. A paper published recently in *Current Biology* [2] suggests that the human kinesin-8 motor, Kif18A, regulates kinetochore microtubule dynamics to promote chromosome congression.

Chromosomes attached to kinetochore microtubules in vertebrate cells typically move with a constant velocity (1–3 μm per min) and make rapid directional changes from poleward to anti-poleward movement, where the direction of movement is described relative to the spindle pole to which the chromosome is attached. The direction of chromosome movement is complemented by kinetochore microtubule plus-end dynamics. The addition of αβ-tubulin dimers at kinetochore microtubule plus-ends correlates with anti-poleward movements and removal of tubulin dimers results