Histone-modifying enzymes, histone modifications and histone chaperones in nucleosome assembly: Lessons learned from Rtt109 histone acetyltransferases

Jayme L Dahlin[1,2], **Xiaoyue Chen**[3], **Michael A. Walters**[4], and **Zhiguo Zhang**[3]

[1]Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic College of Medicine, Rochester, MN, USA

[2]Medical Scientist Training Program, Mayo Clinic College of Medicine, Rochester, MN, USA

[3]Department of Biochemistry and Molecular Biology, Mayo Clinic College of Medicine, Rochester, MN, USA

[4]Institute for Therapeutics Discovery and Development, University of Minnesota, Minneapolis, MN, USA

Abstract

During DNA replication, nucleosomes ahead of replication forks are disassembled to accommodate replication machinery. Following DNA replication, nucleosomes are then reassembled onto replicated DNA using both parental and newly synthesized histones. This process, termed DNA replication-coupled nucleosome assembly (RCNA), is critical for maintaining genome integrity and for the propagation of epigenetic information, dysfunctions of which have been implicated in cancers and aging. In recent years, it has been shown that RCNA is carefully orchestrated by a series of histone modifications, histone chaperones and histone-modifying enzymes. Interestingly, many features of RCNA are also found in processes involving DNA replication-independent nucleosome assembly like histone exchange and gene transcription. In yeast, histone H3 lysine K56 acetylation (H3K56ac) is found in newly synthesized histone H3 and is critical for proper nucleosome assembly and for maintaining genomic stability. The histone acetyltransferase (HAT) regulator of Ty1 transposition 109 (Rtt109) is the sole enzyme responsible for H3K56ac in yeast. Much research has centered on this particular histone modification and histone-modifying enzyme. This *Critical Review* summarizes much of our current understanding of nucleosome assembly and highlights many important insights learned from studying Rtt109 HATs in fungi. We highlight some seminal features in nucleosome assembly conserved in mammalian systems and describe some of the lingering questions in the field. Further studying fungal and mammalian chromatin assembly may have important public health implications, including deeper understandings of human cancers and aging as well as the pursuit of novel anti-fungal therapies.

Address for correspondence: Zhiguo Zhang, Department of Biochemistry and Molecular Biology, 200 First Street SW, Mayo Clinic College of Medicine, Rochester, MN 55905, USA. Tel: 507-778-6074. zhang.zhiguo@mayo.edu.

Keywords

Chromatin; epigenetics; H3K56ac; histone acetyltransferases; nucleosome assembly; replication-coupled nucleosome assembly; Rtt109

Introduction

In eukaryotic cells, DNA is assembled into chromatin, which is composed of repeating structures called nucleosomes consisting of 147 bp of helical DNA wound around a histone octamer, the protein component of the nucleosome containing a $(H3–H4)_2$ tetramer and two H2A–H2B dimers. Chromatin encodes epigenetic information and is the physiological substrate for gene transcription and DNA repair and replication. Therefore, chromatin is dynamically and tightly regulated with respect to DNA-related processes. This regulation is often through the presence or absence of histone post-translational modifications (PTMs) such as methylation, acetylation, phosphorylation, SUMOylation and ubiquitylation. Histone PTMs can be added or removed by histone-modifying enzymes. For histone acetylation, this is done by histone acetyltransferases (HAT) and histone deacetylases, respectively. Furthermore, certain proteins, called histone chaperones, can also regulate the trafficking and assembly of histones into nucleosomes by non-covalent interactions with histones and histone-modifying enzymes, thereby adding yet another layer of complexity and regulation to chromatin.

During the S phase of the cell cycle, nucleosomes located ahead of replication forks are disassembled so that DNA replication machinery can replicate DNA. Once DNA is replicated, it must then be reassembled into nucleosomes using the parental histones that were disassembled ahead of replication forks, as well as newly synthesized histones, in a process called DNA replication-coupled nucleosome assembly (RCNA; Figure 1, top). This process is important for epigenetic inheritance and genomic integrity (Groth *et al.*, 2007; Ransom *et al.*, 2010). During certain DNA damage responses, parental histones must also be removed to pave the way for the DNA repair machinery. This process is thought to have many parallels with RCNA (Morrison & Shen, 2009; Smerdon, 1991). Additionally, nucleosome assembly during gene transcription and histone exchange can occur throughout the cell cycle in a process called DNA replication-independent nucleosome assembly (RINA; Figure 1, bottom). An important question in the epigenetic and chromatin fields is how cells regulate and ultimately carry out the complex processes of nucleosome disassembly and assembly.

In this *Critical Review*, we first summarize the current body of knowledge surrounding nucleosome assembly, describing the importance of several histone chaperones, histone modifications and histone-modifying enzymes to this crucial epigenetic process. While describing the current state of understanding with respect to RCNA and RINA in yeast, we will also describe conserved features in these processes that have been found in mammalian cells. A special emphasis will be placed on critically analyzing the insights learned from studying regulator of Ty1 transposition 109 (Rtt109) HATs and H3K56ac in fungi, as this histone-modifying enzyme and histone PTM are crucial for proper RCNA and for genome

stability in yeast. We go on to synthesize the molecular, enzymatic and structural insights that are helping to explain the function and regulation of Rtt109 HATs and H3K56ac in nucleosome assembly and other cellular processes. We conclude by the describing recent efforts to exploit Rtt109-catalyzed histone acetylation for anti-fungal purposes, an important but challenging bench-to-bedside endeavor.

DNA replication-coupled nucleosome assembly

The incorporation of replicated DNA into nucleosomes has been linked to DNA replication for some time (McKnight & Miller, 1977; Stillman, 1986). By one estimate, RCNA needs to generate ~30 million nucleosomes per replication per human cell (Kadyrova *et al.*, 2013). More recently, lagging-strand DNA (Okazaki fragments) synthesis has been more firmly linked to nucleosome assembly. Not coincidentally, these fragments in yeast have approximately the same length as the repeating unit of nucleosomal DNA (Smith & Whitehouse, 2012). When DNA synthesis and nucleosome assembly are uncoupled by removing nucleosome assembly factors, cells show phenotypes consistent with genomic instability (Tyler *et al.*, 1999). During S phase, nucleosomes already present on genomic DNA (i.e. parental nucleosomes, which are ahead of the replication forks) must be disassembled to make way for replication machinery (Figure 1, top). After DNA replication, parental histones can be recycled and reloaded onto the nascent DNA strands, though many of the mechanistic details of this process are still actively being characterized (Henikoff, 2008). The doubling of genomic content in DNA replication necessitates the doubling of histones, meaning new histone proteins must be synthesized and assembled into nucleosomes (i.e. newly synthesized, or nascent nucleosomes).

Despite its complexity, it is thought the overall process of nucleosome assembly in mammalian cells is conceptually similar to that in yeast, and therefore many features of nucleosome assembly are likely conserved from yeast to human cells. For example, in both yeast and mammalian cell RCNA, nucleosomes must be disassembled by first removing H2A–H2B dimers, followed by removing $(H3–H4)_2$ tetramers. Nucleosome assembly then occurs by the loading of $(H3–H4)_2$ tetramers onto nascent DNA strands – believed to be a random process with respect to strands – which is then followed by the rapid deposition of two H2A–H2B dimers to form a complete nucleosome (Verreault, 2000). A complex system of histone chaperones, histone modifications and histone-modifying enzymes have been shown to coordinate this process (Figure 2) (Burgess & Zhang, 2013). The exact molecular details linking nucleosome assembly to DNA replication are still poorly understood due to complex interactions between the two sets of machinery (Burgess & Zhang, 2013).

In yeast, newly synthesized H3–H4 is bound to antisilencing function protein 1 (Asf1) and is imported from the cytoplasm to the nucleus in the heterotrimeric Asf1-H3–H4 form (English *et al.*, 2006). Newly synthesized histone H3 is marked by H3K56ac prior to nucleosome assembly (Masumoto *et al.*, 2005). H3K56ac is an atypical histone modification because it is located in the globular domain of the histone tetramer (Kenseth & Coldiron, 2004). H3K56ac levels in yeast peak during S phase and decrease in subsequent phases. Our group and others have shown that the acetylation of H3K56 (and other H3 residues as well) is catalyzed by the HAT Rtt109 in yeast, and that this HAT can form a complex with the

histone chaperone vacuolar protein sorting 75 (Vps75) *in vivo* (Driscoll *et al.*, 2007; Han *et al.*, 2007). Rtt109 is unique among the known HATs because it is the sole enzyme responsible for H3K56ac in fungi and utilizes the Asf1-H3–H4 complex as the substrate for H3K56ac (Figure 2). Yeast with defects in Rtt109 or with certain H3K56 mutants are more sensitive to genotoxic stress (Han *et al.*, 2007).

Histone chaperones such as Vps75 and Asf1 are crucial for RCNA, especially as they relate to Rtt109 HATs. Along with its role in stabilizing Rtt109, Vps75 helps traffic Rtt109 to the nucleus (Keck & Pemberton, 2011) and facilitates Rtt109-catalyzed acetylation of N-terminal lysines on H3. While Rtt109 catalyzes H3K56ac via the Asf1-H3-H4 substrate *in vivo*, the role of Vps75 appears to relate more to Rtt109-catalyzed acetylation of the H3 tail. Recently, a study involving the C-terminal carboxy domain of Rtt109 has provided evidence that Vps75 may play some role in promoting H3K56ac *in vivo* (Radovani *et al.*, 2013), observations that demonstrate the complex interactions between Rtt109, histone chaperones and histone substrates.

As it is currently understood, the seminal role of Rtt109 in yeast RCNA is to catalyze the acetylation of H3K56 on newly synthesized H3–H4 during S phase. Defects in H3K56ac lead to reduced cell growth, genotoxic sensitivity and increased Rad52 foci (chromosomal breaks) – phenotypes linked to disruptions in RCNA (Han *et al.*, 2007; Li *et al.*, 2008). Given the critical importance of this modification, what exactly are the functional purposes of H3K56ac? Our group has recently shown H3K56ac promotes the interaction of H3–H4 with the Rtt101[Mms1] E3 ubiquitin ligase *in vivo* and *in vitro* (Han *et al.*, 2013). This interaction in turn promotes ubiquitination at H3K121, H3K122, and H3K125 by Rtt101[Mms1]. Yeast with disruptions in this process show phenotypes consistent with errors in RCNA. Importantly, H3K122ub weakens the Asf1-H3 interaction, which then facilitates the handoff of H3K56ac-H4 from Asf1 to the downstream chaperones regulator of Ty1 transposition 106 (Rtt106) and chromatin assembly factor-1 (CAF-1) (Figure 2) (Su *et al.*, 2012). CAF-1 has well-known roles in targeting newly synthesized histone H3–H4 onto DNA (Krude, 1999; Smith & Stillman, 1991; Verreault *et al.*, 1996). CAF-1 interacts with proliferation cell nuclear antigen (PCNA) via its largest subunit p150, effectively linking DNA replication and nucleosome assembly (Zhang *et al.*, 2000). Based on several lines of evidence, including structural biology, Rtt106 likely promotes nucleosome assembly by aiding in the assembly of (H3–H4)$_2$ tetramers (Fazly *et al.*, 2012; Su *et al.*, 2012; Zunder *et al.*, 2012). Importantly, deposition of newly synthesized (H3–H4)$_2$ tetramers by CAF-1 and Rtt106 should inhibit the formation of mixed nucleosomes containing a parental dimer and another dimer consisting of newly synthesized H3–H4 (Burgess & Zhang, 2013; Xu *et al.*, 2010). Together these studies reveal that deposition of new H3–H4 is a highly regulated process during the S phase of the cell cycle.

After (H3–H4)$_2$ deposition, two H2A–H2B dimers are deposited to complete the nucleosome octomer. *In vitro* experiments show the chaperone FACT can deposit H2A–H2B onto DNA (Belotserkovskaya *et al.*, 2003). However, which histone chaperone(s) are involved in the deposition of histone H2A–H2B dimers (and also linker histone H1) onto newly replicated DNA *in vivo* is still not clear. Sometime during this complicated assembly process, H3K56ac is deacetylated by Hst3/Hst4, histone deacetylases (HDACs) whose

expression peaks in G2/M and are regulated by Mec1 kinase (Celic *et al.*, 2006; Haldar & Kamakaka, 2008; Maas *et al.*, 2006; Thaminy *et al.*, 2007; Yang *et al.*, 2008). For completeness, we note that another HDAC, Sir2, has also been implicated in H3K56ac removal in telomeric heterochromatin (Xu *et al.*, 2007). In addition to Rtt109 and H3K56ac, other HATs like Gcn5 and many other histone PTMs (e.g. H3K27ac, H4K5, 12ac) have been implicated in yeast RCNA, and for brevity, we refer the reader to recent reviews for more thorough discussions (Burgess *et al.*, 2010; Burgess & Zhang, 2013).

RCNA in mammalian cells appears to be more complicated than in yeast. Compared to yeast cells where there is only one histone H3, mammalian cells express both canonical histone H3 (H3.1/H3.2) and the histone H3 variant H3.3. While "only" differing by five amino acids, canonical H3.1 is assembled into nucleosomes during S phase in the context of RCNA. In addition, the histone H3.1–H4 dimer is recruited by Asf1a and Asf1b and is transferred onto CAF-1 for deposition in mammalian cells (Alvarez *et al.*, 2011). Furthermore, Cul4A-DDB1, a paralogue of yeast Rtt101^{Mms1}, appears to also negatively regulate the interaction between Asf1 and histone H3–H4 (Han *et al.*, 2013). Therefore, many features of RCNA are conserved from yeast to human cells.

Although they share many features, one of the differences between yeast and mammalian RCNA involves the associated histone modifications. Compared to yeast, the histone markers essential for mammalian nucleosome assembly are not as well characterized, along with the molecular mechanisms surrounding their role(s) in nucleosome assembly. While detectable, H3K56ac levels appear less abundant in human cells compared to yeast (Jasencakova *et al.*, 2010). A more recent report show global levels of H3K56ac in mammalian cells represent less than 1% of total H3 content (Drogaris *et al.*, 2012), suggesting that this modification may not be globally important, it is highly dynamic, or it has important functions in specialized contexts. Notably, there are conflicting reports as to whether its levels are coupled to the cell cycle (Xie *et al.*, 2009; Yuan *et al.*, 2009). But like in yeast, H3K56ac levels in mammalian cells appear to increase on chromatin following genotoxic treatment and also co-localize with sites of DNA repair (Das *et al.*, 2009), though again there have been conflicting reports (Miller *et al.*, 2010; Tjeertes *et al.*, 2009). These observations suggest the role of H3K56ac in nucleosome assembly is either less critical – or perhaps more complicated, highly localized and/or a transient phenomenon – than that found in simpler model organisms. CAF-1 is necessary to incorporate H3K56ac into chromatin, and as in fungi, Asf1 (Asf1a) is crucial for H3K56ac *in vivo* (Das *et al.*, 2009). The histone-modifying enzymes responsible for H3K56ac and its removal in mammalian cells have also been described. It is reported that the HATs p300 and Gcn5 can acetylate H3K56 in mammalian cells (Das *et al.*, 2009; Tjeertes *et al.*, 2009). There are reports showing H3K56ac is removed by different deacetylases (HDAC1, HDAC2, SIRT1, SIRT2 and SIRT6) in mammalian cells (Michishita *et al.*, 2009; Miller *et al.*, 2010; Toiber *et al.*, 2013; Yang *et al.*, 2009; Yuan *et al.*, 2009).

In human cells, H3K56ac appears to have some relevance to human disease and development. H3K56ac levels are higher in certain human cancers and undifferentiated cells (Das *et al.*, 2009), and they have been linked to unique global epigenetic signatures (Li *et al.*, 2013) and pluripotency factors like NANOG, SOX2, and OCT4 in embryonic stem cells

(Tan *et al.*, 2013; Xie *et al.*, 2009). Recently, several studies have been examining the role of H3K56ac in the context of obesity (Lo *et al.*, 2011). For example, deletion of neural *Sirt6* has been linked to hyperacetylation of H3K56, eventually leading to obesity in mice (Schwer *et al.*, 2010).

A recent study raised concerns about many antibody-based studies (e.g. ChIP, immunofluorescence) focusing on mammalian H3K56ac (Drogaris *et al.*, 2012). The chief concern is that several commercial anti-H3K56ac antibodies, while having specificity towards H3K56ac, also have the potential to recognize similar histone modifications such as H3K9ac, which are in much higher abundance than H3K56ac levels in mammalian cells. We believe these concerns should not be taken lightly, and it may be prudent for the field to reexamine (and hopefully reconfirm) some of the basic findings on H3K56ac in mammalian systems in light of these concerns. In addition, human cells contain both H3.1 and H3.3. It could be possible that these two histones are modified differently at H3K56, which may contribute to the discrepancies in various studies with H3K56ac. Therefore, we believe one of the major tasks in the future will be reconciling several conflicting reports with respect to the roles and regulation of H3K56ac in mammalian nucleosome assembly as well as other cellular processes. This will likely include better confirmation of detection method specificity (and sensitivity), as well as better characterization of H3K56ac localization in the genome, its dependence on the cell cycle and deacetylases and timing, and as discussed later, its role(s) in the DNA damage response.

In addition to H3K56ac, newly synthesized histone H3–H4 in mammalian cells appears to be labeled by acetylation on histone H4 lysines 5 and 12 (catalyzed by HAT1) and by methylation at H3K9 (Loyola *et al.*, 2009; Nagarajan *et al.*, 2013; Verreault *et al.*, 1998). H4K12ac is conserved from yeast to humans, and the loss of HAT1 leads to defects in proliferation and the repair of DNA damage. However, this acetylation also appears dispensable for RCNA (Barman *et al.*, 2006). It is likely that H4K5, K12ac regulate nucleosome assembly in part through modulating nuclear import of newly-synthesized H3.1–H4, as defects in these histone residues are linked to reduced levels of nuclear H3.1–H4 levels (Ejlassi-Lassallette *et al.*, 2011). Additional studies are needed to determine the function of H3K9me1 in nucleosome assembly in human cells.

DNA replication-independent nucleosome assembly (RINA)

As in DNA replication, nucleosomes must be reassembled following gene transcription in a process linked to RINA (Figure 1, bottom). This category of nucleosome assembly also includes a phenomenon known as histone exchange whereby free histones are swapped for intact nucleosomes outside the context of DNA replication. Histone exchange may provide non-dividing cells with opportunities to dynamically regulate chromatin without resorting to replicating DNA, as well as a possible mechanism for replacing damaged histones. In mammalian cells, replication-coupled and RINA utilize distinct histone H3 molecules: canonical H3.1 and histone variant H3.3, respectively.

Many histone proteins are exchanged, including CenH3 (related to centromeres), H3.3 and some mammalian H2A variants. H2A–H2B is thought to exchange more readily than (H3–

$H4)_2$ (i.e. last on, first off), though there is evidence of $(H3–H4)_2$ tetramer splitting and H3–H4 dimer histone exchange at actively-transcribed genes (Katan-Khaykovich & Struhl, 2011). While histone exchange is thought to occur frequently on highly transcribed genes, it has been observed in inactive promoters as well. For a thorough discussion of this important process, we point the reader to a well-written, comprehensive review (Das & Tyler, 2013).

It appears the deposition and exchange of H2A–H2B occurs similarly in both RCNA and in RINA processes such as histone exchange and gene transcription. In contrast to nucleosomal $(H3–H4)_2$ tetramers which are relatively stable, nucleosomal H2A–H2B dimers can undergo rapid exchange with free (unbound) H2A–H2B. This occurs even in S phase, suggesting the deposition of H2A–H2B occurs similarly in the context of DNA replication and gene transcription. One of the most extensively studied H2A–H2B chaperones is Nap1, which preferentially binds H2A–H2B over H3-H4 *in vivo* (Mosammaparast *et al.*, 2002). Nap1 contributes to H2A–H2B deposition and exchange on severallevels, including 1) the shuttling of H2A–H2B into the nucleus from the cytoplasm, 2) the disruption of non-productive histone-DNA interactions and 3) the direct deposition of both H2A–H2B and H3–H4 onto DNA for nucleosome assembly *in vitro* (Andrews *et al.*, 2010; Ito *et al.*, 1997; Mosammaparast *et al.*, 2002). Another histone chaperone implicated in H2A–H2B nucleosome assembly is FACT, which favors binding H2A–H2B over H3–H4 *in vitro*. Based on early *in vitro* experiments, FACT has been proposed to facilitate the removal of H2A–H2B for RNA polymerase II. But more recently, FACT has been implicated in H3–H4 nucleosome assembly following gene transcription (Jamai *et al.*, 2009).

In mammalian cells, histone H3.3 is deposited in RINA (along with H4) by chaperones such as HIRA, Daxx and DEK. HIRA is implicated in the assembly and exchange of histones H3.3–H4 in genic regions, since cells lacking this chaperone have reduced H3.3 levels at repressed and active genes. Daxx is thought to be involved in H3.3 deposition at telomeric regions, while DEK is thought to be involved in maintaining heterochromatin integrity. Therefore, with respect to RINA, H3.3 incorporation is likely regulated by different histone chaperones at different chromatin locations.

Compared to RCNA, there are some notable differences in how H3–H4 is incorporated in RINA. For example, in RCNA, $(H3.1–H4)_2$ tetramers rarely split. However, during S phase, a small percentage of parental $(H3.3–H4)_2$ tetramers split into H3.3–H4 dimers, which then form mixed nucleosomes containing nascent and parental H3.3–H4 dimers (Xu *et al.*, 2010). In yeast, these mixed nucleosomes are concentrated in highly transcribed regions as well as regulatory regions (Katan-Khaykovich & Struhl, 2011). Therefore, unlike H3.1–H4, it may be possible that H3.3–H4 may be deposited in either dimeric or tetrameric form, and future studies will hopefully answer this important question.

Specific histone PTMs have been implicated in RINA. The roles of H3K56ac related to RINA are also intimately related to Asf1, as this chaperone is also known to be important for chromatin disassembly. Disruptions related to Asf1 are linked to reductions in nucleosome remodeling, histone eviction and the incorporation of new H3 at transcription sites (Gkikopoulos *et al.*, 2009; Rufiange *et al.*, 2007). Consistent with the interpretation that Asf1 can facilitate chromatin disassembly via promoting H3K56ac, H3K56Q mutants can

correct defects in chromatin disassembly in *asf1* mutants (Williams *et al.*, 2008). Furthermore, when yeast are unable to acetylate H3K56, the global histone exchange rate decreases (Kaplan *et al.*, 2008). This being said, Asf1 may have roles in chromatin assembly during RINA, independent of its role in chromatin disassembly. It is also hypothesized that the "looser" structure of chromatin enabled by H3K56ac may be important for encouraging multiple transcription rounds at transcribed genes (Dennehey & Tyler, 2014). At the same time, Rtt109 and Asf1 (but interestingly, not H3K56ac) are important for transcription repression in certain instances (Lin & Schultz, 2011), attesting to the complex interplay of histone exchange, gene transcription, histone chaperones and histone PTMs. Future studies may help to detail the contributions of Rtt109 and H3K56 in influencing gene transcription, – whether this is through histone exchange, altering DNA accessibility and/or some other mechanism(s) – and how dependent these mechanisms are to specific genes. There are also other histone PTMs that may affect RINA, specifically the deposition of newly synthesized H3.3–H4. The phosphorylation of histone H4K47 (H4K47ph) by Pak2 promotes the assembly of H3.3–H4 while inhibiting the assembly of H3.1–H4. Future studies will likely implicate more histone PTMs in RINA and gene transcription levels, and it may be important (as well as challenging) to differentiate between the modulation of histone–histone chaperone interactions versus the modulation of histone-DNA or histone-transcription machinery interactions.

Nucleosome assembly in the context of DNA repair

DNA integrity is continuously challenged on multiple fronts, from intrinsic errors in the cellular machinery that handles DNA to electromagnetic radiation to chemicals (e.g. mutagens). Deficiencies in DNA repair are one hallmark of human cancers (Jackson & Bartek, 2009). Several DNA repair pathways exist, notably homologous recombination (HR), non-homologous end joining, nucleotide excision repair (NER) and base excision repair. With respect to chromatin, the current model for DNA repair is "access, repair, restore" (Smerdon, 1991). That is, first chromatin must be disassembled for DNA repair machinery to access the damaged site(s) (*access*), then this machinery must perform the necessary repairs on the DNA (*repair*), and then the repair machinery must detach and the chromatin reassembled (*restore*). Errors in nucleosome assembly associated with the DNA repair process can have far-reaching consequences, as improper assembly could damage epigenetic inheritance (e.g. PTMs) or lead to genomic instability. For instance, DNA damage has the potential to propagate quickly, as nucleo-somes flanking a double-stranded break (DSB) can be disrupted during DNA repair (Tsukuda *et al.*, 2005). While we will discuss nucleosome assembly in the context of DNA repair briefly, we point the reader to recent reviews for a comprehensive summary of this important topic (Adam *et al.*, 2014; Dennehey & Tyler, 2014).

Many of the details regarding the histone chaperones involved in DNA repair are still being characterized (Dennehey & Tyler, 2014). As in RCNA, the details of *how* nucleosomes in the vicinity of DNA damage sites are dismantled are still relatively uncharacterized (Dennehey & Tyler, 2014). DNA-end resection may drive nucleosome disassembly via chromatin remodelers like INO80 (Chambers & Downs, 2012; Morrison *et al.*, 2004), or this process may in fact be more complicated. To add to the complexity, there is also dynamic

exchange of histone variants at sites of DNA damage and repair (Volle & Dalal, 2014). Histone H2A.Z is incorporated near DSBs in human cells (Xu *et al.*, 2012), and defects in its incorporation lead to genotoxic sensitivity and defects in DNA repair. It has been hypothesized that nucleosomes with this histone variant are more easily disassembled, which can allow for better access of repair machinery (Dennehey & Tyler, 2014). Histone H2A.X, which has a random distribution across chromatin, becomes phosphorylated at serine 129/139 in yeast and mammals, respectively, by the DNA damage checkpoint system. This histone variant is therefore involved in signaling the presence of DNA damage, and is removed (via H2A.X–H2B dimers) by FACT and replaced with canonical H2A–H2B dimers (Heo *et al.*, 2008).

The prevailing thought is that nucleosome assembly during DNA repair occurs similarly as compared to RCNA, perhaps because DNA repair involves DNA synthesis. Consistent with this idea, CAF-1 is recruited to sites of UV damage, where it functions to help deposit new H3.1–H4 at DNA repair sites with the aid of PCAF (Adam *et al.*, 2014; Moggs *et al.*, 2000). Recently, the histone H3.3 chaperone HIRA was shown to be enriched at DNA damage sites in human cells, prior to the arrival of CAF-1 (Adam et al., 2013). In yeast, an important component of nucleosome assembly after DNA repair is Asf1-dependent H3K56ac. Supporting this notion is that the H3K56Q mutation, which chemically resembles H3K56ac, removes the requirement for Asf1 in proper nucleosome assembly after DSB in yeast (Chen *et al.*, 2008).

We and others acknowledge there is still much more to learn about nucleosome assembly in the context of DNA damage and repair (Adam *et al.*, 2014). Advances in knowledge related to RCNA will almost certainly help our understanding of nucleosome assembly in the context of DNA repair. Just as the fate of parental histones is elusive in RCNA, their fate in the DNA damage responses will be an important question to address, as it may have important implications for epigenetic memory. We and others are interested to see the differences, if any, between nucleosome assembly in the context of specific types of DNA damage and repair pathways (Adam & Polo, 2012), most notably in mammalian systems.

Relevance of nucleosome assembly to human health

Given the integral roles of nucleosome assembly in gene transcription, DNA replication and DNA repair, it is not surprising that factors in nucleosome assembly have been linked to cancers and other diseases (Aqeilan *et al.*, 2008; Ask *et al.*, 2012; Burgess & Zhang, 2013; May *et al.*, 2013; Polo *et al.*, 2010; Wise-Draper *et al.*, 2009; Yang *et al.*, 2013), aging (Feser *et al.*, 2010), and recently, cell differentiation and neuronal asymmetry (Nakan *et al.*, 2011). For example, mutations in the histone chaperone ATRX, along with DAXX, have been identified in tumors exhibiting alternative lengthening of telomeres (ALT), including pancreatic neuroendocrine tumors and certain sets of pediatric brain tumors (Killela *et al.*, 2013). The loss of wild-type ATRX is thought to promote cancer development by facilitating alternative lengthening of telomeres (ALT) pathway activation (Bower *et al.*, 2012). As another example, the expression levels of the histone chaperone Asf1b has been correlated with the proliferative status of breast cancer cells, a finding which could aid breast cancer diagnosis and prognosis (Corpet *et al.*, 2011). Mutations in Asf1 have also

been predicted to drive cancers by altering DNA replication and by modifying gene expression during cell cycling to increase proliferation (Reimand & Bader, 2013). Other studies have linked factors involved in nucleosome assembly with Beckwith–Wiedemann syndrome, DiGeorge and Velocardiofacial syndromes and congenital dyserythropoietic anemia type I (Catchpool *et al.*, 2000; DAntoni *et al.*, 2004; Renella *et al.*, 2011; Soekarman *et al.*, 1992). Finally, other potential regulators of nucleosome assembly like p300/CBP and HAT-1, enzymes that are responsible for histone modifications linked to nucleosome assembly, have been implicated in developmental disorders and cancers (Nagarajan *et al.*, 2013; Valor *et al.*, 2013; Van Beekum & Kalkhoven, 2007). In the future, additional studies will almost surely identify more links between disruptions in nucleosome assembly and human pathologies. Mechanistic understanding of this critical cellular process ("basic science") and identifying relevant patient subsets (patient stratification) may then provide opportunities for therapeutic interventions (Box 1).

A closer look at Rtt109 HATs: molecular, biochemical and structural insights

Our group and others have discovered and extensively studied Rtt109. These studies have shown Rtt109 HATs are subject to complex regulation, including some rather unique features. This section will synthesize the current knowledge of Rtt109 HATs, including key discoveries in molecular and cellular biology, enzymatic mechanisms and biomolecular structures. It is anticipated that deeper understandings of Rtt109 HATs at the molecular level may enhance our understanding of similar processes in mammalian systems, and may provide additional insights for therapeutically targeting HATs.

Complex chaperone and substrate dynamics

The substrates involved in Rtt109-catalyzed histone acetylation are complex, even in relatively simplified *in vitro* settings. Rtt109 is capable of acetylating H3K56 (Driscoll *et al.*, 2007; Han *et al.*, 2007; Tsubota *et al.*, 2007), located in the globular core of the nucleosome. Rtt109 can also acetylate the N-terminal tail residues H3K9, H3K14, H3K23 and H3K27 *in vitro* (Figure 3A) (Berndsen & Denu, 2008; Berndsen *et al.*, 2008; Burgess *et al.*, 2010; Fillingham *et al.*, 2008). The majority of Rtt109 substrates were determined by Western blotting or mass spectrometry using *in vitro* assays, and have been either qualitative or semi-quantitative in nature, which can make it more difficult to decipher and unambiguously quantify the contributions of various chaperones on specific histone modifications (Fulzele *et al.*, 2012). A welcome advance has been the application of quantitative proteomics to characterize the acetylation patterns of Rtt109-Asf1 and Rtt109-Vps75 *in vitro* (Abshiru *et al.*, 2012). Interestingly, it was found that Rtt109-Vps75 is capable of acetylating H4K12 (and possibly H4K5 and H4K8) *in vitro* (Abshiru *et al.*, 2012), though the contribution of Rtt109 to these modifications *in vivo* is unknown. Future studies with quantitative proteomics may want to compare the effects of each chaperone-Rtt109 combination with Rtt109 itself (i.e. no chaperone present) to better gauge the effect of each chaperone. Rtt109 and its complexes do not significantly acetylate histone H2A, H2B, or H4 *in vitro* (Tsubota *et al.*, 2007). In terms of other *in vitro* substrates, the Rtt109-

Vps75 complex is capable of acetylating histone H1 linker under some conditions *in vitro*, though the functional significance of this ability is unknown (Radovani *et al.*, 2013).

The substrate patterns of Rtt109 are subject to complex modulation by chaperones (Abshiru *et al.*, 2012). *In vitro*, Rtt109-Vps75 activity towards histone H3 is dramatically enhanced by the presence of Asf1 if H4 is also present (Han *et al.*, 2007). Rtt109-Vps75 complexes acetylate non-nucleosomal histone H3, such as $(H3–H4)_2$ tetramers or core histones (H3–H4 – H2A–H2B). However, Rtt109-Vps75 does not acetylate histone H3 efficiently in the context of mono-or dinucleosomes (Han *et al.*, 2007). This observation suggests Rtt109 only acetylates histone H3 *before* it is incorporated into nucleosomes, which is consistent with H3K56ac being observed in newly-synthesized H3 in S phase (Celic *et al.*, 2006; Masumoto *et al.*, 2005; Recht *et al.*, 2006). Therefore, substrate regulation in the form of available nucleosomal or non-nucleosomal H3 is likely another layer of regulating Rtt109 activity. Compared to Asf1, Vps75 seems to confer a broader substrate profile. While Vps75 seems to enhance the substrate profile of Rtt109, this same study showed Asf1 directs Rtt109 towards H3K56 *in vitro* (Abshiru *et al.*, 2012). Together, the collective observations show Rtt109-substrate dynamics are complicated.

The substrate specificity patterns of Rtt109 and its chaperones *in vivo* are difficult to characterize (Figure 3A). Teasing apart the contributions of each chaperone on specific histone modifications is difficult, as the results can be confounded by HATs will overlapping substrates such as Gcn5 in yeast. It is well established that Rtt109 and Asf1 are both required for H3K56ac *in vivo* while Vps75 does not have as appreciable an effect on H3K56ac levels (Berndsen *et al.*, 2008; Celic *et al.*, 2006; Driscoll *et al.*, 2007; Fillingham *et al.*, 2008; Han *et al.*, 2007; Recht *et al.*, 2006; Tsubota *et al.*, 2007). Many studies have also examined the effects of Rtt109, Vps75, Asf1 and Gcn5 deletion on the levels of specific N-terminal histone H3 acetylation modifications (Berndsen *et al.*, 2008; Burgess *et al.*, 2010; Fillingham *et al.*, 2008; Keck & Pemberton, 2011; Tang *et al.*, 2011). Our observation that *gcn5* yeast also lacking *vps75* are completely deficient in H3K27ac levels suggests Vps75 is required for acetylation of the H3 N-terminus (Burgess *et al.*, 2010).

These studies demonstrate the contributions of these chaperones and HATs to individual H3 tail modifications *in vivo* is quite complex, and more studies are needed for clarification. A logical extension of the *in vitro* quantitative proteomics findings could be to apply quantitative proteomics to *in vivo* systems. It may be interesting to get a quantitative characterization of specific histone modifications in response to various chaperone perturbations or environmental stresses *in vivo.* Based on the breadth of data to date, the working model for Rtt109 substrate specificity is that the Rtt109-Vps75 complex is responsible for acetylation of the H3 N-terminus, while Rtt109 and Asf1 are responsible for H3K56ac. As of yet, there have not been any studies testing whether Asf1 is required for the acetylation of the H3 N-terminus *in vivo*.

Characterizing the Rtt109 chaperone interactions

The effects of the histone chaperones Vps75 and Asf1 on Rtt109 activity have been studied extensively. As mentioned previously, Rtt109 forms reversible complexes with the histone chaperone Vps75 (Table 1). Vps75 is a member of the NAP1 histone chaperone family, and

it can also bind either (H3–H4)$_2$ or H2A–H2B dimers (Park *et al.*, 2008; Selth & Svejstrup, 2007). Vps75 forms a self-dimer *in vitro*, and this dimerization enhances Rtt109 HAT activity *in vitro* (Tang *et al.*, 2011). Rtt109 and Vps75 can form a complex *in vitro* and *in vivo* with low nanomolar affinity (Albaugh *et al.*, 2010; Krogan *et al.*, 2006; Tsubota *et al.*, 2007). Additionally, Vps75 protects Rtt109 from proteolysis *in vitro*, most likely by shielding the Vps75-binding loop (residues 130–179) from proteases (Tang *et al.*, 2011). The binding of Vps75 to Rtt109 enhances enzymatic activity *in vitro* (Berndsen *et al.*, 2008; Han *et al.*, 2007; Tsubota *et al.*, 2007), as reflected by a nearly 100-fold increase in k_{cat}, but does not appear to dramatically alter the K_M of either protein or acetyl coenzyme A (acetyl-CoA) substrate, suggesting Vps75 stabilizes a more active conformation of Rtt109 rather than enhancing substrate binding (Tables 1 and 2).

The other chaperone, Asf1, is highly conserved throughout eukaryotes and binds to the H3–H4 heterodimer, as opposed to (H3-H4)$_2$ (Table 1). Rtt109 also interacts with Asf1, but the Rtt109-Asf1 interaction is hard to detect, which contrasts the Rtt109-Vps75 interaction (Han *et al.*, 2007; Tsubota *et al.*, 2007). *In vitro*, Rtt109 and Rtt109-Vps75 cannot pull-down Asf1 without H3–H4 bound to Asf1 (Han *et al.*, 2007). However, different Rtt109 constructs have been reported to bind Asf1 *in vitro* in the absence of histones (Tsubota *et al.*, 2007). Rtt109 and Asf1 do not co-purify *in vivo* without the aid of crosslinking, providing further evidence that this interaction is transient and is mediated in part through their interactions with H3–H4 (Han *et al.*, 2007). These results strongly support a model whereby Rtt109-Vps75 utilizes the Asf1-H3–H4 complex, instead of H3–H4, as the substrate for H3K56ac. This model explains why Asf1 is essential for H3K56 acetylation *in vivo.* Compared to studies with Rtt109-Vps75, there is much less data about the effects of Asf1 on the kinetics of Rtt109 HAT activity (Table 2).

Proposed enzymatic mechanism of action

Understanding of the chemical mechanism of Rtt109-catalyzed histone acetylation can be important for several reasons. In structure-and mechanism-based drug design, transition-state analogs can be potent inhibitors of enzyme activity (Schramm, 2011). This could be important in the design of specific chemical probes to further study nucleosome assembly (Cole, 2008), or for developing Rtt109-specific inhibitors, which as described later on, have been hypothesized as anti-fungal agents. HATs catalyze the transfer of the acetyl moiety from acetyl-CoA to a lysine substrate, though the exact order of substrate binding and the nature of chemical catalysis can vary among HATs (Berndsen & Denu, 2008; Hodawadekar & Marmorstein, 2007; Marmorstein & Trievel, 2009; Roth & Denu, 2001; Vetting *et al.*, 2005; Wang *et al.*, 2008). For instance, in the Theorell-Chance mechanism, acetyltransferases bind both the acetyl-CoA and histone substrates prior to a direct nucleophilic attack by lysine residues to the acetyl moiety on acetyl-CoA (Tanner *et al.*, 1999; Trievel *et al.*, 1999). By contrast, other HATs like yeast Esa1 form an acyl-enzyme intermediate before the acyl moiety is transferred to lysine (i.e. Ping-Pong mechanism) (Yan *et al.*, 2002). A common component of both mechanisms is that lysine, which is normally protonated at physiologic pH, must be deprotonated to sufficiently increase its nucleophilicity towards either the acyl-enzyme intermediate or acetyl-CoA.

Early on, the catalytic mechanism of yeast Rtt109 has been the subject of much investigation. Speculation has surrounded the role of residues D287 and D288 as potential general bases, as double mutations at these two residues can cripple Rtt109 activity *in vitro*, but this possibility has been ruled out. The decrease in activity with D287/D288 mutants has instead been attributed to reduced acetyl-CoA binding and/or suboptimal positioning of acetyl-CoA for enzymatic catalysis, presumably through an interaction with K290ac (Albaugh *et al.*, 2010, 2011). Further examination of the putative Rtt109 active site does not reveal any obvious catalytic residues such as a strong nucleophiles or acids/bases. The search for a general base has even stretched to Vps75, but Rtt109 and Rtt109-Vps75 had similar pH-pK_a profiles (Kolonko *et al.*, 2010). There had also been speculation that the acetylation observed at K290, which happens to be near the acetyl-CoA binding domain, may represent an acyl-enzyme intermediate in a Ping-Pong mechanism. But again, this possibility was excluded by a thorough analysis of K290ac (Albaugh *et al.*, 2011).

In a well-designed series of experiments, Albaugh *et al.* proposed a mechanism of action for the Rtt109-Vps75 complex (Albaugh et al., 2010). They proposed a sequential order of substrate binding where both substrates bind Rtt109-Vps75 to form a ternary complex, with the actual order of substrate binding being either random or not strictly ordered (Figure 4A). This is unlike some other known HATs, where binding has an obligate order (Liu *et al.*, 2008; Tanner *et al.*, 2000). Rapid-quench kinetic analysis was consistent with chemical catalysis being the rate-limiting step, rather than substrate binding or product release. In this proposed mechanism, the lysine substrate, whose primary amine side chain (pK_a ~10) is typically protonated at physiologic pH, must have its positive charge neutralized to increase its nucleophilicity, quaternary amines are insufficiently nucleophilic (Figure 4B). As Rtt109 lacks a discernable general base in its active site, Albaugh *et al.* invoke a Born/desolvation effect to explain how the lysine substrate can become deprotonated and thereby increase its nucleophilicity enough for an acetylation reaction to occur at catalytic rates (Harris & Turner, 2002). In this model, the Rtt109-Vps75 hydrophobic active site perturbs the substrate lysine pK_a from its typical value of 10 downwards to somewhere near 8.5, as it would be energetically unfavorable for a charged species to migrate from a high- to low-dielectric environment. Such a general mechanism of decreasing the lysine pK_a fits with the ability of Rtt109 to acetylate multiple lysine residues, but a more detailed understanding of this proposed mechanism, such as which residues are involved in lysine and transition-state stabilization, will require more studies (Zhang *et al.*, 2014). Several lingering questions remain with respect to the Rtt109 enzymatic mechanism. Due to some well-recognized technical difficulties, these studies were performed with H3 peptides or histones without Asf1, conditions that most likely do not match the key physiological substrate for Rtt109-Vps75 complex. Therefore, it remains to be seen what effects, if any, Asf1 may have on the catalytic mechanism and kinetics of Rtt109 and Rtt109-Vps75 HAT activity (Table 2). Answering these more detailed questions will require careful optimization of experimental conditions, as histones and histone-chaperone complexes like Asf1-H3–H4 are prone to precipitation under many *in vitro* testing conditions (Dahlin JL, Zhang Z, unpublished observations).

Autoacetylation as a regulatory mechanism

An intriguing regulatory feature of yeast Rtt109 activity is lysine 290 acetylation (K290ac), a phenomenon first observed via autoradiography (Driscoll *et al.*, 2007; Han *et al.*, 2007). Several mass spectrometry studies have unambiguously identified K290ac (Lin & Yuan, 2008; Stavropoulos *et al.*, 2008; Tang *et al.*, 2008), and crystal structures show electron densities consistent with acetylation at K290 (Lin & Yuan, 2008; Stavropoulos *et al.*, 2008; Tang *et al.*, 2008). Functionally, K290 mutants show decreased HAT activity *in vitro*, and some are deficient in cellular functions such as genotoxin resistance (Lin & Yuan, 2008; Stavropoulos *et al.*, 2008). Substitution with a thiocarbamate mimic of acetylated lysine can partially restore enzymatic activity *in vitro* (Huang *et al.*, 2010). K290ac is surrounded by a cage of conserved hydrophobic residues, and the formation of this structure likely requires the neutralization of the positive lysine charge by acetylation. K290ac is located approximately 10 Å away from the acetyl-CoA aperture, and it unlikely to be directly involved in enzymatic catalysis. It has currently thought that one role of D288, whose carboxylate forms a hydrogen bond with the K290 amine, is to help properly position K290ac and thereby promote enzymatic activity. Indeed, the body of mutagenic studies for D288 and K290 suggests the precise positioning of the activation domain is crucial for Rtt109 activity *and* more efficient acetyl-CoA binding (Lin & Yuan, 2008; Stavropoulos *et al.*, 2008; Tang *et al.*, 2008).

Additional details concerning K290ac have recently been reported. While the majority of TAP-purified or recombinant Rtt109 is acetylated at K290 (Tang *et al.*, 2008), this modification can be reversed by the histone deacetylase Hst2 *in vitro* (Albaugh *et al.*, 2011). Subsequent experiments have shown K290ac is catalyzed by an intramolecular process (Albaugh *et al.*, 2011). A series of well-designed experiments showed that K290ac is reversible and is critical for Rtt109 HAT activity *in vitro* by increasing the rate of catalysis and by enhancing acetyl-CoA binding, and this modification does not contribute to Rtt109-Vps75 stability or likely represent a catalytic intermediate (Albaugh *et al.*, 2011). Interestingly, both enzymes known to acetylate H3K56 – Rtt109 and p300 – feature autoacetylation, though in p300 this process in intermolecular and contains several autoacetylation sites (Balasubramanyam *et al.*, 2006; Thompson *et al.*, 2004). An early study reported K290ac is not essential for H3K56ac *in vivo* or genotoxic resistance in yeast (Tang *et al.*, 2008), which contrasts with subsequent data (Stavropoulos *et al.*, 2008). It is unclear why this apparent discrepancy exists between the *in vitro* and *in vivo* data. Possible explanations include experimental considerations, or that the role of K290ac with respect to H3K56ac and other cellular functions related to nucleosome assembly are more nuanced than the *in vitro* data suggests. Therefore, additional experiments are likely needed to clarify the role of K290ac in an *in vivo* setting.

Clearly, there are still unanswered questions about the regulation and significance of Rtt109 autoacetylation. It could be that autoacetylation is one of the many layers of Rtt109 regulation *in vivo* (Figure 3B). Analogous to the autophosphorylation seen in protein kinases, autoacetylation may activate the Rtt109 enzyme. It has been hypothesized that Rtt109 may exist between two states, an inactive state without K290ac, and an active state with K290ac (Stavropoulos *et al.*, 2008). The enzymes responsible for its deacetylation *in*

vivo are unknown, though Hst3 and/or Hst4 may be responsible (Albaugh *et al.*, 2011). This could explain how cells completing S phase nucleosome assembly decrease in both H3K56ac levels and Rtt109 activity. Another intriguing connection brought up by Albaugh *et al.* is that K290ac lowers the K_M for acetyl-CoA, which may prime Rtt109 for the low levels of acetyl-CoA found during its peak expression in G1/S phase yeast. Autoacetylation appears to be a conserved feature among Rtt109 HATs, as K290 is highly conserved among fungal Rtt109s. Signs of autoacetylation has been observed *in vitro* by autoradiography in several other fungal Rtt109 HATs, including *Schizosaccharomyces pombe, Candida albicans, Pneumocystis carinii, Pneumocystis jirovecii* and *Aspergillus fumigatus* (Dahlin JL, Zhang Z, unpublished results). The continued understanding of Rtt109 autoacetylation may also have implications for HATs in general, as acetylation has been found in other HATs (Kadlec *et al.*, 2011; Karanam *et al.*, 2006; Santos-Rosa *et al.*, 2003; Thompson *et al.*, 2004; Wang & Chen, 2010).

Structural overview of yeast Rtt109

Several crystal structures have been solved for Rtt109 (Stavropoulos *et al.*, 2008; Su *et al.*, 2011; Tang *et al.*, 2008, 2011) and many structural features have now been linked to specific protein functions. The overall structure revolves around a central plate-like eight-strand β-sheet (the "acetyltransferase domain"), which is surrounded by nine a-helices and some additional loops (Figure 5A). Borrowing the nomenclature from Tang *et al.*, the helices α_1–α_4 link the bottom strands of Rtt109. Helices α_5–α_7 (which form part of the ~45-residue "activation domain") and helices α_8–α_9 bundle along opposite ends of the protein. Two linkers connect the acetyltransferase domain to the activation domain, which sits atop the acetyltransferase domain. The acetyl, β-mercaptoethylamine and pantothenic acid moieties of acetyl-CoA are surrounded by the β_4–α_2 and β_5–α_4 segments on the interior side of the binding domain, and are encapsulated by the 21-residue L_1 loop that connects β_5 to α_4. The adenosine diphosphate moiety of acetyl-CoA is solvent-exposed, while the acetyl moiety extends towards a narrow aperture approximately 6 Å wide. In terms of electrostatics, the histone-binding region proximal to the proposed lysine-binding site is relatively apolar with a nearby basic patch (Figure 5A). Many of the structures contain a truncated Vps75-binding loop (Stavropoulos *et al.*, 2008; Tang *et al.*, 2008), which normally consists of residues 130–179 but is intrinsically disordered and non-amenable to crystallization without Vps75 present (Su *et al.*, 2011). Many of the active site residues that define the acetyl-CoA active site are conserved among putative Rtt109 orthologues (Tang *et al.*, 2008).

Differences in Rtt109-Vps75 structures

Two series of crystal structures have been published of the Rtt109-Vps75 complex (Su *et al.*, 2011; Tang *et al.*, 2011), along with an excellent review on these structures (D'Arcy & Luger, 2011). Given that Vps75 increases the efficiency of Rtt109-catalyzed histone acetylation but not its binding affinity towards histones or acetyl-CoA, it has been thought that Vps75 stabilizes or induces an active Rtt109 conformation. In both series, the addition of Vps75 did not cause a significant shift in the conformation of the Rtt109 active sites, which suggests either Vps75 does not induce such a conformation or that this active conformation is not represented in the solved crystal structures (Figure 5B). The two series of structures have some notable differences. In one series of structures by Tang *et al.*,

Rtt109-Vps75 was crystallized with acetyl-CoA, and showed a 2:2 Rtt109: Vps75 ratio whereas the Su *et al.* structures show a 1:2 Rtt109:Vps75 ratio and do not model acetyl-CoA. The two series revealed two and three Rtt109-Vps75 interactions, respectively (Figure 5C). In both structures, Vps75 adopts a homodimeric headphone structure, with the earmuff domains binding to Rtt109, forming a central pore that presumably accommodates a histone substrate (Figure 5C).

The most significant differences between the two sets of structures is the Rtt109:Vps75 stoichiometry and the resulting remaining interfaces. Interface I involves the Rtt109 residues 130–175, which are susceptible to proteolysis and important for Rtt109 stabilization (Fillingham *et al.*, 2008; Keck & Pemberton, 2011). This disordered loop was truncated in earlier Rtt109 structures, but becomes ordered when bound to Vps75 (Tang *et al.*, 2011). In the Su *et al.* structures, there are two additional interfaces, II and III, while the Tang *et al.* structures contain only interfaces I and II. Interface II is between helices α_8 and α_9 of Rtt 109 and chain A of the Vps75 earmuff domain and involves a mix of hydrophobic contacts and electrostatic interactions (Figure 5C) (Su *et al.*, 2011). Interface III is between the K290ac helix of Rtt109 and chain B in the Vps75 earmuff domain, as well as the N-terminal tail of chain A. With the exception of one salt bridge, Interface III is composed almost entirely of hydrophobic interactions (Figure 5C) (Su *et al.*, 2011).

Comparison of the two structures shows this stoichiometry has a potentially significant effect on the modeled size and nature of the putative histone-binding region located in the pore formed by the proteins (Figure 5C). The differences between these two sets of structures may arise from different purification and crystallization procedures, or different protein constructs, as the Tang *et al.* structures used a truncated form of Vps75. Another possibility is that each set of structures may represent a different enzymatic state of the Rtt109-Vps75 complex (i.e. acetylation of H3K56 versus acetylation of H3 N-termini residues). Clearly, more experiments are needed to further address these questions.

The next structural questions that could be addressed involve the interactions among Rtt109, its chaperones, and histones. Detailed understanding of how chaperones and histones interact with Rtt109 and how this confers acetylation specificity is still a key unanswered question. Another key question is how Vps75 *and* Asf1 interact with respect to Rtt109? Fortunately, there is already structural information for chaperones and histones, including crystal structures for yeast Asf1 (Daganzo *et al.*, 2003; Padmanabhan *et al.*, 2005), yeast Asf1 bound to histone substrates (Antczak *et al.*, 2006; Chavez *et al.*, 2012; English *et al.*, 2006) and yeast Vps75 (Tang *et al.*, 2008). However, there remains no crystal or solution structures modeling Rtt109-Asf1 interactions, which may be due to the transient nature of this interaction, or perhaps experimental considerations.

The nature of the Rtt109-histone interaction is now beginning to be probed. In the Su *et al.* structures, the three proteins form a cylindrical enclosure ~30×35 Å, a spatial arrangement which is unlikely to accommodate a full (H3–H4)$_2$ tetramer. The fact that the histone-binding pore domain is probably too small to accommodate a full (H3–H4)$_2$ tetramer (and therefore H3K56) has led us to also hypothesize that Asf1 may function to dissociate (H3–H4)$_2$ tetramers, which would presumably allow for H3K56 to be properly positioned for

Rtt109-catalyzed acetylation. Given some of the difficulties in obtaining crystal structures with Rtt109-protein complexes, NMR-based methods may be useful for further probing Rtt109-related protein-protein interactions and protein dynamics (Box 2).

Therapeutic applications targeting Rtt109 and H3K56ac levels

The crucial roles of Rtt109 HATs and H3K56ac in yeast nucleosome assembly and genotoxic resistance may provide exploitable opportunities for therapeutics in humans. One of the recognized traits of good drug targets is that chemical and/or genetic modulation leads to a clear phenotype with a proven link to the pathophysiology of a disease (Gashaw *et al.*, 2012). Indeed, the growth defects and increased sensitivities of yeast to genotoxins caused by mutant H3K56 or deletion of *rtt109* demonstrate functional consequences of this pathway modulation. Another key observation is that Rtt109 HATs are not found in mammals, which several groups – including ours – have tried to exploit for anti-fungal purposes.

Anti-fungal hypothesis

Opportunistic fungal infections represent a significant source of morbidity and mortality in organ transplant patients, cancer patients and other immunocompromised populations (Fei *et al.*, 2009; Marr *et al.*, 2002; Steinbach, 2010). Mortality from these infections can exceed 50% in many human studies (Maschmeyer *et al.*, 2007; Salman *et al.*, 2011). The difficulty in successfully treating opportunistic fungal infections is due to several factors. First, fungi are eukaryotes, and many of their biologically crucial genes are conserved in humans. As a result, it has proven difficult to find fungi-specific therapeutic targets to help minimize toxicity to humans. Second, fungal populations can become resistant to therapeutics (Linden *et al.*, 2011). Third, detection and diagnosis of opportunistic fungal infections can be difficult in the clinic (Carmona & Limper, 2011; Thornton, 2010). Finally, fungal pathogenesis is characterized by complicated host-pathogen interactions (Cramer *et al.*, 2011; Thomas Jr & Limper, 2007). Therefore, there is still an unmet clinical need for novel, efficacious and minimally toxic anti-fungal treatments.

Several key observations have led to the hypothesis that modulating Rtt109-catalyzed histone acetylation may constitute an effective anti-fungal strategy. First, Rtt109 does not show any obvious primary sequence homology to mammalian HATs (Tang *et al.*, 2008). Second, Rtt109 appears to utilize a different catalytic mechanism than p300/CBP, the closest known homologue found in mammals (Table 3). Third, potent inhibitors of p300 activity *in vitro* such as Lys-CoA are relatively ineffective inhibitors of Rtt109 activity *in vitro* (Tang *et al.*, 2008). Based on the growing availability of sequenced fungal genomes, Rtt109 HATs appear to be conserved across the fungal kingdom (Figure 6). This conservation is also observed for the key histone chaperones Vps75 and Asf1 (Dahlin & Zhang, unpublished observations). This suggests that inhibitors targeting Rtt109 may be adaptable for use against different fungal species. For instance, small-molecule Rtt109 inhibitors developed against one fungal species may inhibit Rtt109 from other fungal species, depending on their mechanism(s) of inhibition, the degree of sequence and structural homology with other fungal species, as well as other species-specific factors. Based on available Rtt109 primary sequences, several features appear to be highly conserved in fungal Rtt109 HATs such as the autoacetylation site (K290 in *Saccharomyces cerevisiae*) and key catalytic residues (D89,

W221, W222, D287 and D288 in *S. cerevisiae*; Figure 6). Interestingly, some fungal species do not appear to contain the known Vps75-binding motif found in yeast (Figure 6).

Rtt109 has already been linked to virulence in the opportunistic fungi *C. albicans*, which is responsible for significant morbidity and mortality in certain immunocompromised populations (Baell *et al.*, 2013; Tomaši & Maši , 2012). Genetic deletion of *rtt109* sensitizes *C. albicans* to genotoxic stress and select echinocandin anti-fungals, and leads to altered cellular phenotypes (Lopes da Rosa *et al.*, 2010; Wurtele *et al.*, 2010). In one of these studies, an *rtt109 C. albicans* strain was more susceptible to ROS-mediated macrophage clearance. Most significantly, mice infected with *rtt109*-deficient *C. albicans* showed decreased fungal burden, attenuated immune responses and increased survival compared to wild-type virulent strains (Lopes da Rosa *et al.*, 2010; Wurtele *et al.*, 2010), strongly suggesting Rtt109 contributes to fungal pathogenesis and is therefore a promising therapeutic target in *C. albicans* and perhaps other fungi. Interestingly, hyperacetylation of H3K56 via Hst3 histone deacetylase modulation also appears to be toxic for *C. albicans* (Wurtele *et al.*, 2010), suggesting that cellular H3K56ac levels are subject to important regulations in order to maintain cell viability in this organism. Modulation of Hst3 activity with nicotinamide was cytotoxic to several fungal species, suggesting H3K56 hyperacetylation toxicity may be applicable to fungi in general. However, the concentrations used for these particular *in vitro* and *in vivo* experiments may not be therapeutically useful and may also contribute to off-target effects. It will be interesting to see whether this "opposing" approach targeting H3K56ac *removal* can also be exploited for anti-fungal purposes, as H3K56 hyperacetylation can also be detrimental to cell health (Celic *et al.*, 2008). For this strategy, minimizing potential toxicity in humans would likely necessitate fungal H3K56ac deacetylase(s) be sufficiently structurally divergent from mammalian HDACs. Additionally, modulators of Rtt109-cataylzed histone acetylation could be used synergistically with other therapeutics (Pfaller *et al.*, 2009; Singh & Pursell, 2008), as *rtt109* deletion can increase yeast sensitivity to genotoxins.

Rtt109 HATs possess many other properties associated with good therapeutic targets. Multiple robust methods have been developed to assay HAT activity, as "assayability" is an important consideration for high-throughput screen (HTS) campaigns (Aherne *et al.*, 2002). Additionally, multiple protein structures have been solved for yeast Rtt109, Rtt109-Vps75 and their protein substrates, which enable structure-based "druggability" assessments and pave the way for structure-based lead-finding and lead-optimization strategies. Lessons learned from studying nucleosome assembly and targeting Rtt109 HATs may also provide important insights relevant to human cancers and aging.

Rtt109 in clinically relevant fungal pathogens

Most studies of Rtt109 HATs have been performed in yeast, which are excellent model organisms but are generally not a significant source of deadly opportunistic fungal infections in humans compared to other pathogens like *Pneumocystis* and *Aspergillus.* To move towards more clinically relevant targets, our group has been steadily characterizing the Rtt109 system in *Pneumocystis* and other fungi. *Pneumocystis* pneumonia (PCP) is caused by the opportunistic pathogen *P. jirovecii* and is a significant source of mortality, especially

in patients infected with HIV (Thomas Jr & Limper, 2007). *Pneumocystis* research is challenging because each host species is susceptible to a specific *Pneumocystis* species, and *Pneumocystis* cannot be continuously propagated by cell culture. This means most basic research is performed *in vivo* using surrogate organisms like *P. carinii* or *Pneumocystis murina*, which are responsible for PCP in rats and mice, respectively. A natural consequence is that it is difficult to perform many standard molecular biology techniques like genetic manipulation. However, using heterologous expression in budding yeast and *in vitro* HAT assays, we have shown that *P. carinii* contains a functional Rtt109 orthologue, PcRtt109, whose activity can be enhanced by the histone chaperones Vps75 and Asf1 derived from either yeast or *P. carinii* (Kottom *et al.*, 2011; Pupaibool *et al.*, 2013). With the recent availability of a *P. jirovecii* genome (Cissé *et al.*, 2012), we have shown that *P. jirovecii* also contains a functional Rtt109 orthologue, PjRtt109, with similar *in vitro* properties as PcRtt109 (Dahlin *et al.*, 2013). This supports the use of *P. carinii* as a surrogate model for *P. jirovecii* with regards to the Rtt109 system. Despite these studies, detailed knowledge of non-yeast Rtt109 HATs is relatively sparse. Towards the goals of developing species-specific or broad-spectrum Rtt109-based anti-fungal therapies, we believe additional studies are needed to clarify the inter-species variation in fungal Rtt109 HAT structures, function and regulation. Additionally, further examination of fungal H3K56ac HDACs and the nature of H3K56ac hyperacetylation toxicity may also be worthwhile pursuits as an alternative therapeutic strategy.

Medicinal chemistry considerations of Rtt109 HATs

In principle, there are multiple ways Rtt109-catalyzed histone acetylation could be modulated by a small-molecule or peptide. Compounds could bind in the acetyl-CoA binding domain (Figures 7 and 8), the histone-lysine binding region (Figure 8A, middle), or occupy both, as is the case for the p300 bi-substrate inhibitor Lys-CoA (see Table S1 for a listing these interaction surfaces). In yeast Rtt109, the acetyl-CoA active site is relatively hydrophobic and narrow at its interior region, which corresponds to the binding domain for the acetyl-pantetheine portion of acetyl-CoA. Anti-fungal agents that selectively target fungal Rtt109 over important human HATs such as p300 requires substrate selectivity. Figure 7 (middle) shows that in both human p300 and yeast Rtt109, the pantetheine portion of bound ligands are almost structurally identical, suggesting that targeting this portion of the enzyme would present selectivity challenges. Further removed from the active site, the 3′-phosphoadenosine-5′-diphosphate moiety does not appear to be rigidly bound in either of these enzymes. Its binding region appears to be relatively flat, hinting that binding here is fairly conserved between these two acetyl-CoA-utilizing enzymes and simply serves to direct the acetyl-CoA into the acetyltranferase active site (Figure 7, left). Probably the greatest challenge with targeting the CoA binding region is that CoA is used as a co-factor in up to 4% of all human enzymes. Therefore, developing selective inhibitors solely targeting this portion of the enzyme might be challenging. Avoiding potentially ubiquitous co-factor binding domains has also been applied to the somewhat related histone methyltransferases (Kubicek *et al.*, 2007).

The fact that Rtt109 can acetylate multiple lysine residues suggests the histone-binding region(s) can accommodate a range of substrates and potential inhibitors. A detailed

description of the histone-binding region is difficult, as there are currently no structures of histones or peptides crystallized in a physiologically relevant manner. However, we can infer the histone-binding region is located on the interior surface of the Rtt109-Vps75 complex (Figure 8A, right, and 8B) through which the lysine residue penetrates to access the poised acetyl group of the acetyl-CoA. This surface is highly conserved (Tang *et al.*, 2008, 2011), and is lined by amino acids on non-contiguous chains of the Rtt109 enzyme. One prominent feature of this surface is a binding region that is predicted to be "druggable" by DoGSiteScorer (Volkamer *et al.*, 2012), though the utility of targeting this pocket is still highly speculative (Figure 8B). Nevertheless, the histone-binding region of Rtt109, with its attendant "druggable pocket", may be the best target for the preparation of selective fungal Rtt109 inhibitors.

Compounds could also bind to an allosteric site on Rtt109, though the prediction of such sites (if any) may be difficult *a priori* given the complexity of Rtt109 biochemistry (Fuller *et al.*, 2009). Compounds could also modulate a key protein-protein interaction in the Rtt109-Vps75-Asf1-H3–H4 system. However, the utility of disrupting these interactions is difficult to gauge, given that Rtt109 can still be active even in the absence of chaperones *in vitro* and *in vivo*. Useful compounds inhibiting Rtt109 would most likely be reversible inhibitors, as none of the cysteine residues in Rtt109 has been implicated directly in enzymatic catalysis. However, we have found Rtt109-Vps75 activity *in vitro* is still sensitive to nanomolar concentrations of non-specific thiol-reactive electrophiles (Dahlin, Zhang, Walters, forthcoming results), as well as low micromolar concentrations of chemical aggregators (Dahlin *et al.*, 2013).

HTS campaigns targeting yeast Rtt109

With the goal of identifying potential anti-fungal compounds, two HTS s to identify small-molecule inhibitors of Rtt109-catalyzed histone acetylation were recently reported (Dahlin *et al.*, 2013; Lopes da Rosa et al., 2013). Both screens utilized a thiol-sensitive fluorescence probe, 7-diethylamino-3-(4′-maleimidyl-phenyl)-4-methylcoumarin (CPM), to quantify the amount of CoA by-product from the HAT reaction. Both screens utilized the Rtt109-Vps75 as the enzymatic component, though the screens differed in their substrates: yeast Asf1 complexed to full-length *Drosophila* H3-H4 versus an H3 peptide (and higher concentrations of acetyl-CoA). In the former screen, the majority of active compounds from the HTS were assay artifacts resulting from a combination of chemical aggregation, redox-activity, fluorescence quenching, compound-reagent interactions and non-specific thiol-reactivity (Dahlin *et al.*, 2013). The few remaining active compounds could inhibit Rtt109-catalyzed histone acetylation in orthogonal assays for HAT activity, but are still undergoing further characterization to evaluate their selectivity, efficacy and mechanism(s) of inhibition (Dahlin, Walters, Zhang, unpublished results). In the other screen, performed using a different academic screening library, one small-molecule was reported to inhibit Rtt109-Vps75-catalyzed histone acetylation, but not the activity of related HATs p300 or Gcn5, at nanomolar compound concentrations *in vitro* (Figure 8C) (Lopes da Rosa *et al.*, 2013). From a medicinal chemistry perspective, this hydantoin derivative does not contain any conspicuous structural alerts and is synthetically accessible. Surprisingly, for such a potent inhibitor, this compound did not affect cellular H3K56ac levels or increase genotoxic

sensitivity in either *S. cerevisiae* or *C. albicans.* Additional close structural analogues of this compound were relatively inactive.

At first glance, this result may appear damning with respect to finding Rtt109 inhibitors that could be developed into useful therapeutics. However, we suggest caution when jumping to this conclusion. The lack of anti-fungal activity of this apparently potent and selective compound (Figure 8D) could be explained by several factors that are not directly related to the Rtt109-catalyzed histone acetylation, such as compound metabolism, cell permeability, compound efflux by membrane transporters, or even compound degradation in the cellular setting (i.e. lack of target engagement *in vivo).* Despite its potent *in vitro* activity (unusually potent for a non-optimized screening hit and the target class), and its apparent enzymatic selectivity, the lack of interpretable structure-activity relationship using close structural analogues, the relatively low standards reported for compound purity (>80%), and the absence of activity re-confirmation by HPLC-purified commercial samples *and* independent compound re-synthesis, raises questions about the validity of this reported inhibitor as a chemical probe (Baell, 2010; Dahlin & Walters, 2014). Inhibitory activity and apparent enzymatic selectivity may arise from variable intrinsic susceptibilities of enzymes to certain forms of non-specific enzymatic inhibition, such as thiol-reactive compounds or trace metal impurities. Until further studies are done on this compound and purified analogues, one should not exclude the possibility that this compound inhibits Rtt109 activity *in vitro* via a therapeutically uninteresting mechanism (Baell, 2010). For instance, an impurity such as a degradation product or a remnant from its chemical synthesis could disrupt enzymatic activity, though isolating and characterizing such impurities can be notoriously difficult (Beasley *et al.*, 2003; Imbert *et al.*, 2007).

Identifying compounds capable of inhibiting Rtt109-catalyzed histone acetylation, both *in vitro* and *in vivo*, is still an unmet need. Identifying HAT inhibitors may simply be an intrinsically difficult task with the current technology and screening libraries. For instance, a well-designed HTS versus a human MYST HAT only identified one small-molecule inhibitor, though its structure and any *in vivo* activity have not yet been reported due to intellectual property reasons (Falk *et al.*, 2011; Laplante *et al.*, 2013). While there are potent HDAC inhibitors (Carafa *et al.*, 2013; Marson, 2009; Pan *et al.*, 2012) and histone methyltransferase inhibitors available (Wagner & Jung, 2012), there is still an overall lack of *bona fide* HAT inhibitors reported in the scientific literature or patent applications (Furdas *et al.*, 2011). Of course, the small numbers of reported HAT inhibitors could also result from the comparatively small amounts of academic and industrial resources directed towards HATs. Garcinol, a natural product with reported activity against several HATs (Balasubramanyam *et al.*, 2004; Dahlin *et al.*, 2013), can also inhibit Rtt109-Vps75 activity at low micromolar compound concentration *in vitro* (Dahlin *et al.*, 2013). Several other reported HAT inhibitors showed weak activity against Rtt109 across several orthogonal assays (Dahlin, Walters, Zhang, unpublished results). However, many of these reported HAT inhibitors are likely promiscuous inhibitors, assay artifacts and/or therapeutically intractable chemotypes based on their chemical structures and available biological evaluations (Baell, 2010; Baell & Holloway, 2010). Protein-protein interactions like those found in the Rtt109-Vps75-Asf1 system may also be difficult to target using standard

screening libraries, as their chemotypes may be biased towards more conventional drug discovery targets like GPCRs and protein kinases (Wells & McClendon, 2007). Therefore, targeting Rtt109-catalyzed histone acetylation with a small-molecule is likely a difficult endeavor given the size and composition of conventional academic screening libraries.

We can only speculate that success in identifying and optimizing a tractable therapeutic may require the use of a combination of different screening methodologies and alternative chemical matter. Given the propensity for CPM-based assays to select thiol-reactive compounds, along with lower signal-to-background ratios compared to other methods, any future cell-free assays targeting Rtt109 HATs should consider quantifying the modified protein product(s) rather than the CoA by-product. Such methods could include antibody-based assays like AlphaScreen and TR-FRET, or high-throughput electrophoresis (Caliper technology). Another concern we foresee with testing Rtt109 inhibitors for anti-fungal purposes is the cell-based readouts themselves. In most settings, cytotoxicty is a relatively non-specific readout, so candidate compounds will have to be rigorously evaluated for actual target engagement in a cellular context. In summary, the hypothesis that Rtt109 inhibitors can function as useful antifungal agents is still unresolved. HATs like Rtt109 may be difficult targets, but they represent a potential solution to an urgent, unmet clinical need.

Conclusions and future perspectives

Nucleosome assembly is a complex and highly regulated process in eukaryotes. Proper nucleosome disassembly and assembly is crucial for maintaining genomic integrity. Nucleosome assembly requires an intricate regulation of histone-modifying enzymes, histone chaperones and histone modifications. Many important discoveries pertaining to nucleosome assembly have originated initially using the SV40 DNA replication-coupled system. In recent years, yeast-based experiments have provided new insights into how assembly of new H3–H4 is regulated through histone modifications and chaperones. Not surprisingly for such a biologically critical process, there are many similarities between yeast and mammalian nucleosome assembly pathways, underscoring the continued utility of model organisms such as yeast. Despite these efforts, it remains largely unknown how parental H3–H4 are assembled into nucleosomes.

The HAT Rtt109 is an important fixture in fungal RCNA and genotoxic resistance. Rtt109 is the sole HAT responsible for H3K56ac in fungi. The roles of H3K56ac in mammalian systems are still being actively investigated, and it will be interesting to verify which features relating to Rtt109 and H3K56ac are conserved in mammalian cells, and which features are replaced or omitted. One of the main unanswered questions for Rtt109 HATs is how the histone chaperone Asf1 directs Rtt109 substrate specificity, particularly from a structural standpoint. Another remaining question with pro-found clinical relevance is whether potent and specific inhibitors of Rtt109-catalyzed histone acetylation can exploit the absence of Rtt109 HATs in metazoans to selectively inhibit fungal growth while sparing mammalian cells from cytotoxicity. Deeper understanding of the basic mechanisms of epigenetic processes such as nucleosome assembly will require continued investigations into histone-modifying enzymes, histone chaperones, histone PTMs and also patterns of molecular recognition (histone readers). This combined knowledge may have profound

implications on human health, as epigenetic alterations have been associated with fungal virulence, cancers and aging (Box 3).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

References

Abshiru N, Ippersiel K, Tang Y, et al. Chaperone-mediated acetylation of histones by Rtt109 identified by quantitative prote-omics. J Proteomics. 2012; 81:80–90. [PubMed: 23036725]

Adam S, Polo S, Almouzni G. Transcription recovery after DNA damage requires chromatin priming by the H3.3 histone chaperone HIRA. Cell. 2013; 155:94–106. [PubMed: 24074863]

Adam S, Polo SE, Almouzni Gv. How to restore chromatin structure and function in response to DNA damage – let the chaperones play. FEBS J. 2014; 281:2315–23. [PubMed: 24673849]

Adam S, Polo SE. Chromatin dynamics during nucleotide excision repair: histones on the move. Int J Mol Sci. 2012; 13:11895–911. [PubMed: 23109890]

Aherne GW, Rowlands MG, Stimson L, Workman P. Assays for the identification and evaluation of histone acetyltransferase inhibitors. Methods. 2002; 26:245–53. [PubMed: 12054880]

Albaugh BN, Arnold KM, Lee S, Denu JM. Autoacetylation of the histone acetyltransferase Rtt109. J Biol Chem. 2011; 286:24694–701. [PubMed: 21606491]

Albaugh BN, Kolonko EM, Denu JM. Kinetic mechanism of the Rtt109-Vps75 histone acetyltransferase-chaperone complex. Biochemistry. 2010; 49:6375–85. [PubMed: 20560668]

Alvarez F, Munoz F, Schilcher P, et al. Sequential establishment of marks on soluble histones H3 and H4. J Biol Chem. 2011; 286:17714–21. [PubMed: 21454524]

Andrews AJ, Chen X, Zevin A, et al. The histone chaperone Nap1 promotes nucleosome assembly by eliminating nonnucleosomal histone DNA interactions. Mol Cell. 2010; 37:834–42. [PubMed: 20347425]

Antczak AJ, Tsubota T, Kaufman PD, Berger JM. Structure of the yeast histone H3-Asf1 interaction: implications for chaperone mechanism, species-specific interactions, and epigenetics. BMC Struct Biol. 2006; 6:26. [PubMed: 17166288]

Aqeilan RI, Hassan MQ, Bruin Ad, et al. The WWOX tumor suppressor is essential for postnatal survival and normal bone metabolism. J Biol Chem. 2008; 283:21629–39. [PubMed: 18487609]

Arif M, Kumar GVP, Narayana C, Kundu TK. Autoacetylation induced specific structural changes in histone acetyltransferase domain of p300: probed by surface enhanced Raman spectroscopy. J Phys Chem B. 2007; 111:11877–79. [PubMed: 17894486]

Ask K, Jasencakova Z, Menard P, et al. Codanin-1, mutated in the anaemic disease CDAI, regulates Asf1 function in S-phase histone supply. EMBO J. 2012; 31:2013–23. [PubMed: 22407294]

Baell JB, Ferrins L, Falk H, Nikolakopoulos G. PAINS: relevance to tool compound discovery and fragment-based screening. Aust J Chem. 2013; 66:1483–94.

Baell JB, Holloway GA. New substructure filters for removal of pan assay interference compounds (PAINS) from screening libraries and for their exclusion in bioassays. J Med Chem. 2010; 53:2719–40. [PubMed: 20131845]

Baell JB. Observations on screening-based research and some concerning trends in the literature. Future Med Chem. 2010; 2:1529–46. [PubMed: 21426147]

Balasubramanyam K, Altaf M, Varier R, et al. Polyisoprenylated benzophenone, garcinol, a natural histone acetyltransferase inhibitor, represses chromatin transcription and alters global gene expression. J Biol Chem. 2004; 279:33716–26. [PubMed: 15155757]

Balasubramanyam K, Jiang L, Wang L, et al. Kinetic and mass spectrometric analysis of p300 histone acetyltransferase domain autoacetylation. J Biol Chem. 2006; 281:40292–301. [PubMed: 17065153]

Barlev NA, Liu L, Chehab NH, et al. Acetylation of p53 activates transcription through recruitment of coactivators/histone acetyltransferases. Mol Cell. 2001; 8:1243–54. [PubMed: 11779500]

Barman H, Takami Y, Ono T, et al. Histone acetyltransferase 1 is dispensable for replication-coupled chromatin assembly but contributes to recover DNA damages created following replication blockage in vertebrate cells. Biochem Biophys Res Commun. 2006; 345:1547–57. [PubMed: 16735025]

Beasley JR, Dunn DA, Walker TL, et al. Evaluation of compound interference in immobilized metal ion affinity-based fluorescence polarization detection with a four million member compound collection. Assay Drug Dev Technol. 2003; 1:455–59. [PubMed: 15090182]

Belotserkovskaya R, Oh S, Bondarenko V, et al. FACT facilitates transcription-dependent nucleosome alteration. Science. 2003; 301:1090–93. [PubMed: 12934006]

Berndsen CE, Denu JM. Catalysis and substrate selection by histone/protein lysine acetyltransferases. Curr Opin Struc Biol. 2008; 18:682–89.

Berndsen CE, Tsubota T, Lindner SE, et al. Molecular functions of the histone acetyltransferase chaperone complex Rtt109-Vps75. Nat Struct Mol Biol. 2008; 15:948–56. [PubMed: 19172748]

Bower K, Napier C, Cole S, et al. Loss of wild-type ATRX expression in somatic cell hybrids segregates with activation of alternative lengthening of telomeres. PLOS ONE. 2012; 7:e55602.

Burgess RJ, Zhang Z. Histone chaperones in nucleosome assembly and human disease. Nat Struct Mol Biol. 2013; 20:14–22. [PubMed: 23288364]

Burgess RJ, Zhou H, Han J, Zhang Z. A role for Gcn5 in replication-coupled nucleosome assembly. Mol Cell. 2010; 37:469–80. [PubMed: 20188666]

Carafa V, Miceli M, Altucci L, Nebbioso A. Histone deacetylase inhibitors: a patent review (2009 – 2011). Expert Opin Ther Pat. 2013; 23:1–17. [PubMed: 23094822]

Carmona EM, Limper AH. Update on the diagnosis and treatment of *Pneumocystis* pneumonia. Ther Adv Respir Dis. 2011; 5:41–59. [PubMed: 20736243]

Catchpool ED, Smallwood A, Joyce J, et al. Mutation analysis of H19 and NAP1L4 (hNAP2) candidate genes and IGF2 DMR2 in Beckwith–Wiedemann syndrome. J Med Genet. 2000; 37:212–15. [PubMed: 10777363]

Cebrat M, Kim CM, Thompson PR, et al. Synthesis and analysis of potential prodrugs of coenzyme A analogues for the inhibition of the histone acetyltransferase p300. Bioorg Med Chem. 2003; 11:3307–13. [PubMed: 12837541]

Celic I, Masumoto H, Griffith WP, et al. The sirtuins Hst3 and Hst4p preserve genome integrity by controlling histone H3 lysine 56 deacetylation. Curr Biol. 2006; 16:1280–9. [PubMed: 16815704]

Celic I, Verreault A, Boeke JD. Histone H3 K56 hyperacetyla-tion perturbs replisomes and causes DNA damage. Genetics. 2008; 179:1769–84. [PubMed: 18579506]

Chambers AL, Downs JA. The RSC and INO80 chromatin-remodeling complexes in DNA double-strand break repair. Prog Mol Biol Transl Sci. 2012; 110:229–61. [PubMed: 22749148]

Chan HM, Thangue NBL. p300/CBP proteins: HATs for transcriptional bridges and scaffolds. J Cell Sci. 2001; 114:2363–73. [PubMed: 11559745]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Chavez MS, Scorgie JK, Dennehey BK, et al. The conformational flexibility of the C-terminus of histone H4 promotes histone octamer and nucleosome stability and yeast viability. Epigenetics Chromatin. 2012; 5:5. [PubMed: 22541333]

Chen CC, Carson JJ, Feser J, et al. Acetylated lysine 56 on histone H3 drives chromatin assembly after repair and signals for the completion of repair. Cell. 2008; 134:231–43. [PubMed: 18662539]

Chen H, Fan M, Pfeffer LM, Laribee RN. The histone H3 lysine 56 acetylation pathway is regulated by target of rapamycin (TOR) signaling and functions directly in ribosomal RNA biogenesis. Nucleic Acids Res. 2012

Cissé OH, Pagni M, Hauser PM. *De novo* assembly of the *Pneumocystis jirovecii* genome from a single bronchoalveolar lavage fluid specimen from a patient. mBio. 2012; 4:e00428–12. [PubMed: 23269827]

Cole PA. Chemical probes for histone-modifying enzymes. Nat Chem Biol. 2008; 4:590–97. [PubMed: 18800048]

Corpet A, De Koning L, Toedling J, et al. Asf1b, the necessary Asf1 isoform for proliferation, is predictive of outcome in breast cancer. EMBO J. 2011; 30:480–93. [PubMed: 21179005]

Cramer RA, Rivera A, Hohl TM. Immune responses against *Aspergillus fumigatus*: what have we learned? Curr Opin Infect Dis. 2011; 24:315–22. [PubMed: 21666456]

Daganzo SM, Erzberger JP, Lam WM, et al. Structure and function of the conserved core of histone deposition protein Asf1. Curr Biol. 2003; 13:2148–58. [PubMed: 14680630]

Dahlin JL, Kottom TJ, Han J, et al. *Pneumocystis jirovecii* expresses a functional ortholog of *Pneumocystis carinii* Rtt109. Mol Microbiol. 2013a in press.

Dahlin JL, Sinville R, Solberg J, et al. A cell-free fluorometric high-throughput screen for inhibitors of Rtt109-catalyzed histone acetylation. PLOS ONE. 2013b; 8:e78877. [PubMed: 24260132]

Dahlin JL, Walters MA. The essential roles of chemistry in highthroughput screening triage. Future Med Chem, Ahead of Print. 2014

D'Antoni S, Mattina T, Di Mare P, et al. Altered replication timing of the HIRA/Tuple1 locus in the DiGeorge and Velocardiofacial syndromes. Gene. 2004; 26:111–19. [PubMed: 15177686]

D'Arcy S, Luger K. Understanding histone acetyltransferase Rtt109 structure and function: how many chaperones does it take? Curr Opin Struc Biol. 2011; 21:1–7.

Das C, Lucia M, Hansen K, Tyler J. CBP/p300-mediated acetylation of histone H3 on lysine 56. Nature. 2009; 459:113–17. [PubMed: 19270680]

Das C, Tyler JK. Histone exchange and histone modifications during transcription and aging. Biochem Biophys Acta. 2013; 1819:332–42. [PubMed: 24459735]

Dennehey, BK.; Tyler, J. Histone chaperones in the assembly and disassembly of chromatin. In: Workman, JL.; Abmayr, SM., editors. Fundamentals of chromatin. New York: Springer; 2014. p. 29-67.

Di Tommaso P, Moretti S, Xenarios I, et al. T-Coffee: a web server for the multiple sequence alignment of protein and RNA sequences using structural information and homology extension. Nucleic Acids Res. 2011; 39:W13–17. [PubMed: 21558174]

Driscoll R, Hudson A, Jackson SP. Yeast Rtt109 promotes genome stability by acetylating histone H3 on lysine 56. Science. 2007; 315:649–52. [PubMed: 17272722]

Drogaris P, Villeneuve V, Pomiès C, et al. Histone deacetylase inhibitors globally enhance H3/H4 tail acetylation without affecting H3 lysine 56 acetylation. Sci Rep. 2012; 2

Dutta D, Ray S, Home P, et al. Regulation of angiogenesis by histone chaperone HIRA-mediated incorporation of lysine 56-acetylated histone H3.3 at chromatin domains of endothelial genes. J Biol Chem. 2010; 285:41567–77. [PubMed: 21041298]

Dyda F, Klein DC, Hickman AB. GCN5-related *N*-acetyltransferases: a structural overview. Annu Rev Biophys Biomol Struct. 2000; 29:81–103. [PubMed: 10940244]

Ejlassi-Lassallette A, Mocquard E, Arnaud MC, Thiriet C. H4 replication-dependent diacetylation and Hat1 promote S-phase chromatin assembly *in vivo*. Mol Biol Cell. 2011; 22:245–55. [PubMed: 21118997]

English CM, Adkins MW, Carson JJ, et al. Structural basis for the histone chaperone activity of Asf1. Cell. 2006; 127:495–508. [PubMed: 17081973]

Falk H, Connor T, Yang H, et al. An efficient high-throughput screening method for MYST family acetyltransferases, a new class of epigenetic drug targets. J Biomol Screen. 2011; 16:1196–205. [PubMed: 22086725]

Fazly A, Li Q, Hu Q, et al. Histone chaperone Rtt106 promotes nucleosome formation using (H3-H4)$_2$ tetramers. J Biol Chem. 2012; 287:10735–60.

Fei M, Kim E, Sant C, et al. Predicting mortality from HIV-associated *Pneumocystis* pneumonia at illness presentation: an observational cohort study. Thorax. 2009; 64:1070–76. [PubMed: 19825785]

Feser J, Truong D, Das C, et al. Elevated histone expression promotes life span extension. Mol Cell. 2010; 39:724–35. [PubMed: 20832724]

Fillingham J, Recht J, Silva A, et al. Chaperone control of the activity and specificity of the histone H3 acetyltransferase Rtt109. Mol Cell Biol. 2008; 28:4342–53. [PubMed: 18458063]

Fuller JC, Burgoyne NJ, Jackson RM. Predicting druggable binding sites at the protein-protein interface. Drug Discov Today. 2009

Fulzele A, Malgundkar S, Govekar R, et al. Keratins in oral cancer: necessity of mass spectrometry for validation of antibody based identifications. J Proteomics. 2012; 75:2404–16. [PubMed: 22387131]

Furdas SD, Kannan S, Sippl W, Jung M. Small molecule inhibitors of histone acetyltransferases as epigenetic tools and drug candidates. Archiv der Pharmazie. 2011; 345:7–21. [PubMed: 22234972]

Gashaw I, Ellinghaus P, Sommer A, Asadullah K. What makes a good drug target? Drug Discov Today. 2012; 17

Girdwood D, Bumpass D, Vaughan OA, et al. P300 transcriptional repression is mediated by SUMO modification. Mol Cell. 2003; 11:1043–54. [PubMed: 12718889]

Gkikopoulos T, Havas K, Dewar H, Owen-Hughes T. SWI/SNF and Asf1p cooperate to displace histones during induction of the saccharomyces cerevisiae HO promoter. Mol Cell Biol. 2009; 29:4057–66. [PubMed: 19470759]

Gouet P, Robert X, Courcelle E. ESPript/ENDscript: extracting and rendering sequence and 3D information from atomic structures of proteins. Nucleic Acids Res. 2003; 31:3320–3. [PubMed: 12824317]

Groth A, Rocha W, Verreault A, Almouzni G. Chromatin challenges during DNA replication and repair. Cell. 2007; 128:721–33. [PubMed: 17320509]

Haldar D, Kamakaka RT. *Schizosaccharomyces pombe* Hst4 functions in DNA damage response by regulating histone H3 K56 acetylation. Eukaryotic Cell. 2008; 7:800–13. [PubMed: 18344406]

Han J, Zhang H, Zhang H, et al. A Cul4 E3 ubiquitin ligase regulates histone hand-off during nucleosome assembly. Cell. 2013; 155:817–29. [PubMed: 24209620]

Han J, Zhou H, Horazdovsky B, et al. Rtt109 acetylates histone H3 lysine 56 and functions in DNA replication. Science. 2007a; 315:653–5. [PubMed: 17272723]

Han J, Zhou H, Li Z, et al. The Rtt109-Vps75 histone acetyltransferase complex acetylates non-nucleosomal histone H3. J Biol Chem. 2007b; 282:14158–64. [PubMed: 17369253]

Han J, Zhou H, Li Z, et al. Acetylation of lysine 56 of histone H3 catalyzed by Rtt109 and regulated by Asf1 is required for replisome integrity. J Biol Chem. 2007c; 282:28587–96. [PubMed: 17690098]

Harris TK, Turner GJ. Structural basis of perturbed *pKa* values of catalytic groups in enzyme active sites. IUBMB Life. 2002; 53:85–98. [PubMed: 12049200]

Henikoff S. Nucleosome destabilization in the epigenetic regulation of gene expression. Nat Rev Genet. 2008; 9:15–26. [PubMed: 18059368]

Heo K, Kim H, Choi S, et al. FACT-mediated exchange of histone variant H2AX regulated by phosphorylation of H2AX and ADP-ribosylation of Spt16. Mol Cell. 2008; 30:86–97. [PubMed: 18406329]

Hodawadekar S, Marmorstein R. Chemistry of acetyl transfer by histone modifying enzymes: structure, mechanism and implications for effector design. Oncogene. 2007; 26:5528–40. [PubMed: 17694092]

Huang R, Holbert MA, Tarrant MK, et al. Site-specific introduction of an acetyl-lysine mimic into peptides and proteins by cysteine alkylation. J Am Chem Soc. 2010; 132:9986–7. [PubMed: 20608637]

Ide S, Saka K, Kobayashi T. Rtt109 prevents hyper-amplification of ribosomal RNA genes through histone modification in budding yeast. PLoS Genet. 2013; 9:e1003410. [PubMed: 23593017]

Imbert P-E, Unterreiner V, Siebert D, et al. Recommendations for the reduction of compound artifacts in time-resolved fluorescence resonance energy transfer assays. Assay Drug Dev Technol. 2007; 5:363–72. [PubMed: 17638536]

Ito T, Bulger M, Pazin MJ, et al. ACF, an ISWI-containing and ATP-utilizing chromatin assembly and remodeling factor. Cell. 1997; 90:145–55. [PubMed: 9230310]

Jackson SP, Bartek J. The DNA-damage response in human biology and disease. Nature. 2009; 461:1071–8. [PubMed: 19847258]

Jamai A, Puglisi A, Strubin M. Histone chaperone spt16 promotes redeposition of the original H3-H4 histones evicted by elongating RNA polymerase. Mol Cell. 2009; 35:377–83. [PubMed: 19683500]

Jasencakova Z, Scharf AND, Ask K, et al. Replication stress interferes with histone recycling and predeposition marking of new histones. Mol Cell. 2010; 37:736–43. [PubMed: 20227376]

Johnston M, Hillier L, Riles L, et al. The nucleotide sequence of *Saccharomyces cerevisiae* chromosome XII. Nature. 1997; 387:87–90. [PubMed: 9169871]

Kadlec J, Hallacli E, Lipp M, et al. Structural basis for MOF and MSL3 recruitment into the dosage compensation complex by MSL1. Nat Struct Mol Biol. 2011; 18:142–9. [PubMed: 21217699]

Kadyrova LY, Blanko ER, Kadyrov FA. Human CAF-1-dependent nucleosome assembly in a defined system. Cell Cycle. 2013; 12:3286–97. [PubMed: 24036545]

Kaplan T, Liu C, Erkmann J, et al. Cell cycle-and chaperone-mediated regulation of H3K56ac incorporation in yeast. PLoS Genet. 2008; 4:e1000270. [PubMed: 19023413]

Karanam B, Jiang L, Wang L, et al. Kinetic and mass spectrometric analysis of p300 histone acetyltransferase domain autoacetylation. J Biol Chem. 2006; 281:40292–301. [PubMed: 17065153]

Katan-Khaykovich Y, Struhl K. Splitting of H3-H4 tetramers at transcriptionally active genes undergoing dynamic histone exchange. Proc Natl Acad Sci USA. 2011; 108:1296–301. [PubMed: 21220302]

Keck KM, Pemberton LF. Interaction with the histone chaperone Vps75 promotes nuclear localization and HAT activity of Rtt109 *in vivo*. Traffic. 2011; 12:826–39. [PubMed: 21463458]

Kenseth JR, Coldiron SJ. High-throughput characterization and quality control of small-molecule combinatorial libraries. Curr Opin Chem Biol. 2004; 8:418–23. [PubMed: 15288253]

Killela P, Reitman Z, Jiao Y, et al. TERT promoter mutations occur frequently in gliomas and a subset of tumors derived from cells with low rates of self renewal. Proc Natl Acad Sci USA. 2013; 110:6021–6. [PubMed: 23530248]

Kolonko EM, Albaugh BN, Lindner SE, et al. Catalytic activation of histone acetyltransferase Rtt109 by a histone chaperone. Proc Natl Acad Sci USA. 2010; 107:20275–80. [PubMed: 21057107]

Kottom TJ, Han J, Zhang Z, Limper AH. *Pneumocystis carinii* expresses an active Rtt109 histone acetyltransferase. Am J Resp Cell Mol Biol. 2011; 44:768–76.

Krogan N, Cagney G, Yu H, et al. Global landscape of protein complexes in the yeast *Saccharomyces cerevisiae*. Nature. 2006; 440:637–43. [PubMed: 16554755]

Krude T. Chromatin assembly during DNA replication in somatic cells. Eur J Biochem. 1999; 263:1–5. [PubMed: 10429179]

Kubicek S, O'Sullivan RJ, August EM, et al. Reversal of H3K9me2 by a small-molecule inhibitor for the G9a histone methyltransferase. Mol Cell. 2007; 25:473–81. [PubMed: 17289593]

Laplante SR, Carson R, Gillard J, et al. Compound aggregation in drug discovery: implementing a practical NMR assay for medicinal chemists. J Med Chem. 2013; 56:5142–50. [PubMed: 23730910]

Li B, Su T, Ferrari R, Li J-Y, Kurdistani SK. A unique epigenetic signature is associated with active DNA replication loci in human embryonic stem cells. Epigenetics. 2013; 9:257–67. [PubMed: 24172870]

Li Q, Zhou H, Wurtele H, et al. Acetylation of histone H3 lysine 56 regulates replication-coupled nucleosome assembly. Cell. 2008; 134:244–55. [PubMed: 18662540]

Lin C, Yuan YA. Structural insights into histone H3 lysine 56 acetylation by Rtt109. Structure. 2008; 16:1503–10. [PubMed: 18707894]

Lin L, Schultz M. Promoter regulation by distinct mechanisms of functional interplay between lysine acetylase Rtt109 and histone chaperone Asf1. Proc Natl Acad Sci USA. 2011; 108:19599–604. [PubMed: 22106264]

Linden, JWMvd; Snelders, E.; Kampinga, GA., et al. Clinical implications of azole resistance in *Aspergillus fumigatus*, The Netherlands, 2007–2009. Emerging Infect Dis. 2011; 17:1846–54. [PubMed: 22000354]

Liu X, Wang L, Zhao K, et al. The structural basis of protein acetylation by the p300/CBP transcriptional coactivator. Nature. 2008; 451:846–50. [PubMed: 18273021]

Liu Y, Wang D-L, Chen S, et al. Oncogene Ras/phosphatidy-linositol 3-kinase signaling targets histone H3 acetylation at lysine 56. J Biol Chem. 2012; 287:41469–80. [PubMed: 22982396]

Lo KA, Bauchmann MK, Baumann AP, et al. Genome-wide profiling of H3K56 acetylation and transcription factor binding sites in human adipocytes. PLOS ONE. 2011; 6:e19778. [PubMed: 21655096]

Lopes da Rosa J, Bajaj V, Spoonamore J, Kaufman PD. A small molecule inhibitor of fungal histone acetyltransferase Rtt109. Bioorg Med Chem Lett. 2013; 23:2853–59. [PubMed: 23587423]

Lopes da Rosa J, Boyartchuk VL, Zhu LJ, Kaufman PD. Histone acetyltransferase Rtt109 is required for *Candida albicans* pathogenesis. Proc Natl Acad Sci USA. 2010; 107:1594–99. [PubMed: 20080646]

Loyola A, Tagami H, Bonaldi T, et al. The HP1alpha-CAF1-SetDB1-containing complex provides H3K9me1 for Suv39-mediated K9me3 in pericentric heterochromatin. EMBO Rep. 2009; 10:769–75. [PubMed: 19498464]

Maas NL, Miller KM, DeFazio LG, Toczyski DP. Cell cycle and checkpoint regulation of histone H3 K56 acetylation by Hst3 and Hst4. Mol Cell. 2006; 23:109–19. [PubMed: 16818235]

Malapeira J, Khaitova LC, Mas P. Ordered changes in histone modifications at the core of the *Arabidopsis* circadian clock. Proc Natl Acad Sci USA. 2012; 109:21540–5. [PubMed: 23236129]

Manning ET, Ikehara T, Ito T, et al. p300 forms a stable, template-committed complex with chromatin: role for the bromo-main. Mol Cell Biol. 2001; 21:3876–87. [PubMed: 11359896]

Marmorstein R, Trievel RC. Histone modifying enzymes: structures, mechanisms, and specificities. Biochem Biophys Acta. 2009; 1789:58–68. [PubMed: 18722564]

Marr KA, Carter RA, Boeckh M, et al. Invasive aspergillosis in allogeneic stem cell transplant recipients: changes in epidemiology and risk factors. Blood. 2002; 100:4358–66. [PubMed: 12393425]

Marson C. Histone deacetylase inhibitors: design, structure-activity relationships and therapeutic implications for cancer. Anticancer Agents Med Chem. 2009; 9:661–92. [PubMed: 19601748]

Maschmeyer G, Haas A, Cornely OA. Invasive aspergillosis: epidemiology, diagnosis and management in immunocompromised patients. Drugs. 2007; 67:1567–601. [PubMed: 17661528]

Masumoto H, Hawke D, Kobayashi R, Verreault A. A role for cell-cycle-regulated histone H3 lysine 56 acetylation in the DNA damage response. Nature. 2005; 436:294–8. [PubMed: 16015338]

May T, Yang J, Shoni M, et al. BRCA1 expression is epigenetically repressed in sporadic ovarian cancer cells by overexpression of C-terminal binding protein 2. Neoplasia. 2013; 15:600–08. [PubMed: 23730208]

McKnight SL, Miller OL. Electron microscopic analysis of chromatin replication in the cellular blastoderm *Drosophilia melano-gaster* embryo. Cell. 1977; 12:795–804. [PubMed: 411576]

McManus KJ, Hendzel MJ. Quantitative analysis of CBP-and P300-induced histone acetylations *in vivo* using native chromatin. Mol Cell Biol. 2003; 23:7611–27. [PubMed: 14560007]

Michishita E, McCord RA, Boxer LD, et al. Cell cycle-dependent deacetylation of telomeric histone H3 lysine K56 by human SIRT6. Cell Cycle. 2009; 8:2664–6. [PubMed: 19625767]

Miller KM, Tjeertes JV, Coates J, et al. Human HDAC1 and HDAC2 function in the DNA-damage response to promote DNA nonhomologous end-joining. Nat Struct Mol Biol. 2010; 17:1144–U15. [PubMed: 20802485]

Moggs J, Grandi P, Quivy J, et al. A CAF-1-PCNA-mediated chromatin assembly pathway triggered by sensing DNA damage. Mol Cell Biol. 2000; 20:1206–18. [PubMed: 10648606]

Morrison A, Highland J, Krogan N, et al. INO80 and gamma-H2AX interaction links ATP-dependent chromatin remodeling to DNA damage repair. Cell. 2004; 119:767–75. [PubMed: 15607974]

Morrison A, Shen X. Chromatin remodelling beyond transcription: the INO80 and SWR1 complexes. Nat Rev Mol Cell Biol. 2009; 10:373–84. [PubMed: 19424290]

Mosammaparast N, Ewart C, Pemberton L. A role for nucleosome assembly protein 1 in the nuclear transport of histones H2A and H2B. EMBO J. 2002; 21:6527–38. [PubMed: 12456659]

Nagarajan P, Ge Z, Sirbu B, et al. Histone acetyltransferase 1 is essential for mammalian development, genome stability, and the processing of newly synthesized histones H3 and H4. PLoS Genet. 2013; 9:e1003518. [PubMed: 23754951]

Nakan S, Stillman B, Horvitz HR. Replication-coupled chromatin assembly generates a neuronal bilateral asymmetry in *C. elegans*. Cell. 2011; 147:1525–36. [PubMed: 22177093]

Notredame C, Higgins D, Heringa J. T-Coffee: a novel method for fast and accurate multiple sequence alignment. J Mol Biol. 2000; 302:205–17. [PubMed: 10964570]

Ogryzko VV, Schiltz RL, Russanova V, et al. The transcriptional coactivators p300 and CBP are histone acetyltransferases. Cell. 1996; 87:953–9. [PubMed: 8945521]

Padmanabhan B, Kataoka K, Umehara T, et al. Structural similarity between histone chaperone Cia1p/Asf1p and DNA-binding protein NF-kappaB. J Biochem. 2005; 138:821–29. [PubMed: 16428312]

Pan H, Cao J, Xu W. Selective histone deacetylase inhibitors. Anticancer Agents Med Chem. 2012; 12:247–70. [PubMed: 22263804]

Park YJ, Sudhoff KB, Andrews AJ, et al. Histone chaperone specificity in Rtt109 activation. Nat Struct Mol Biol. 2008; 15:957–64. [PubMed: 19172749]

Pfaller MA, Messer SA, Georgopapadakou N, et al. Activity of MGCD290, a Hos2 histone deacetylase inhibitor, in combination with azole antifungals against opportunistic fungal pathogens. J Clin Microbiol. 2009; 47:3797–804. [PubMed: 19794038]

Polo SE, Theocharis SE, Grandin L, et al. Clinical significance and prognostic value of chromatin assembly factor-1 overexpression in human solid tumours. Histopath. 2010; 57:716–24.

Pupaibool J, Kottom TJ, Bouchonville K, Limper AH. Characterization of the *Pneumocystis carinii* histone acetyltranferase chaperone proteins PcAsf1 and PcVps75. Infect Immun. 2013; 81:2268–75. [PubMed: 23569117]

Radovani E, Cadorin M, Shams T, et al. The carboxyl terminus of Rtt109 functions in chaperone control of histone acetylation. Eukaryotic Cell. 2013; 12:654–64. [PubMed: 23457193]

Ransom M, Dennehey B, Tyler J. Chaperoning histones during DNA replication and repair. Cell. 2010; 140:183–95. [PubMed: 20141833]

Recht J, Tsubota T, Tanny JC, et al. Histone chaperone Asf1 is required for histone H3 lysine 56 acetylation, a modification associated with S phase in mitosis and meiosis. Proc Natl Acad Sci USA. 2006; 103:6988–93. [PubMed: 16627621]

Reimand J, Bader G. Systematic analysis of somatic mutations in phosphorylation signaling predicts novel cancer drivers. Mol Syst Biol. 2013; 9:1–17.

Renella R, Roberts NA, Brown JM, et al. Codanin-1 mutations in congenital dyserythropoietic anemia type 1 affect HP1{alpha} localization in erythroblasts. Blood. 2011; 117:6928–38. [PubMed: 21364188]

Rishton G. Reactive compounds and in vitro false positives in HTS. Drug Discov Today. 1997; 2:382–4.

Roth S, Denu J. Histone acetyltransferases. Annu Rev Biochem. 2001; 70:81–120. [PubMed: 11395403]

Rufiange A, Jacques P, Bhat W, et al. Genome-wide replication-independent histone H3 exchange occurs predominantly at promoters and implicates H3 K56 acetylation and Asf1. Mol Cell. 2007; 27:393–405. [PubMed: 17679090]

Sagar V, Zheng W, Thompson PR, Cole PA. Bisubstrate analogue structure-activity relationships for p300 histone acetyltransferase inhibitors. Bioorg Med Chem. 2004; 12:3383–90. [PubMed: 15158807]

Sakaguchi K, Herrera JE, Saito S, et al. DNA damage activates p53 through a phosphorylation-acetylation cascade. Genes Dev. 1998; 12:2831–41. [PubMed: 9744860]

Salman N, Törün SH, Budan B, Somer A. Invasive aspergillosis in hematopoietic stem cell and solid organ transplantation. Exp Rev Ant-Infect Ther. 2011; 9:307–15.

Santos-Rosa H, Valls E, Kouzarides T, Martínez-Balbás M. Mechanisms of P/CAF auto-acetylation. Nucleic Acids Res. 2003; 31:4285–92. [PubMed: 12888487]

Schramm V. Enzymatic transition states, transition-state analogs, dynamics, thermodynamics, and lifetimes. Annu Rev Biochem. 2011; 80:703–32. [PubMed: 21675920]

Schwer B, Schumacher B, Lombard DB, et al. Neural sirtuin 6 (Sirt6) ablation attenuates somatic growth and causes obesity. Proc Natl Acad Sci USA. 2010; 107:21790–4. [PubMed: 21098266]

Selth L, Svejstrup JQ. Vps75, a new yeast member of the NAP histone chaperone family. J Biol Chem. 2007; 282:12358–62. [PubMed: 17344218]

Singh N, Pursell KJ. Combination therapeutic approaches for the management of invasive aspergillosis in organ transplant recipients. Mycoses. 2008; 51:99–108. [PubMed: 18254745]

Smerdon M. DNA repair and the role of chromatin structure. Curr Opin Cell Biol. 1991; 3:422–8. [PubMed: 1892653]

Smith DJ, Whitehouse I. Intrinsic coupling of lagging-strand synthesis to chromatin assembly. Nature. 2012; 483:434–8. [PubMed: 22419157]

Smith S, Stillman B. Stepwise assembly of chromatin during DNA replication *in vitro*. EMBO J. 1991; 10:971–80. [PubMed: 1849080]

Soekarman D, Lindern Mv, Daenen S, et al. The translocation (6;9) (p23;q34) shows consistent rearrangement of two genes and defines a myeloproliferative disorder with specific clinical features. Blood. 1992; 79:2990–7. [PubMed: 1586743]

Stavropoulos P, Nagy V, Blobel G, Hoelz A. Molecular basis for the autoregulation of the protein acetyl transferase Rtt109. Proc Natl Acad Sci USA. 2008; 105:12236–41. [PubMed: 18719104]

Steinbach WJ. Invasive aspergillosis in pediatric patients. Curr Med Res Opin. 2010; 26:1779–87. [PubMed: 20513209]

Stillman B. Chromatin assembly during SV40 DNA replication *in vitro*. Cell. 1986; 45:555–65. [PubMed: 3011272]

Su D, Hu Q, Li Q, et al. Structural basis for recognition of H3K56-acetylated histone H3-H4 by the chaperone Rtt106. Nature. 2012; 483:104–07. [PubMed: 22307274]

Su D, Hu Q, Zhou H, et al. Structure and histone binding properties of the Vps75-Rtt109 chaperone-lysine acetyltransferase complex. J Biol Chem. 2011; 286:15625–9. [PubMed: 21454705]

Tan Y, Xue Y, Song C, Grunstein M. Acetylated histone H3K56 interacts with Oct4 to promote mouse embryonic stem cell pluripotency. Proc Natl Acad Sci USA. 2013; 110:11493–8. [PubMed: 23798425]

Tanaka A, Tanizawa H, Sriswasdi S, et al. Epigenetic regulation of condensin-mediated genome organization during the cell cycle and upon DNA damage through histone H3 lysine 56 acetylation. Mol Cell. 2012

Tang Y, Holbert M, Wurtele H, et al. Fungal Rtt109 histone acetyltransferase is an unexpected structural homolog of metazoan p300/CBP. Nat Struct Mol Biol. 2008a; 15:738–45. [PubMed: 18568037]

Tang Y, Holbert MA, Delgoshaie N, et al. Structure of the Rtt109-AcCoA/Vps75 complex and implications for chaperone-mediated histone acetylation. Structure. 2011; 19:221–31. [PubMed: 21256037]

Tang Y, Meeth K, Jiang E, et al. Structure of Vps75 and implications for histone chaperone function. Proc Natl Acad Sci USA. 2008b; 105:12206–11. [PubMed: 18723682]

Tanner K, Langer M, Kim Y, Denu J. Kinetic mechanism of the histone acetyltransferase GCN5 from yeast. J Biol Chem. 2000a; 275:22048–55. [PubMed: 10811654]

Tanner K, Trievel R, Kuo M, et al. Catalytic mechanism and function of invariant glutamic acid 173 from the histone acetyltransferase GCN5 transcriptional coactivator. J Biol Chem. 1999; 274:18157–60. [PubMed: 10373413]

Tanner KG, Langer MR, Denu JM. Kinetic mechanism of human histone acetyltransferase P/CAF. Biochemistry. 2000b; 39:15652. [PubMed: 11112555]

Thaminy S, Newcomb B, Kim J, et al. Hst3 is regulated by Mec1-dependent proteolysis and controls the S phase checkpoint and sister chromatid cohesion by deacetylating histone H3 at lysine 56. J Biol Chem. 2007; 282:37805–14. [PubMed: 17977840]

Thomas CF Jr, Limper AH. Current insights into the biology and pathogenesis of *Pneumocystis* pneumonia. Nat Rev Microbiol. 2007; 5:298–308. [PubMed: 17363968]

Thompson PR, Wang D, Wang L, et al. Regulation of the p300 HAT domain via a novel activation loop. Nat Struct Mol Biol. 2004; 11:308–15. [PubMed: 15004546]

Thornton CR. Detection of invasive aspergillosis. Adv App Microbiol. 2010; 70:187–216.

Tjeertes J, MIller K, Jackson S. Screen for DNA-damage-responsive histone modifications identifies H3K9Ac and H3K56Ac in human cells. EMBO J. 2009; 28:1878–89. [PubMed: 19407812]

Toiber D, Erdel F, Bouazoune K, et al. SIRT6 recruits SNF2H to DNA break sites, preventing genomic instability through chromatin remodeling. Mol Cell. 2013; 51:454–68. [PubMed: 23911928]

Tomašiť T, Mašiť LP. Rhodanine as a scaffold in drug discovery: a critical review of its biological activities and mechanisms of target modulation. Exp Opin Drug Discov. 2012

Trievel RC, Rojas JR, Sterner DE, et al. Crystal structure and mechanism of histone acetylation of the yeast GCN5 transcriptional coactivator. Proc Natl Acad Sci USA. 1999; 96:8931–6. [PubMed: 10430873]

Tsubota T, Berndsen CE, Erkmann JA, et al. Histone H3-K56 acetylation is catalyzed by histone chaperone-dependent complexes. Mol Cell. 2007; 25:703–12. [PubMed: 17320445]

Tsukuda T, Fleming AB, Nickoloff JA, Osley MA. Chromatin remodelling at a DNA double-strand break site in *Saccharomyces cerevisiae*. Nature. 2005; 438:379–83. [PubMed: 16292314]

Tyler JK, Adams CR, Chen SR, et al. The RCAF complex mediates chromatin assembly during DNA replication and repair. Nature. 1999; 402:555–60. [PubMed: 10591219]

Valor L, Viosca J, Lopez-Atalaya J, Barco A. Lysine acetyltransferases CBP and p300 as therapeutic targets in cognitive and neurodegenerative disorders. Curr Pharm Des. 2013; 19:5051–64. [PubMed: 23448461]

Van Beekum O, Kalkhoven E. Aberrant forms of histone acetyltransferases in human disease. Subcell Biochem. 2007; 41:233–62. [PubMed: 17484131]

Verreault A, Kaufman PD, Kobayashi R, Stillman B. Nucleosome assembly by a complex of CAF-1 and acetylated histones H3/H4. Cell. 1996; 87:95–104. [PubMed: 8858152]

Verreault A, Kaufman PD, Kobayashi R, Stillman B. Nucleosomal DNA regulates the core-histone-binding subunit of the human Hat1 acetyltransferase. Curr Biol. 1998; 8:96–108. [PubMed: 9427644]

Verreault A. *De novo* nucleosome assembly: new pieces in an old puzzle. Genes Dev. 2000; 14:1430–8. [PubMed: 10859162]

Vetting MW, Carvalho LPSd, Yu M, et al. Structure and functions of the GNAT superfamily of acetyltransferases. Arch Biochem Biophys. 2005; 433:212–26. [PubMed: 15581578]

Volkamer A, Kuhn D, Grombacher T, et al. Combining global and local measures for structure-based druggability predictions. J Chem Inf Model. 2012; 52:360–72. [PubMed: 22148551]

Volle C, Dalal Y. Histone variants: the tricksters of the chromatin world. Curr Opin Genet Dev. 2014; 25:8–14. [PubMed: 24463272]

Wagner T, Jung M. New lysine methyltransferase drug targets in cancer. Nat Biotechnol. 2012; 30:622–3. [PubMed: 22781684]

Wang J, Chen J. SIRT1 regulates autoacetylation and histone acetyltransferase activity of TIP60. J Biol Chem. 2010; 285:11458–64. [PubMed: 20100829]

Wang L, Tang Y, Cole PA, Marmorstein R. Structure and chemistry of the p300/CBP and Rtt109 histone acetyltransferases: implications for histone acetyltransferase evolution and function. Curr Opin Struc Biol. 2008; 18:741–7.

Wells JA, McClendon CL. Reaching for high-hanging fruit in drug discovery at protein-protein interfaces. Nature. 2007; 450:1001–9. [PubMed: 18075579]

Williams S, Truong D, Tyler J. Acetylation in the globular core of histone H3 on lysine-56 promotes chromatin disassembly during transcriptional activation. Prco Natl Acad Sci USA. 2008; 105:9000–5.

Wise-Draper TM, Mintz-Cole RA, Morris TA, et al. Overexpression of the cellular DEK protein promotes epithelial transformation *in vitro* and *in vivo*. Cancer Res. 2009; 69:1792–9. [PubMed: 19223548]

Wurtele H, Tsao S, Lépine G, et al. Modulation of histone H3 lysine 56 acetylation as an antifungal therapeutic strategy. Nat Med. 2010; 16:774–80. [PubMed: 20601951]

Xie W, Song C, Young NL, et al. Histone H3 lysine 56 acetylation is linked to the core transcriptional network in human embryonic stem cells. Mol Cell. 2009; 33:417–27. [PubMed: 19250903]

Xu F, Zhang Q, Zhang K, et al. Sir2 deacetylates histone H3 lysine 56 to regulate telomeric heterochromatin structure in yeast. Mol Cell. 2007; 27:890–900. [PubMed: 17889663]

Xu M, Long C, Chen X, et al. Partitioning of histone H3–H4 tetramers during DNA replication-dependent chromatin assembly. Science. 2010; 328:94–8. [PubMed: 20360108]

Xu Y, Ayrapetov M, Xu C, et al. Histone H2A.Z controls a critical chromatin remodeling step required for DNA double-strand break repair. Mol Cell. 2012; 48:723–33. [PubMed: 23122415]

Yan Y, Harper S, Speicher D, Marmorstein R. The catalytic mechanism of the ESA1 histone acetyltransferase involves a self-acetylated intermediate. Nat Struct Biol. 2002; 9:862–9. [PubMed: 12368900]

Yang B, Miller A, Kirchmaier AL. HST3/HST4-dependent deacetylation of lysine 56 of histone H3 in silent chromatin. Mol Biol Cell. 2008; 19:4993–5005. [PubMed: 18799617]

Yang B, Zwaans BMM, Eckersdorff M, Lombard DB. The sirtuin SIRT6 deacetylates H3 K56Ac *in vivo* to promote genomic stability. Cell Cycle. 2009; 8:2662–3. [PubMed: 19597350]

Yang X, Song JH, Cheng Y, et al. Long non-coding RNA HNF1A-AS1 regulates proliferation and migration in oesophageal adenocarcinoma cells. Gut. 2013

Yuan J, Pu M, Zhang Z, Lou Z. Histone H3-K56 acetylation is important for genomic stability in mammals. Cell Cycle. 2009; 8:1747–53. [PubMed: 19411844]

Zhang X, Ouyang S, Kong X, et al. Catalytic mechanism of histone acetyltransferase p300: from the proton transfer to acetylation reaction. J Phys Chem B. 2014a; 118:2009–19. [PubMed: 24521098]

Zhang Z, Shibahara K, Stillman B. PCNA connects DNA replication to epigenetic inheritance in yeast. Nature. 2000; 408:221–5. [PubMed: 11089978]

Zhang Z, Yang Q, Sun G, et al. Histone H3K56 acetylation is required for quelling-induced small RNA production through its role in homologous recombination. J Biol Chem. 2014b; 289:9365–71. [PubMed: 24554705]

Zhou R, Yang F, Chen D-F, et al. Acetylation of chromatin-associated histone H3 lysine 56 inhibits the development of encysted Artemia embryos. PLOS ONE. 2013; 8:e68374. [PubMed: 23840851]

Zunder RM, Antczak AJ, Berger JM, Rine J. Two surfaces on the histone chaperone Rtt106 mediate histone binding, replication, and silencing. P Natl Acad Sci USA. 2012; 109:E144–53.

Box 1

Other recent findings related to Rtt109 and H3K56

Several recent studies are linking Rtt109 and H3K56 to other important cellular processes. For example, in *Neurospora*, H3K56ac is required homologous recombination and interestingly, this modification has been implicated in quelling-induced small RNA production (Zhang *et al.*, 2014). Other reports have expanded the links between Rtt109 and/or H3K56ac to diverse biological processes such as *Artemia* development (Zhou *et al.*, 2013), rRNA hyper-amplification (Ide *et al.*, 2013), retrotransposon regulation (Tanaka *et al.*, 2012), the Ras-PI3K pathway (Liu *et al.*, 2012), angiogenesis (Dutta *et al.*, 2010), circadian rhythms (Malapeira *et al.*, 2012) and mTOR signaling and rRNA biogenesis (Chen *et al.*, 2012). Some of these biological functions may be inherently linked to the role of H3K56ac in nucleosome assembly. Future studies are needed to address these possibilities.

Box 2

Comparison of Rtt109 with p300

An intriguing component of Rtt109 HATs lies in its comparison to other HATs. There are three major families of HATs: MYST, GNAT and the p300/CBP family, of which Rtt109 is a member. For a more thorough comparison of HATs, we point the reader to several well-written reviews (Berndsen & Denu, 2008; Dyda *et al.*, 2000; Roth & Denu, 2001). In fungi, H3K56ac is catalyzed solely by Rtt109, while in mammalian cells this process may be carried out by p300 and/or Gcn5. Interestingly, organisms with p300 do not contain Rtt109 and vice-versa. It appears Rtt109 is an evolutionary precursor to p300. While both enzymes catalyze the formation of H3K56ac, there are some significant differences between Rtt109 and p300 (Table 3). Rtt109 does not have significant primary structure homology to p300, but its core ($\beta2$–$\beta4$ and α_2) does share a general shape similarity with p300 (Tang *et al.*, 2008). Both enzymes have a similar secondary structure arrangement featuring an ab core and helical bundles at opposing ends. Another common structural feature is the L1 and L2 loops that help bury the acetyl-CoA substrate. The electrostatic surface potentials of Rtt109 and p300 are also different in that the histone-binding region of p300 is predominately electronegative, while the same region is relatively apolar with an adjacent basic patch in Rtt109, making it more alike to Gcn5 (Tang *et al.*, 2008). Rtt109 can bind to and co-crystallize with Vps75, whereas p300 does not feature such a chaperone. Like Rtt109, p300 has a hydrophobic active site, is autoacetylated, and requires an ionizible group at pH 8.4 (Karanam *et al.*, 2006; Liu *et al.*, 2008). Further understanding of the structural divergences between these two classes of enzymes – as well as other HATs – should enhance the overall understanding of histone-acetylation pathways, and as will be discussed below, has the potential to be exploited for anti-fungal purposes.

Box 3

Executive summary | Rtt109 HATs and chromatin assembly

- Nucleosomes must be disassembled to make way for replication machinery and then reassembled afterwards during DNA replication (replication-coupled nucleosome assembly, RCNA). Many of the same principles of RCNA apply to replication-independent (RINA) processes like histone exchange and transcription, as well as DNA damage and repair.

- Proper RCNA is essential for maintaining genome stability, and dysfunctions in RCNA have been associated with aging as well as various human pathologies like cancers.

- In yeast, the HAT Rtt109 is best known for its catalysis of H3K56ac, which is a PTM found abundantly on newly synthesized histones and is critical for proper RCNA.

- H3K56ac enhances the Rtt101^{Mms1}-catalyzed ubiquitination of these histones, which is thought to facilitate the formation of $(H3–H4)_2$ tetramers and ultimately nascent nucleosome formation.

- Rtt109 HATs represent a unique class of HATs that are conserved in fungi and are essential for the production of H3K56ac in fungi.

- H3K56ac levels are less abundant in mammalian cells, and the roles of this modification in mammalian RCNA and the DNA damage response are still being characterized.

- Rtt109 substrate specificity and enzymatic activity are subject to complex regulation by the histone chaperones Vps75 and Asf1 both *in vitro* and *in vivo*.

- Inhibitors of Rtt109-catalyzed histone acetylation are hypothesized as minimally-toxic anti-fungal agents, but this therapeutic hypothesis is still unresolved.

- Future work regarding Rtt109 HATs may focus on the structural determinants of substrate specificity and its roles in other fungi like opportunistic pathogens.

- More work is needed in mammalian cells to clarify the role(s), if any, of H3K56ac in RCNA and RINA, especially with respect to DNA damage and repair.
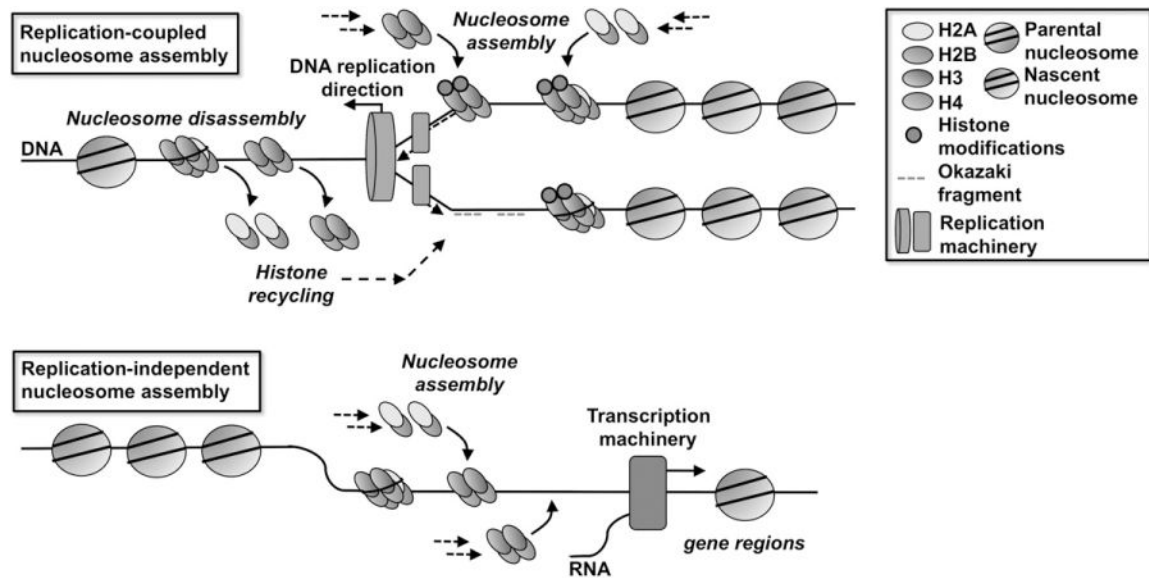
Figure 1.

General schematic of RCNA and RINA. (Top) Replication-coupled nucleosome assembly (RCNA). Nucleosomes must be disassembled to make way for DNA replication machinery. Nucleosomes are then (re)assembled in close concert with DNA replication on the leading and lagging strands. (Bottom) Replication-independent nucleosome assembly (RINA). Many of the same core principles of nucleosome disassembly, DNA access and nucleosome assembly are likely applicable to replication-independent processes like gene transcription.
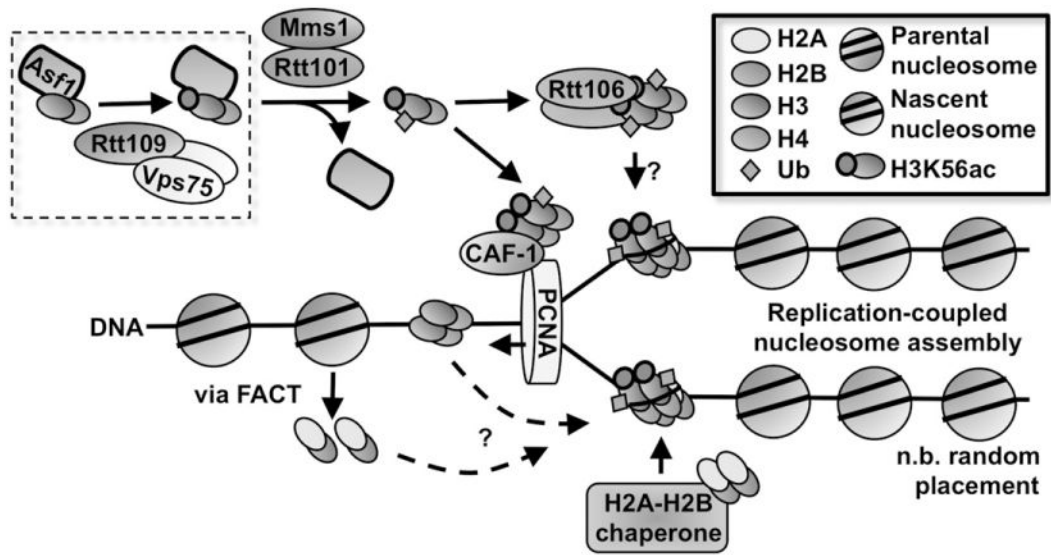
Figure 2.
Fungal RCNA pathways. Shown is a schematic of RCNA in yeast. Note that the placement of parental and nascent nucleosomes is random with respect to strands.
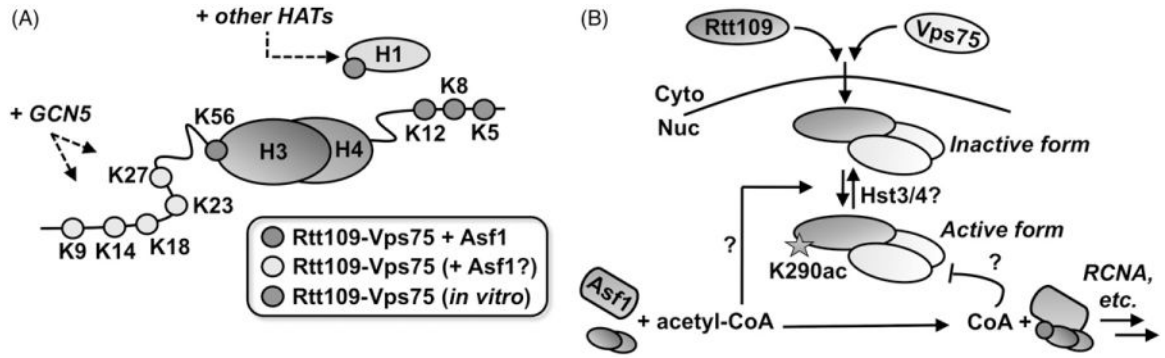
Figure 3.
Molecular biology of Rtt109 HATs. (A) Simplified substrate profile for Rtt109. (B) Model of Rtt109 regulation *in vivo*. Vps75 is linked to trafficking Rtt109 to the nucleus. We caution that the exact cellular location of Rtt109 autoacetylation and its removal has not been confirmed. The cellular levels of acetyl-CoA has been hypothesized as one regulator of Rtt109 autoacetylation, and thus Rtt109 activity, *in vivo*. Inhibition by product accumulation (CoA, acetylated histones) may also regulate Rtt109 activity.
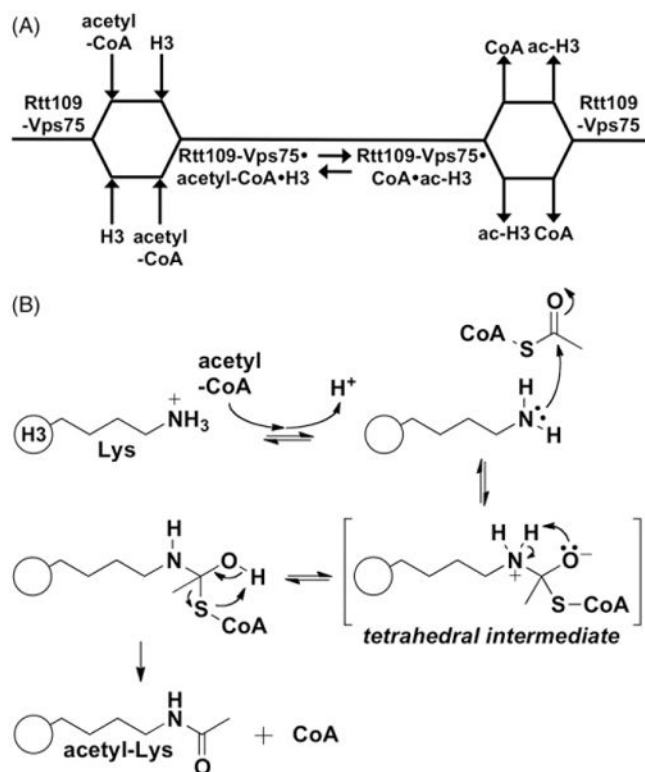
Figure 4.
Current model for enzymatic mechanism of yeast Rtt109. (A) Cleland diagram for Rtt109-Vps75-catalyzed histone acetylation depicting the proposed order of substrate binding and product release. H3 is depicted as the substrate, but it can most likely include other substrates. Protein substrate and acetyl-CoA appear to bind and depart Rtt109-Vps75 in a non-obligate order. (B) Proposed catalytic model of Rtt109-catalyzed histone acetylation. The lysine substrate (pK_a ~10) becomes deprotonated during the course of binding. In the absence of a general base catalyst, the lysine side chain is likely positioned at an optimal distance and orientation with respect to bound acetyl-CoA to undergo a nucleophilic attack at the acetyl carbonyl carbon. A tetrahedral intermediate would then be formed, followed by product release. The protein and/or solvent component(s) that likely stabilize the transition states are unknown.
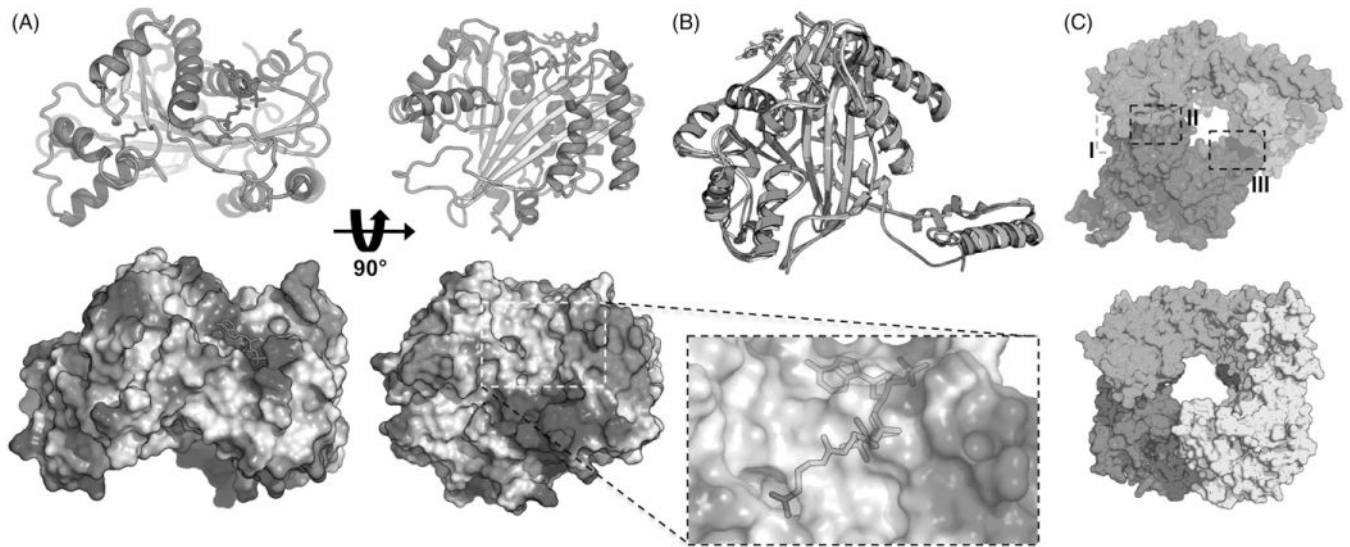
Figure 5.
Structural overview of yeast Rtt109. (A) Secondary structure features (top) and electrostatic potentials (bottom) of yeast Rtt109 (PDB ID 3QMO). The inset highlights the acetyl-CoA binding mode. α-helices, red; β-sheets, yellow; loops, green; acetyl-CoA, magenta sticks; K2090ac, blue sticks. (B) Presence of Vps75 does not significantly alter secondary structure of Rtt109 in crystal structures. PDB IDs: green, 3Q33; yellow, 3QMO; magenta, 3Q66. (C) Comparison of yeast Rtt109-Vps75 crystal structures. (top) Su *et al.* structure, PDB ID 3Q66. Green and cyan, Vps75; magenta, Rtt109. I–III denotes the locations of the three protein-protein interfaces. (bottom) Tang *et al.* structure, PDB ID 3Q33. Yellow and green, Vps75; magenta and blue, Rtt109. All molecular representations were constructed using PyMOL.
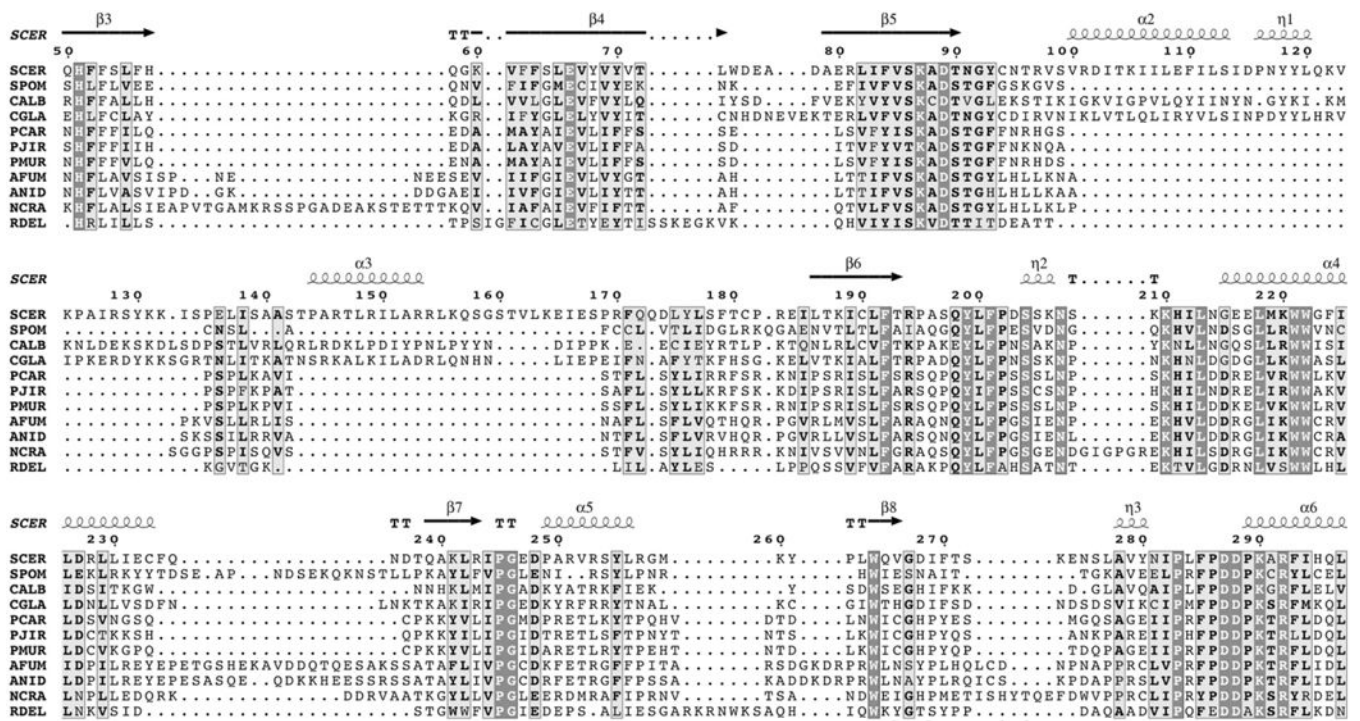
Figure 6.

Multiple-sequence alignment of Rttl09 from several fungal species. Sequence numbering and secondary structure annotation is based on *S. cerevisiae* Rttl09. Sequences were accessed from GenBank (NCBI). Alignment was performed with the T-Coffee algorithm (Di Tommaso *et al.*, 2011; Notredame *et al.*, 2000) and rendered with ENDscript (http://endscript.ibcp.fr) (Gouet *et al.*, 2003). Secondary structure annotations were assigned based on the structure of PDB ID 3Q66. Organism abbreviations: SCER, *S. cerevisiae;* SPOM, *S. pombe;* CALB, *C. albicans;* CGLA, *C. glabrata;* PCAR, *P. carinii;* PJIR, *P. jirovecii;* PMUR, *P. murina;* AFUM, *A. fumigatus;* ANID, *Aspergillus nidulans;* NCRA, *Neurospora crassa;* RDEL, *Rhizopus delemar*.
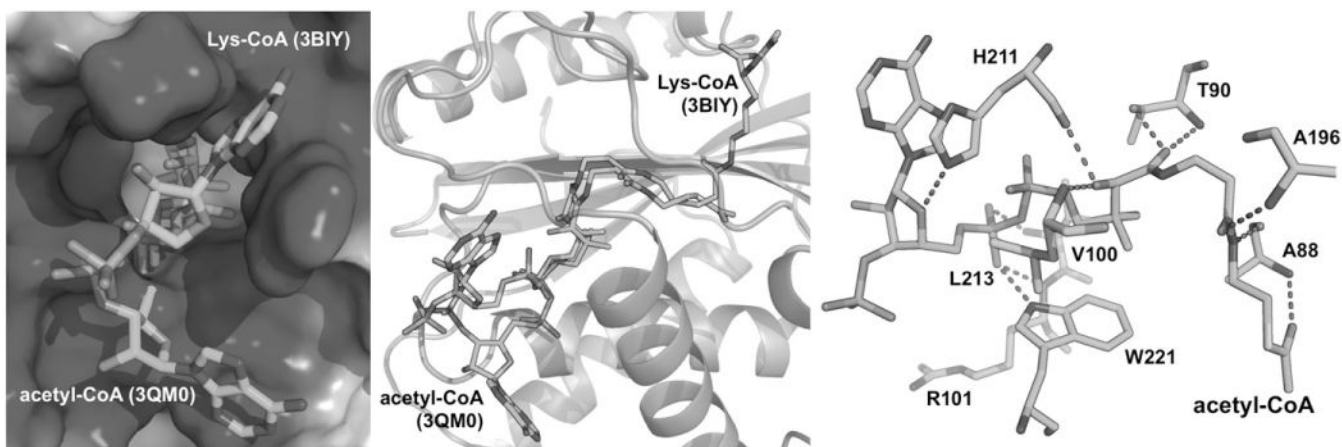
Figure 7.
Binding mode analysis of acetyl-CoA to yeast Rtt109. (Left) Comparison of the site and modes of binding of the 3′-phosphoadenosine-5′-phosphate moiety in the related HATs Rtt109 and p300. Shown are the bisubstrate inhibitor Lys-CoA (PDB ID 3BIY, light blue sticks) and acetyl-CoA (PDB ID 3QM0, green sticks). The electrostatic surface (blue, positive potential) is p300 (3BIY). (Middle) Another view of the bound structures of acetyl-CoA and Lys-CoA. Tertiary structure shown is p300 (3BIY, light green). (Right) Polar contacts between acetyl-CoA (red dashed lines) and Rtt109 (acetyl-CoA, green sticks; contact residues in blue sticks; PDB ID 2ZFN). All biomolecular representations were constructed using PyMOL.
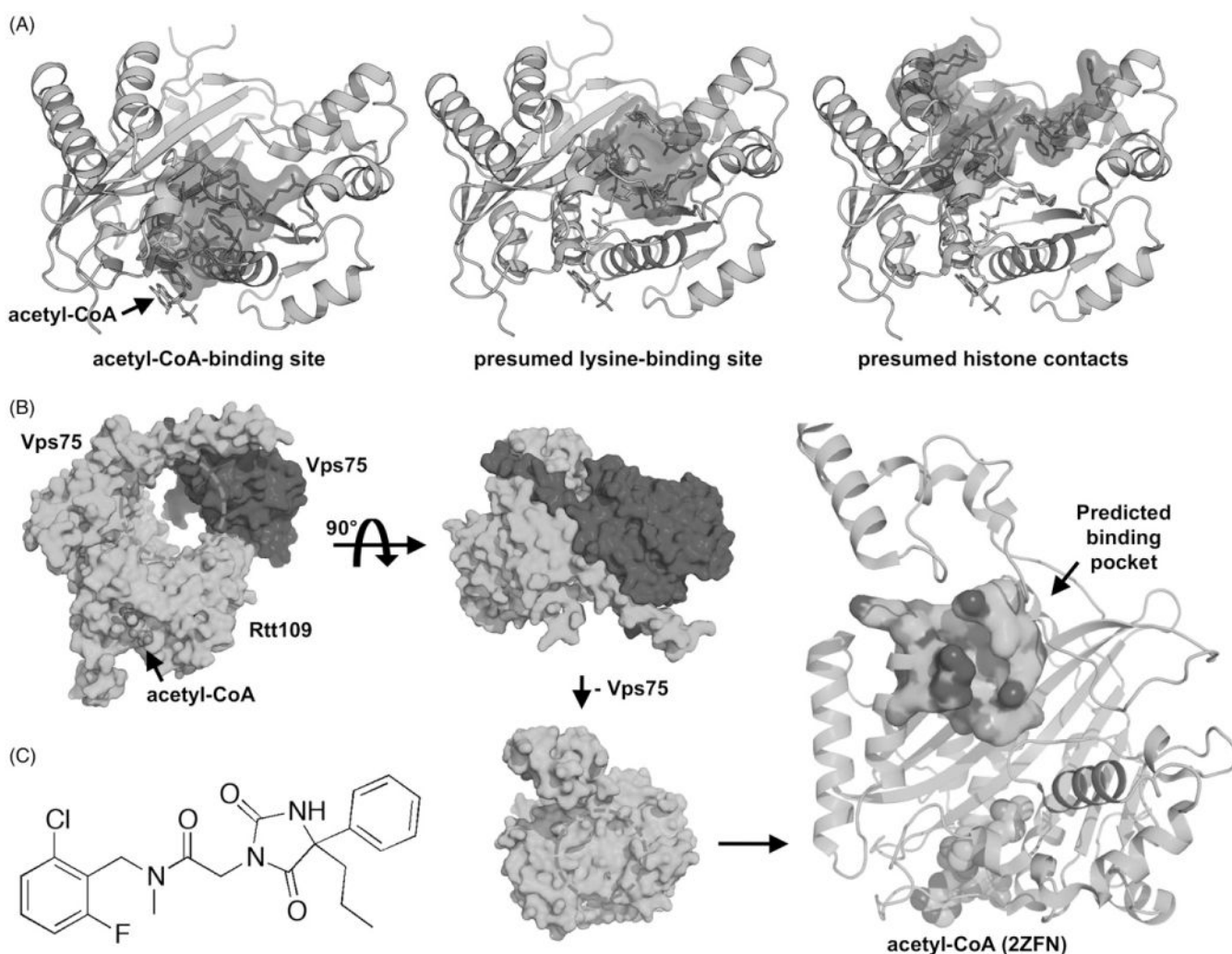
Figure 8.
Medicinal chemistry considerations of Rtt109 HATs. (A) Crystallographic binding sites of
acetyl-CoA (left) and the presumed binding and contact sites (purple surfaces) of histone-
lysine (middle) and histone substrates (right) using PDB ID 2ZFN (cartoon, light blue). (B)
Structure of Rtt109-Vps75 complex (left, PDB ID 3Q66). Red circle demarks the putative
H3-H4-binding pocket. Tertiary structure of Rtt109 (far right) with druggable binding site
predicted by DoGSiteScorer. Volkamer A, Kuhn D, Grombacher T, *et al.* (2012).
Combining global and local measures for structure-based druggability predictions. J Chem
Inf Model 52:360–72. Shown as solid surface colored by atom type: N, dark blue; O, red; C,
light blue. (C) Chemical structure of a reported inhibitor of Rtt109 HAT activity *in vitro*,
identified by a CPM-based HTS (Lopes da Rosa *et al.*, 2013). All biomolecular
representations were constructed using PyMOL.

Table 1

Summary of select Rtt109-related protein–protein interactions.

Protein 1	Protein 2	$^{app}K_d$ (nM)	Technical notes	Reference
Rtt109	Vps75	10 ± 2	FP; acetyl-CoA present	(Albaugh *et al.*, 2010)
Rtt109	Vps75	13 ± 3	Steady-state kinetic method; acetyl-CoA and H3 present	(Kolonko *et al.*, 2010)
Rtt109	Vps75	10; 2700	K_{d1} and K_{d2}; modeled as Vps75 and Rtt109 dimers	(Tang *et al.*, 2008)
Rtt109	H3–H4	150 ± 10	FT	(Park *et al.*, 2008)
Rtt109	H3	17 ± 8	FP	(Berndsen *et al.*, 2008)
Vps75	H3–H4	25 ± 3	FT	(Park et al., 2008)
Vps75$_{1-223}$	H3–H4	13 ± 1	FT; truncated C-terminal acidic domain	(Park *et al.*, 2008)
Vps75	H3	120 ± 10	FT	(Park *et al.*, 2008)
Vps75	H3	56 ± 8	FP	(Berndsen *et al.*, 2008)
Vps75	H2A–H2B	4.5 ± 0.3	FT	(Park *et al.*, 2008)
Asf1	H3–H4	10 ± 1	FT	(Park *et al.*, 2008)
H3–H4	Asf1	2.5 ± 0.7	FQ	(Rishton, 1997)

FP, fluorescence polarization; FT, fluorescence titration method; FQ, fluorescence quenching method.

Table 2

Summary of Rtt109 kinetic parameters.

Enzyme form	Protein substrate	Protein K_M (μM)	k_{cat} (s^{-1})	acetyl-CoA K_M (μM)	Technical notes	Reference
Rtt109	H3$_{1-20}$	83 ± 29	0.0017 ± 0.0001	0.3 ± 0.1	acetyl-CoA + protein substrate pairing unclear	(Berndsen et al., 2008)
	H3	8.1 ± 0.1	0.0033 ± 0.0003	0.3 ± 0.1	acetyl-CoA + protein substrate pairing unclear	(Berndsen et al., 2008)
	H3-H4	2.9 ± 0.6	0.0044 ± 0.0009	0.3 ± 0.1	acetyl-CoA + protein substrate pairing unclear	(Berndsen et al., 2008)
Rtt109-Vps75	H3$_{1-20}$	75 ± 15	0.13 ± 0.04	1.0 ± 0.2	acetyl-CoA + protein substrate pairing unclear	(Berndsen et al., 2008)
	H3$_{1-20}$	112 ± 11	0.11 ± 0.01	0.3 ± 0.1	–	(Albaugh et al., 2010)
	H3	5.8 ± 0.8	0.21 ± 0.04	1.0 ± 0.2	acetyl-CoA + protein substrate pairing unclear	(Berndsen et al., 2008)
	H3	–	0.19 ± 0.01	1.0 ± 0.2	–	(Tsubota et al., 2007)
	H3	3.9 ± 2	0.069 ± 0.001	NR	0.2 eq. Vps75	(Kolonko et al., 2010)
	H3	6.5 ± 2	0.62 ± 0.02	NR	8 eq. Vps75	(Kolonko et al., 2010)
	H3	7 ± 1	0.63 ± 0.02	NR	–	(Albaugh et al., 2010)
	H3	8.5 ± 0.7	0.375 ± 0.027	8.0 ± 2.0	–	(Tang et al., 2008)
	H3-H4	1.4 ± 0.4	0.11 ± 0.05	1.0 ± 0.2	acetyl-CoA-protein substrate pairing unclear	(Berndsen et al., 2008)
	H3-H4	2.12 ± 0.63	0.068 ± 0.004	NR	6 eq. Vps75	(Tsubota et al., 2007)
	H3-H4	0.84 ± 0.28	0.34 ± 0.04	NR	–	(Tang et al., 2011)
	H3-H4	0.5 ± 0.3	0.041 ± 0.004	NR	0.2 eq. Vps75	(Kolonko et al., 2010)
	H3-H4	0.1 ± 0.1	0.13 ± 0.007	NR	8 eq. Vps75	(Kolonko et al., 2010)
	yH3-H4	1.4 ± 0.4	0.41 ± 0.05	NR	Tetramer	(Kolonko et al., 2010)
	yH3(A110E)-H4	2.4 ± 0.7	0.39 ± 0.06	NR	Dimeric form	(Kolonko et al., 2010)
Rtt109-Asf1	H3-H4	1.19 ± 0.34	0.021 ± 0.002	Low micromolar	–	(Tsubota et al., 2007)
	H3-H4	1.84 ± 0.81	0.015 ± 0.002	NR	–	(Tang et al., 2011)

In general, the K_M for protein substrate decreases from the peptide to the full-length H3 to the H3–H4 tetramer form, with or without Vps75 present. In general, the addition of Vps75 leads to an increase in k_{cat} regardless of protein substrate, while the K_M for acetyl-CoA is relatively constant. NR, no report.

Table 3

Generalized comparisons of Rtt109 and p300.

	Rtt109	p300	Key references
Catalyzes H3K56ac	Y	Y	(Das *et al.*, 2009; Han *et al.*, 2007)
Found in mammalian cells	N	Y	–
Found in fungi	Y	N	–
Proposed enzymatic mechanism	Sequential (random)	Theorell-Chance	(Albaugh *et al.*, 2010; Liu *et al.*, 2008)
Substrate binding order	Non-obligate	Obligate	(Albaugh *et al.*, 2010; Liu *et al.*, 2008)
Multiple histone residue substrates	Y	Y	(McManus & Hendzel, 2003)
Known non-histone substrates	N	Y	(Barlev *et al.*, 2001; Sakaguchi *et al.*, 1998)
Inhibition by Lys-CoA	N	Y	(Cebrat *et al.*, 2003; Sagar *et al.*, 2004; Tang *et al.*, 2008)
Acetylates nucleosomes	N	Y	(Han *et al.*, 2007b; Ogryzko *et al.*, 1996)
Contains bromodomain	N	Y	(Manning *et al.*, 2001)
Autoacetylation	Y	Y	(Albaugh *et al.*, 2010, 2011; Arif *et al.*, 2007; Karanam *et al.*, 2006)
Intra/intermolecular autoacetylation	Intra-	Inter-	(Albaugh *et al.*, 2010, 2011; Karanam *et al.*, 2006)
Other post-translational modifications	N	Y	(Girdwood *et al.*, 2003)
Size (aa)	436	2414	(Chan & Thangue, 2001; Johnston *et al.*, 1997)