DNA Repair 9 (2010) 414–420

Contents lists available at ScienceDirect

DNA Repair

journal homepage: www.elsevier.com/locate/dnarepair

The Rtt109 histone acetyltransferase facilitates error-free replication to prevent CAG/CTG repeat contractions

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ARTICLE INFO

Article history:
Received 29 October 2009
Received in revised form
23 December 2009
Accepted 29 December 2009
Available online 18 January 2010

Keywords:
Trinucleotide repeat
Rtt109
H3K56 acetylation
Replication
Nucleosome assembly
Genome instability

ABSTRACT

Lysine 56 is acetylated on newly synthesized histone H3 in yeast, *Drosophila* and mammalian cells. All of the proteins involved in histone H3 lysine 56 (H3K56) acetylation are important for maintaining genome integrity. These include Rtt109, a histone acetyltransferase, responsible for acetylating H3K56, Asf1, a histone H3/H4 chaperone, and Hst3 and Hst4, histone deacetylases which remove the acetyl group from H3K56. Here we demonstrate a new role for Rtt109 and H3K56 acetylation in maintaining repetitive DNA sequences in *Saccharomyces cerevisiae*. We found that cells lacking *RTT109* had a high level of CAG/CTG repeat contractions and a twofold increase in breakage at CAG/CTG repeats. In addition, repeat contractions were significantly increased in cells lacking *ASF1* and in an *hst3Δhst4Δ* double mutant. Because the Rtt107/Rtt101 complex was previously shown to be recruited to stalled replication forks in an Rtt109-dependent manner, we tested whether this complex was involved. However, contractions in *rtt109Δ* cells were not due to an inability to recruit the Rtt107/Rtt101 complex to repeats, as absence of these proteins had no effect on repeat stability. On the other hand, Dnl4 and Rad51-dependent pathways did play a role in creating some of the repeat contractions in *rtt109Δ* cells. Our results show that H3K56 acetylation by Rtt109 is important for stabilizing DNA repeats, likely by facilitating proper nucleosome assembly at the replication fork to prevent DNA structure formation and subsequent slippage events or fork breakage.

1. Introduction

Microsatellites are DNA sequences of 1–6 nucleotides that are repeated multiple times and are abundant in most genomes [1]. About 3% of the human genome is composed of microsatellites, which are present in both coding and non-coding regions [2]. Nearly 30 human neurological disorders are caused by expansions of these simple DNA repeats, many of which are trinucleotide repeat (TNR) diseases, including Huntington's disease, fragile X syndrome and myotonic dystrophy type 1 [3]. Previous studies have established that the occurrence of these diseases is repeat length-dependent, and longer repeats are associated with increased disease severity and earlier age of onset [3].

In order to understand the molecular mechanism of triplet repeat instability, studies have been done in *Escherichia coli*, yeast, mouse, and mammalian cells containing repetitive sequences [1,3]. Due to the unusual secondary structures that TNRs can form, expansions and contractions occur during processes in which single-stranded DNA is produced, such as DNA replication, repair, and transcription [3]. CAG/CTG repeats are natural replication pause sites on plasmids in yeast or bacterial cells and are sites of replication fork reversal on a yeast chromosome and *in vitro*, indicating that they pose a challenge for replication fork progression [4–7]. Indeed, disruption of replication through CAG/CTG repeats can cause repeat instability [8,9]. TNR sequences can also interfere with DNA repair: base excision repair, nucleotide excision repair and mismatch repair can all contribute to CAG/CTG repeat expansions [10]. Studies in yeast and *E. coli* indicate that mistakes during double-strand break (DSB) repair are another mechanism of generating repeat instability [11]. For example, both contractions and expansions were observed when CAG/CTG repeats were used as a template to repair an induced DSB [12]. Also, a Rad52-dependent process has been shown to be responsible for repeat expansions and contractions that occur in yeast cells lacking either the Mre11–Rad50–Xrs2 (MRX) complex or the Srs2 helicase [6,13].

H3K56, which is located in the globular domain of histone H3, was found to be acetylated by mass spectrometry, and this modification has been shown to exist in yeast (both *Saccharomyces cerevisiae* and *Saccharomyces pombe*), *Drosophila*, and human cells [14,15]. Acetylation of H3K56 is catalyzed by the histone acetyltransferase (HAT) Rtt109 in budding yeast and CBP/p300 in

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doi:10.1016/j.dnarep.2009.12.022

Drosophila and human cells [16–19]. In addition to Rtt109, binding of histone chaperone Asf1 to histones H3 and H4 promotes efficient acetylation of H3K56 [20]. Deacetylation of H3K56 depends on two histone deacetylases (HDACs): Hst3 and Hst4 in yeast, and SIRT1 and SIRT2 in mammalian cells [15,21]. Interestingly, cells lacking an acetylatable lysine at H3K56 show hypersensitivity to a variety of DNA-damaging agents, such as methyl methanesulfonate (MMS), hydroxyurea (HU), and camptothecin (CPT), which suggests a critical role for H3K56 acetylation in maintenance of genome stability [21].

Several lines of evidence indicate that H3K56 acetylation plays an important role in DNA replication. Acetylation of H3K56 is a mark of newly synthesized histone H3 and the acetylation level increases during S phase [22]. Additionally, histone H3 acetylated on K56 is incorporated into replicating DNA by chromatin assembly factor 1 (CAF-1) and the histone chaperone Rtt106 [23]. This modification increased the binding affinity of CAF-1 and Rtt106 for H3 *in vitro* and *in vivo*, and enhanced replication-coupled nucleosome assembly in cell extract experiments [23]. Finally, both Rtt109 and Asf1 are required for the association of replication proteins Rfc3, PCNA, and Polε on a stalled replication fork, and this process is H3K56 acetylation-dependent [24,25]. Taken together, these data show that H3K56 acetylation maintains genome stability during replication by enhancing nucleosome assembly on newly replicated DNA and by stabilizing the replisome on stalled replication forks. Acetylation of H3K56 is also required for chromatin reassembly during repair of an HO-induced DSB [26]. Strains lacking Asf1 or Rtt109 no longer reassemble their chromatin at the DSB site even after proper DNA repair, and this defect can be rescued by using the acetylated mimic H3K56Q [26].

Notably, repetitive sequences can affect nucleosome assembly, and CAG/CTG repeat sequences are one of the strongest known natural nucleosome positioning elements [27]. Therefore, nucleosome repositioning or histone modification during DNA processes such as replication or repair could be particularly important at long CAG/CTG repeat tracts. At several loci, highly expanded TNRs are associated with a silent chromatin state that affects transcription of nearby genes and contributes to the disease phenotype [27]. However, the role of histone modifications on the stability of repetitive DNA sequences has not been directly investigated.

Here, *S. cerevisiae* strains containing 85 CAG/CTG repeats on a yeast artificial chromosome (YAC) were used to examine the role of H3K56 acetylation in maintaining repeat stability. We found that contractions of CAG/CTG repeats were significantly increased in strains lacking *RTT109* and other genes involved in H3K56 acetylation and deacetylation. Our data support a model whereby defects in replisome integrity and inefficient chromatin assembly on newly replicated DNA allows DNA structure formation on the template strand, leading to repeat contractions.

2. Materials and methods

2.1. Yeast strains and plasmids

Yeast strains used in this study (Table S2) were made by one-step gene replacement [28] in a BY4705 yeast strain background containing YAC CF1 with either no CAG repeat (CAG-0) or 85 CAG repeats (CAG-85) [8,29]. *rtt101Δ* and *rtt107Δ* strains were from the Stanford deletion set in the isogenic BY4742 background [30] and the YAC CF1 was introduced by cytoduction as described in Callahan et al. [8].

To test the effect of mutating the H3K56 residue, the *HHT2–HHF2* locus (one of two H3–H4 gene loci in yeast) was first replaced by the *KanMX6* gene [29] in yeast cells containing YAC CF1 with CAG-85. Plasmids with wild-type or mutated *HHT2* (H3 copy 2 gene) and wild-type *HHF2* (H4 copy 2 gene) provided by the Kirchmaier lab [31] were then transformed into the *hht2Δ–hhf2Δ* cells [32]. Lastly, the other H3–H4 gene locus (*HHT1* and *HHF1*) on yeast chromosome II was replaced by *His3MX6* [29].

2.2. CAG stability assay

For each yeast strain, four individual colonies with a starting tract length of CAG-85 were inoculated separately in YC-Leu liquid cultures. After 16-h incubation at 30 °C, a dilution of each culture was plated onto YC-Leu-Ura for single colonies. Approximately 20 daughter colonies from each plate, at least 80 daughter colonies total, were tested for repeat tract length by colony PCR. CAG repeat sequences were amplified with primers newCAGforprimer (CCTCAGCCTGGCCGAAAGAAAGAAA) and newCAGrevprimer (CAGTCACGACGTTGT AAAACGACGG) using Taq polymerase (Phoenix Lab, Tufts Medical Center). Amplified products were separated on a 2% Metaphor gel (Lonza) for 80 min at 80 V, and repeat size was estimated by comparison with Hyladder 1 kb marker (Denville Scientific Inc.). This method can detect tract length changes of three repeats (9 bp) or greater.

2.3. CAG fragility assay

A single starting colony from each strain background with the correct tract length as determined by colony PCR was suspended in 1 ml YC-Leu media and used to inoculate 5 or 10 separate cultures. After growth in liquid media for 6–7 doublings at 30 °C, cultures were plated onto FOA-Leu and YC-Leu plates as previously described [13]. The rate of FOA resistance was calculated by the method of the maximum-likelihood using SALVADOR software [33]. Assays were repeated 3–6 times for each tract length in each strain background and statistical significance was determined by a pooled variance *t* test using Systat software.

3. Results

3.1. CAG/CTG repeat contractions are significantly increased in cells with abnormal H3K56 acetylation

Since long CAG/CTG repeats interfere with DNA replication, and Rtt109 and Asf1-dependent H3K56 acetylation is important for replisome integrity at a stalled replication fork [24,25], we tested whether proteins involved in H3K56 acetylation and deacetylation help to protect CAG/CTG repeats from tract length changes. An expanded CAG/CTG repeat originally from a myotonic dystrophy type 1 patient was cloned into a YAC, orientated such that the CAG repeat tract is on the lagging strand template during replication [8]. The repeat is less contraction-prone and more expansion-prone in this orientation compared to when the CTG repeat tract is on the lagging strand template, although contractions still predominate in yeast [11]. Cells containing 85 CAG/CTG repeats on the YAC (abbreviated as CAG) were used to study repeat instability in mutants that affect H3K56 acetylation.

The frequency of CAG repeat contractions was significantly increased in cells lacking *RTT109* or *ASF1*, where no acetylation of H3K56 is detectable [16–18]. The *rtt109Δ* strain showed 36% contractions compared to 11% for a CAG-85 tract in the wild-type (WT) background, a 3.3-fold increase ($p = 2.8 \times 10^{-8}$; Fig. 1A). CAG contractions were also increased about twofold in *asf1Δ* cells. Expansions were slightly increased in both mutant backgrounds, but the frequency was not significantly different from the WT strain (Table S1). To test whether contractions were occurring through the same pathway in *rtt109Δ* and *asf1Δ* mutants, a *rtt109Δasf1Δ* double mutant was made. Repeat contractions were significantly