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Rad26p, a Transcription-Coupled Repair Factor, Promotes the Eviction and Prevents the Reassociation of Histone H2A−H2B Dimer during Transcriptional Elongation in Vivo

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Supporting Information

ABSTRACT: We have recently demonstrated the formation of an atypical histone H2A−H2B dimer-enriched chromatin at the coding sequence of the active gene in the absence of Rad26p in vivo. However, the mechanisms for such a surprising observation remain unknown. Here, using a ChIP assay, we demonstrate that Rad26p promotes the eviction of histone H2A−H2B dimer and prevents the reassociation of the dimer with naked DNA in the wake of elongating RNA polymerase II at the coding sequence of the active *GAL1* gene. Thus, the absence of Rad26p leads to the generation of an atypical histone H2A−H2B dimer-enriched chromatin at the active coding sequence in vivo.

Numerous damaging agents of endogenous and exogenous origin continuously attack DNA. An extremely cytotoxic ramification of DNA damage exists in cases where single-strand DNA damage in the coding sequence of an active gene results in stalling of the transcription elongation machinery, eventually leading to a number of diseases. Thus, the efficient removal of the single-strand damage from the coding sequence of the active gene is essential for proper cellular functions. Fortunately, cells employ a specific repair mode termed as transcription-coupled repair (TCR). In yeast, Rad26p is a TCR factor and is conserved among eukaryotes. Its human homologue, Cockayne syndrome B (CSB), plays an important role in TCR, and thus, the mutations in CSB are associated with severe growth retardation, progressive neurological dysfunction, mental retardation, cataracts, hearing and vision impairments, retinal pigmentation, and mild sun sensitivity.[1,2] Both Rad26p and CSB have Swi/Snf-type DNA-dependent ATPase activity[3,4] and are involved in regulation of chromatin structure.[5,6] Further, CSB has been shown to recognize a DNA lesion in the presence of RNA polymerase II for repair.[7] Similarly, in yeast, Rad26p recognizes a DNA lesion in an elongating RNA polymerase II-dependent manner.[8] Thus, the requirement of RNA polymerase II for recognition of the lesion by Rad26p and CSB makes them TCR-specific factors, and hence, the lesions in the inactive genes are not repaired by Rad26p or CSB. In addition to their roles in TCR, both CSB and Rad26p promote transcriptional elongation in humans and yeast, respectively.[9−11] Consistently, we have recently demonstrated that Rad26p is present at the coding sequences of active genes and promotes the association of elongating RNA polymerase II.[8,12] We have further shown that an impaired association of RNA polymerase II with the active coding sequence in the Δ*rad26* strain is correlated with a significantly high occupancy of histone H2A−H2B dimer, but not histone H3−H4 tetramer.[12] These results provide important insight into how Rad26p regulates transcriptional elongation. However, how the absence of Rad26p led to the generation of an atypical histone H2A−H2B dimer-enriched chromatin at the active coding sequence remained a puzzle. To address this, we have analyzed here the role of Rad26p in regulation of the occupancies of histone H2A−H2B dimer and histone H3−H4 tetramer at the coding sequence of the galactose-inducible *GAL1* gene immediately following transcriptional induction, using a formaldehyde-based in vivo cross-linking and chromatin immunoprecipitation (ChIP) assay (Supporting Information), as presented below.

During chromatin disassembly, histone H2A−H2B dimer is evicted first followed by histone H3−H4 tetramer. To determine the role of Rad26p in chromatin disassembly during transcriptional elongation, we have analyzed the eviction of histone H2A−H2B dimer from the coding sequence of the *GAL1* gene following transcriptional induction in galactose-containing growth medium (inducible conditions). In this manner, we grew both the wild-type and Δ*rad26* strains expressing Flag-tagged histone H2B (Supporting Information) in raffinose-containing growth medium (noninducible conditions) at 30 °C until the OD_{600} reached 0.9 and then transferred the strains to galactose-containing growth medium for different induction periods to analyze the eviction of histone H2A−H2B dimer from the *GAL1* coding sequence in the Δ*rad26* and wild-type strains, using the ChIP assay. We find that histone H2A−H2B dimer was evicted normally from the *GAL1* coding sequence following transcriptional induction in the wild-type strain (Figure 1A), consistent with previous studies.[12,13] Intriguingly, histone H2A−H2B dimer was not efficiently evicted at the 10 min time point following transcriptional induction in the Δ*rad26* strain (Figure 1A). However, at the 20 min induction time point, histone H2A-H2B dimer was evicted normally from the *GAL1* coding sequence in the Δ*rad26* strain, like the wild-type equivalent (Figure 1A). These results support the finding that the eviction of histone H2A−H2B dimer from the *GAL1* coding sequence is

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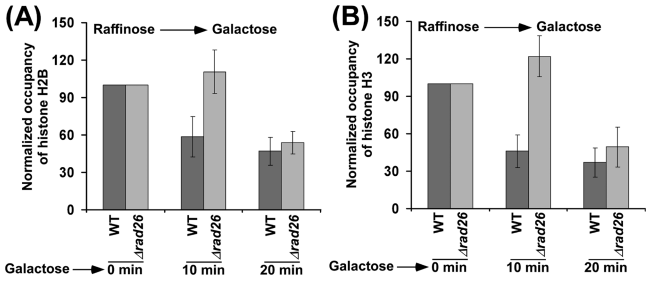

Figure 1. Regulation of the occupancies of histone H2A-H2B dimer (A) and histone H3−H4 tetramer (B) at the *GAL1* coding sequence by Rad26p.

significantly decelerated following transcriptional induction in the Δ*rad26* strain (Figure 1A), thus implicating Rad26p in the promotion of the eviction of histone H2A−H2B dimer.

As mentioned above, chromatin disassembly is initiated with the eviction of histone H2A−H2B dimer followed by histone H3−H4 tetramer. Because the eviction of histone H2A−H2B dimer is decelerated in the absence of Rad26p (Figure 1A), one would thus expect to observe a significantly decreased rate of eviction of histone H3−H4 tetramer at 10 min following transcriptional induction, as an impaired eviction of histone H2A−H2B dimer would indirectly slow the eviction of histone H3−H4 tetramer. To test this, we next analyzed the occupancy of histone H3−H4 tetramer at the *GAL1* coding sequence following transcriptional induction. Indeed, we find that the eviction of histone H3−H4 tetramer was impaired at the 10 min time point following transcriptional induction in the Δ*rad26* strain (Figure 1B). However, histone H3−H4 tetramer was evicted normally from the *GAL1* coding sequence at the 10 min transcriptional induction time point in the wild-type strain (Figure 1B), consistent with previous studies.[12,13] Basically, we find that the eviction of both histone H2A−H2B dimer and histone H3−H4 tetramer from the *GAL1* coding sequence was impaired in the Δ*rad26* strain at 10 min following transcriptional induction (Figure 1A,B). However, when histone H2A−H2B dimer was evicted normally in the Δ*rad26* strain at the 20 min time point following transcriptional induction (Figure 1A), histone H3−H4 tetramer was also evicted normally in the absence of Rad26p (Figure 1B). Collectively, our data support the finding that Rad26p promotes chromatin disassembly at the *GAL1* coding sequence following transcriptional induction in vivo.

Because histone H2A−H2B dimer is evicted prior to histone H3−H4 tetramer during chromatin disassembly, the defect in histone H3−H4 tetramer eviction would have a minimal effect on the eviction of histone H2A−H2B dimer. Indeed, our previous studies[13] have demonstrated that an impairment of histone H3−H4 tetramer eviction in the absence of Rtt109p does not dramatically alter the eviction of histone H2A−H2B dimer. On the other hand, the defect in the eviction of histone H2A−H2B dimer will have a dramatic effect on histone H3−H4 tetramer eviction, as the eviction of histone H3−H4 tetramer during chromatin disassembly is dependent on prior removal of histone H2A−H2B dimer. Therefore, we observed a dramatic decrease in the eviction of histone H3−H4 tetramer from the *GAL1* coding sequence, when histone H2A−H2B dimer eviction was impaired 10 min following transcriptional induction in the Δ*rad26* strain (Figure 1A,B). Hence, Rad26p appears to directly regulate the eviction of histone H2A−H2B dimer and indirectly histone H3−H4 tetramer eviction. However, there is a possibility of a direct role of Rad26p in eviction of histone H3−H4 tetramer, in addition to its function in histone H2A−H2B dimer eviction. This possibility is less likely on the basis of our recent publication[12] that demonstrated the role of Rad26p in regulation of the occupancy of histone H2A−H2B dimer, but not histone H3−H4 tetramer, at the active gene.

Because both histone H2A−H2B dimer and histone H3−H4 tetramer in the Δ*rad26* strain were evicted normally from the *GAL1* coding sequence at levels similar to those in the wild-type strain at 20 min following transcriptional induction, one would thus expect to observe a significantly low occupancy of histone H2A−H2B dimer and histone H3−H4 tetramer at the *GAL1* coding sequence at the 30 min transcriptional induction time point in the absence of Rad26p. Indeed, we find a low level of histone H3−H4 tetramer at the *GAL1* coding sequence 30 min following transcriptional induction in the Δ*rad26* strain, similar to the wild-type equivalent (Figure 2A). Surprisingly, we

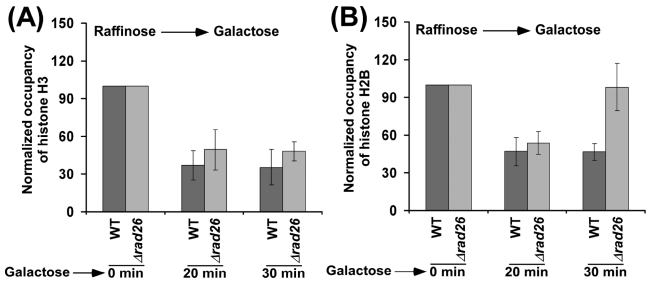

Figure 2. Regulation of the occupancies of histone H3−H4 tetramer (A) and histone H2A−H2B dimer (B) at the *GAL1* coding sequence by Rad26p.

find a significantly high level of histone H2A−H2B dimer at the *GAL1* coding sequence in the Δ*rad26* strain as compared to the wild-type equivalent at the 30 min transcriptional induction time point (Figure 2B). Such a surprisingly high level of histone H2A−H2B dimer at the *GAL1* coding sequence at the 30 min induction time point (Figure 2B) following its normal eviction at 20 min in the Δ*rad26* strain (Figure 1A) supports the reassociation of histone H2A−H2B dimer with naked DNA in the wake of elongating RNA polymerase II in the absence of Rad26p in vivo. Such an association of histone H2A−H2B dimer with naked DNA is further supported by the fact that histone H2A−H2B dimer has a high affinity for naked DNA.[14]

During chromatin reassembly, histone H3−H4 tetramer is deposited first on the DNA, and subsequently, histone H2A−H2B dimer joins to complete the formation of a canonical nucleosome. Thus, the reassociation of histone H2A−H2B dimer with naked DNA in the absence of Rad26p would impair the formation of the canonical nucleosome in the wake of elongating RNA polymerase II, hence leading to the formation of histone H2A−H2B dimer-enriched chromatin. Such an atypical chromatin is likely to be inefficiently evicted by the next wave of elongating RNA polymerase II, because of the high affinity of histone H2A−H2B dimer for naked DNA.[14] Therefore, we observed an atypical histone H2A−H2B dimer-enriched chromatin at the *GAL1* coding sequence at the 30 min transcriptional induction time point in the absence of Rad26p (Figure 2B). Similarly, histone H2A−H2B dimer-enriched chromatin was also observed at the *GAL1* coding sequence at later induction time points (e.g., 60 and 90 min) in the Δ*rad26* strain in our recent study.[12]

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Collectively, our results support a model in which Rad26p promotes the eviction of histone H2A−H2B dimer and hence chromatin disassembly (Figure 1 of the Supporting Information). Such a facilitated chromatin disassembly enhances the passage of elongating RNA polymerase II through the nucleosome at the coding sequence and, hence, transcription.[12] The nucleosome is subsequently reassembled in the wake of elongating RNA polymerase II. Rad26p prevents the reassociation of histone H2A−H2B dimer with naked DNA and allows histone H3−H4 tetramer to interact with DNA toward formation of the canonical nucleosome in the wake of elongating RNA polymerase II (Figure 1 of the Supporting Information). Such a canonical nucleosome is rapidly disassembled by the next wave of elongating RNA polymerase II, and thus, histone H3−H4 tetramer-enriched chromatin is not observed in the wild-type strain (Figures 1B and 2A). Chromatin reassembly and dynamics play important roles in regulating transcriptional elongation. If chromatin is not reassembled efficiently or properly in the wake of elongating RNA polymerase II, transcription will be altered. In the absence of Rad26p, the reassociation of histone H2A−H2B dimer with naked DNA is favored, competing out histone H3−H4 tetramer and hence disallowing the formation of the canonical nucleosome (Figure 1 of the Supporting Information). Therefore, histone H2A−H2B dimer-enriched chromatin was observed at the *GAL1* coding sequence in the Δ*rad26* strain at 30 min (Figure 2A,B) and later transcriptional induction time points.[12]

Because our data support the role of Rad26p in the eviction of histone H2A−H2B dimer, and prevention of its reassociation with naked DNA, Rad26p is thus likely to interact with histone H2A−H2B dimer. Therefore, one would expect to find Rad26p throughout the genome via its interaction with histone H2A−H2B dimer. However, our recent studies[8,12] have demonstrated that Rad26p associates predominantly with the coding sequences of active genes, but not active promoters or inactive genes. These studies[8,12] support the possibility that Rad26p does not interact with histone H2A−H2B dimer or chromatin in the absence of active transcription machinery. Likewise, previous studies[15] have demonstrated the interaction of a histone chaperone, Asf1p, with chromatin in a transcription-dependent manner. Therefore, similar to Asf1p, Rad26p is likely to interact with histone H2A−H2B dimer or chromatin in the presence of transcription factor(s) or machinery.

In summary, this study demonstrates an important role of Rad26p in promoting the eviction of histone H2A−H2B dimer and preventing its reassociation with naked DNA in the wake of elongating RNA polymerase II to favor efficient chromatin disassembly and reassembly at the active gene. Such regulation of chromatin structure by Rad26p not only regulates transcription but also likely controls the recruitment of DNA repair factors (and hence TCR), because chromatin structure has been implicated in altering the access of DNA repair factors to the lesion. Further, TCR is also controlled by Rad26p-mediated transcriptional regulation. Overall, this study provides novel insight into how Rad26p regulates chromatin structure to ultimately control transcription and TCR, thus significantly advancing our knowledge of the regulation of chromatin structure by Rad26p in vivo. Because Rad26p is highly conserved from yeast to humans, its homologue CSB is likely to play similar regulatory roles in humans. Hence, the results of this study hint at the possible etiological mechanisms of CS disease. However, such mechanisms remain to be further elucidated in humans. Nonetheless, this study describes for the first time how the yeast homologue of human CSB functions to regulate chromatin structure (and hence transcription and TCR), thus unveiling a novel and important function of this key TCR factor toward an etiological understanding of CS disease.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Materials and methods and Figure 1. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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