Published in final edited form as:

Science. 2018 September 28; 361(6409): 1386–1389. doi:10.1126/science.aat8849.

A mechanism for preventing asymmetric histone segregation onto replicating DNA strands

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Abstract

How parental histone (H3–H4)₂ tetramers, the primary carriers of epigenetic modifications, are transferred onto leading and lagging strands of DNA replication forks for epigenetic inheritance remains elusive. Here, we show that parental (H3–H4)₂ tetramers are assembled into nucleosomes onto both leading and lagging strands, with a slight preference for lagging strands. The lagging strand preference increases markedly in cells lacking Dpb3 and Dpb4, two subunits of the leading strand DNA polymerase, Pol δ , due to the impairment of parental (H3–H4)₂ transfer to leading strands. Dpb3–Dpb4 binds H3–H4 *in vitro* and participates in the inheritance of heterochromatin. These results indicate that different proteins facilitate the transfer of parental (H3–H4)₂ onto leading vs lagging strands, and that Dpb3–Dpb4 plays a significant role in this poorly understood process.

Posttranslational modifications (PTMs) on histones in eukaryotic chromatin including those in response to environmental stimuli and during development have a profound impact on gene expression states. Recently, it has been shown that at least some of these PTMs such as methylation of histone H3 lysine 9 and lysine 27 are inheritable traits during mitotic cell division and even through meiosis (1–4). As the “first” and rate-limiting step of transmission of these PTMs, it was proposed that parental histone (H3–H4)₂ tetramers, the primary carriers of epigenetic modifications, are randomly and equally distributed to leading and lagging strands of DNA replication forks (5, 6), and serve as the “template” for copying epigenetic modifications onto newly synthesized (H3–H4)₂ tetramers, which do not mix

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with parental (H3–H4)₂ (7) and have distinct PTMs from parental histones. This dogmatic view has not been tested *in vivo* due to challenges in monitoring histone segregation onto leading and lagging strands. Moreover, while significant insights into nucleosome assembly of newly synthesized (H3–H4)₂ tetramers have gained over the years (8, 9), the molecular mechanisms underlying the transfer of parental histone (H3–H4)₂ tetramers onto replicating DNA strands remain largely unknown.

We modified the eSPAN (enrichment and sequencing of protein-associated nascent DNA) method, which can detect whether a protein is enriched on leading or lagging strands at a genome-wide scale (10) to monitor the segregation of newly synthesized and parental histone H3, which should represent (H3–H4)₂ tetramers, onto replicating DNA strands (Fig. 1A–B and fig. S1A). Briefly, we released G1-arrested yeast cells for 45 min into medium containing BrdU, in order to label newly synthesized DNA, and hydroxyurea (HU) to facilitate analysis by slowing DNA synthesis but having no apparent effect on the initiation of DNA replication and nucleosome assembly from early replication origins (11). Chromatin from G1 and early S phase cells was digested with micrococcal nuclease (MNase), which cleaves DNA between nucleosomes, and was used for deep sequencing (MNase-Seq). The digested chromatin was also analyzed by chromatin immunoprecipitation (ChIP) using antibodies (fig. S1B) against acetylation of histone H3 lysine 56 (H3K56ac), a mark of newly synthesized H3 (12), and trimethylation of histone H3 lysine 4 (H3K4me3, a surrogate mark for parental H3, see below) and subsequent strand-specific (ChIP-ssSeq) and eSPAN analysis.

Analysis of MNase-Seq of G1- and early S-phase chromatin revealed well-positioned nucleosomes surrounding early replication origins (fig. S2A–C), consistent with published results (13–15). Moreover, H3K56ac ChIP-ssSeq peaks co-localized with BrdU-IP peaks (fig. S2A and fig. S2D–E), demonstrating that H3K56ac can be used as a surrogate mark for newly synthesized (H3–H4)₂ tetramers. We chose H3K4me3 as the surrogate mark for parental H3 for the following reasons. First, H3K4me3 was not detected on newly synthesized H3 (16). Second, we observed that the H3K4me3 level at newly replicated regions, but not the total level during early S phase, was reduced compared to the G1 phase of the cell cycle (fig. S1C–D), likely reflecting the dilution of parental H3K4me3 as well as a delay in writing this mark on newly synthesized H3 during the short-time frame of our experiments. Finally, while at reduced occupancy, the positioning of H3K4me3-containing nucleosomes on newly replicated chromatin, as detected by H3K4me3 ChIP-ssSeq, was similar to that of G1 phase (fig. S2F–G).

We calculated the log₂ ratio of H3K56ac and H3K4me3 eSPAN sequence reads of the Watson over Crick strand at 20 individual nucleosomes, which on average span the replicated region, surrounding each of the 134 early replication origins. If H3K56ac and H3K4me3 were equally distributed onto leading and lagging strands, one would expect that this ratio were close to zero (Fig. 1B). Instead, we observed that H3K56ac eSPAN peaks exhibited a small but consistent leading-strand bias at most nucleosomes except –1 and +1 nucleosomes (Fig. 1C–D and fig. S3A), whereas H3K4me3 eSPAN peaks showed a lagging strand bias at newly replicating chromatin regions (Fig. 1E–F and fig. S3B). These bias patterns were more consistent at Group 1 origins than at less efficient Group 2 origins (Fig.

1C, 1E and fig. S3C–D). Based on the average bias ratio from three independent experiments, we estimated that about 8% more H3K56ac-H4 tetramers were deposited onto leading strands than the corresponding lagging strands, whereas at least 23% more parental H3K4me3-H4 tetramers were transferred to lagging strands than the corresponding leading strands. The differential enrichment of parental and newly synthesized (H3–H4)₂ tetramers at lagging and leading strands (23% vs 8%) observed here and Figs. 2 and 3 below, likely suggest that nucleosomes formed from parental (H3–H4)₂ tetramers are more stable and resistant to MNase digestion than those formed from newly synthesized tetramers. Thus, parental (H3–H4)₂ tetramers are transferred onto both leading and lagging strands, with a slight preference for lagging strands.

Unexpectedly, we discovered that deletion of *DPB3* and *DPB4*, encoding two non-essential subunits of leading strand DNA polymerase, Pol ϵ (17), significantly increased the bias pattern of H3K56ac and H3K4me3 eSPAN peaks compared to wild type cells (fig. S4 and fig. S5), while having no apparent effect either on the levels of H3K56ac and H3K4me3 (fig. S1B) or the overall nucleosome occupancy and positioning of G1- and early S-phase chromatin (fig. S6). We estimated that ~41% more H3K56ac-H4 were deposited onto leading strands than the corresponding lagging strands, whereas 120% more parental H3K4me3-H4 were transferred to lagging strand than the corresponding leading strand in *dpb3* or *dpb4* mutant cells.

We used the Recombination-Induced Tag Exchange (RITE) system (18), which marks newly synthesized and parental histone H3 with the T7 and HA tag, respectively (Fig. 2A and fig. S7A), as an independent approach to analyze the impact of *DPB3* and *DPB4* deletion on histone segregation. The H3-T7 and H3K56ac eSPAN peaks showed a strong leading strand bias (Fig. 2B–C and fig. S7B–C), whereas H3-HA and H3K4me3 eSPAN peaks exhibited strong lagging strand bias in *dpb3* mutant cells (Fig. 2D–E and fig. S7D–E). We also analyzed the distribution of H3K56ac and H3K4me3 at replicating chromatin in *dpb3* cells during normal S phase without HU using the eSPAN and observed the same effect (Fig. 3 and fig. S8). These results demonstrate that deletion of *DPB3* and *DPB4* alters the distribution pattern of new and parental (H3–H4)₂ tetramers at leading and lagging strands.

To determine how depletion of *DPB3* and *DPB4* affects the distribution pattern of H3K4me3 and H3K56ac, we calculated the relative amount of these histones at leading and lagging strands in *dpb3* or *dpb4* over wild type cells (Fig. 4A). Compared to wild type, the H3K4me3 at leading strands at both sides of the origins in *dpb3* and *dpb4* cells were reduced significantly (Fig. 4B and fig. S9A). In contrast, the H3K4me3 at lagging strands in these two mutants increased slightly compared to wild type (Fig. 4B and fig. S9A). Moreover, deletion of *DPB3* and *DPB4* had minor effects on H3K56ac deposition at leading and lagging strands compared to wild type (fig. S9B–C). These results indicate that the transfer of parental (H3–H4)₂ tetramers marked by H3K4me3 to leading strands is compromised the most in *dpb3* and *dpb4* cells (fig. S9D–E). Thus, Dpb3 and Dpb4 facilitate the transfer of parental (H3–H4)₂ onto leading strands. Supporting this idea, we observed that Dpb3 and Dpb4 were enriched at leading strands (Fig. 4C–D), in a manner similar to the catalytic subunit of Pols (10). Moreover, *in vitro*, Dpb3-Dpb4 dimers, which are structurally similar to H2A-H2B (19) and co-purify with H4 in cells (20), interacted with

(H3–H4)₂ tetramers (Fig. 4E). These results support the model that once nucleosomes ahead of DNA replication forks are disassembled, Dpb3-Dpb4 molecules serve as “receptors/chaperones” for parental (H3–H4)₂ tetramers and facilitate their assembly onto leading strands (Fig. 4G).

Cells lacking Dpb3 or Dpb4 are defective in heterochromatin silencing (19, 21). Using the assay that monitors transient losses of heterochromatin silencing at the mating type loci (22), we observed a significant increase in the switching of the heterochromatin state at the *HML* locus in *dpb3* or *dpb4* cells (Fig. 4F), suggesting that a defect in parental (H3–H4)₂ transfer in *dpb3* or *dpb4* compromises epigenetic inheritance.

In summary, parental (H3–H4)₂ tetramers are distributed onto both leading and lagging strands, with a slight preference for the lagging strands. Dpb3-Dpb4 facilitates their assembly on leading strands, thereby providing a mechanism for preventing a large asymmetric distribution of parental (H3–H4)₂ at replicating DNA strands. Our results suggest that different proteins likely promote nucleosome assembly of parental (H3–H4)₂ tetramers onto leading vs lagging strands. It is known that Mcm2, a subunit of the MCM helicase, contains a histone binding motif that binds (H3–H4)₂. Moreover, it was proposed that Mcm2 is involved in nucleosome assembly of parental (H3–H4)₂ tetramers (23). In addition, DNA polymerase α and DNA polymerase clamp (PCNA) also have chromatin-related functions (24, 25). In the future, it would be interesting to determine whether any of these and other DNA replication proteins have a direct role in reassembly of parental histones. The utilization of different proteins for the parental histone transfer also hints a strategy for eukaryotic cells to alter histone distribution pattern and possibly chromatin states in response to developmental stimuli, thereby endowing eukaryotic cells the ability both to maintain epigenetic states and to generate epigenetic plasticity such as the generation of neuronal bilateral asymmetry in *Caenorhabditis elegans* during development (26).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank Drs. Songtao Jia, Gary Struhl and Bruce Stillman for comments on this manuscript, and Drs Fred van Leeuwen, Helle D Ulrich and Jasper Rine for yeast strains. This study was supported by the following grants: NIH R35GM118015 (Z.Z.); NSFC 31521002, MOST 2017YFA0103304 and CAS XDB08010100 (RMX); and Cancerfonden and the Swedish Research Council (E.J., A.C). The deep sequencing datasets were deposited in Gene Expression Omnibus (GEO) database (GSE112522).

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Fig. 1. Newly synthesized (H3K56ac) and parental histone (H3K4me3) show a slight preference for leading and lagging strands, respectively.

(A–B) An outline of experimental procedures (A) and a diagram for the hypothetical eSPAN outcome assuming four parental and four new (H3–H4)₂ tetramers are equally distributed to leading and lagging strand (B). (C) Heatmap showing the bias pattern of H3K56ac eSPAN peaks at each of the 20 individual nucleosomes surrounding 134 early DNA replication origins. The individual nucleosome position is numbered from (–10 to +10) and represented by a circle (top panel). Each row represents the average log₂ ratio Watson/Crick H3K56ac eSPAN sequence reads at one of the 134 origins and is clustered based on hierarchical clustering analysis. (D) The average bias ratio of H3K56ac eSPAN peaks at each of the 20 individual nucleosomes of the 134 early replication origins from three independent experiments. (E–F) H3K4me3 eSPAN peaks at newly replicated chromatin exhibit a slight lagging strand bias.

Fig. 2. Analyzing nucleosome assembly of new and parental histone H3 in *dpb3* cells using the Recombination-Induced Tag Exchange (RITE) system.

(A) Schematic outline for marking the parental and newly synthesized H3 with the HA epitope (H3-HA) and T7 (H3-T4), respectively. (B) Heat map showing H3-T7 eSPAN bias pattern in *dpb3* cells at each of 134 individual origins ranked from top to bottom based on the replication efficiency. (C) The average bias pattern of H3-T7 eSPAN peaks at 134 early replication origins. (D–E) H3-HA eSPAN peaks in *dpb3* cells show a strong lagging strand bias.

Fig. 3. Deletion of *DPB3* results in a marked increase in the bias ratio of H3K56ac and H3K4me3 eSPAN peaks under normal cell cycle progression.

(A) A snapshot of BrdU IP-ssSeq, H3K56ac and H3K4me3 eSPAN peaks at individual nucleosomes surrounding *ARS1309* in *dpb3* cells. (B–D) Analysis of the average bias pattern of H3K56ac eSPAN peaks (B–C) and H3K4me3 eSPAN peaks (D–E) in *dpb3* cells at normal S phase (n=2). H3K56ac and H3K4me3 eSPAN were performed using *dpb3* cells released from the G1 block into fresh media without HU at 25°C for 30 min.

Fig. 4. Deletion of *DPB3* and *DPB4* compromises the parental (H3–H4)₂ transfer to leading strands.

(A) A diagram for calculating the relative amount of histone (H3–H4)₂ at leading and lagging strand in *dpb3Δ* cells compared to wild type using the eSPAN datasets. Grey circles: parental (H3–H4)₂; orange circles: new (H3–H4)₂; Black line: parental DNA; Red and Green lines: newly synthesized Watson and Crick strand, respectively. (B) Analysis of the relative levels of H3K4me3 in *dpb3Δ* cells compared to wild type at each of the 134 individual origins. 0: no difference between mutant and wild type (black); 0.03C0 and >0 represent less (green color) and more (red color) H3K4me3 eSPAN sequence reads in *dpb3Δ* cells than in wild type cells, respectively. Bottom panel: the average amount of H3K4me3 at lagging and leading strands of 134 replication origins in *dpb3Δ* cells over wild type. (C) Dpb3 and Dpb4 eSPAN peaks at the *ARS1309* origin. (D) The average bias of Dpb3 and Dpb4 eSPAN peaks at 134 early replication origins. (E) GST-Dpb3-Dpb4 pull down assays show that Dpb3-Dpb4 dimers bind recombinant (H3–H4)₂ tetramers *in vitro*. (F) *dpb3Δ* and *dpb4Δ* cells exhibit an increased loss of silencing at the *HML* locus. (G) A role for Dpb3-Dpb4 in the transfer of parental histone (H3–H4)₂ onto leading strands.