Histone methylation sets the stage for meiotic DNA breaks

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Covalent post-translational modifications of histones have important functions in transcription, replication, repair, and other aspects of eukaryotic chromosome dynamics. Trimethylation of lysine-4 on histone H3 is enriched at actively transcribed loci in many organisms. The impact of this histone modification on transcription has been extensively studied, but less is known about its effects on other chromosomal processes. An intriguing new study in this issue of EMBO Journal demonstrates that H3 lysine-4 trimethylation is critical in budding yeast for formation of the programmed DNA double-strand breaks that initiate homologous recombination during meiosis. These findings have important implications for elucidating the previously recognized but little understood connections between meiotic break formation and transcriptional promoters in this organism.

Figure 1 Integration of multiple histone modifications promotes the formation of meiotic DSBs. It is likely that a combination of histone modifications is established at promoter regions prior to entry into meiosis. Ubiquitination of lysine-123 of H2B promotes subsequent Set1 activity upon lysine-4 of H3. H3K4me3 is then read by a putative trimethyl-lysine reader either contained as part of the pol II machinery or as a component of the DSB protein complex allowing for the formation of the majority of DSBs within promoters. Other subsets of DSBs occur within alternative chromatin environments and occur within ORFs, intergenic, and heterochromatic regions. Frequencies of meiotic DSBs are depicted by a smoothed histogram along a fictitious segment of yeast chromosome. Purple shading signifies promoters with nucleosomes enriched with the combination of H2B ub and H3K4me3, whereas ORFs are indicated by blue arrows.
Eukaryotic genomes are packaged in a relatively compact form through incorporation of DNA into arrays of repeating nucleosomes. Each nucleosome consists of an octamer of histone proteins, usually two H2A–H2B dimers and an (H3–H4), tetramer, wrapped nearly twice around by 150 bp of DNA. One of the ways that cells control both the higher order folding of nucleosome arrays and the ability of other proteins to access the DNA is through chromatin through the placement and removal of covalent modifications on histones. One such modification is trimethylation of the lysine-4 residue of histone H3 (H3K4m3). This modification is enriched around the promoters and 5’ ends of actively transcribed genes. In the budding yeast Saccharomyces cerevisiae, H3K4 trimethylation is catalysed by the Set1/COMPASS methyltransferase complex (also known as KMT2) associated with the RNA polymerase II transcriptional machinery (Shilatifard, 2008). H3K4m3 thought to be involved in nucleosome remodelling and transcriptional elongation. In this issue of EMBO Journal, a new link is exposed between H3K4m3 and the formation of DNA double-strand breaks (DSBs) during meiosis (Borde et al., 2009).

Meiosis is the specialized cell division that reduces the genome complement by half to generate cells for sexual reproduction. During meiosis in most organisms, homologous maternal and paternal chromosomes pair and recombine with one another so that they can be accurately segregated during the first meiotic division (Borde et al., 2009). Recombination in which certain combinations of histone modifications create an environment favourable to DSB formation. It is clear, however, that different histone modifications influence particular hotspots to different extent. For example, although set1 deletion reduced or eliminated a majority of DSBs across the genome, 22 DSB sites were refractory to this decrease, even showing an increase in DSBs in the set1 mutant (Borde et al., 2009). These refractory sites lacked H3K4m3 in wild-type cells. These findings dramatically illustrate the fact that not all DSB sites are created equal, and that the ‘rules’ that dictate hotspot activity differ from one hotspot to another (Petes, 2001).

How then is the DSB-forming machinery influenced by H3K4m3 and other modifications? One possibility is that one or more of the proteins required for DSB formation ‘read’ H3K4m3 by direct binding, thereby recruiting Spo11 and/or other factors to sites enriched for this modification. In S. cerevisiae, such hotspots most often occur within promoter regions, consistent with the interpretation that DSB formation is facilitated by the open chromatin state characteristic of yeast promoters. Not all promoters are subject to significant DSB formation, however, and current data do not support a direct relationship between the level of transcription and formation of DSBs (Hunter, 2006).

In this issue, Borde et al. (2009) demonstrate that deletion of the Set1 methyltransferase in S. cerevisiae leads to a dramatic decrease in the number of DSBs at the hottest hotspots examined, with 70% of these sites exhibiting a greater than two-fold reduction. This finding prompted the authors to more closely investigate the occurrence of H3K4m3 at these DSB sites. Using chromatin immunoprecipitation, they found that this modification was indeed highly enriched at DSB hotspots in promoters. Interestingly, the correlation between the presence of H3K4m3 and DSB hotspot activity was independent of the steady-state level of RNA from the genes. This result suggests that the connection between this histone modification and DSB formation is not tied directly to the transcriptional status. Moreover, H3K4m3 enrichment at hotspots is already seen during vegetative growth, that is, before cells enter meiosis, indicating that this modification is a pre-existing mark for regions that are especially permissive for DSB formation.

This study provides new insight into chromatin features that influence the distribution of meiotic recombination events. These results also tie in nicely with a previous analysis of H2B ubiquitination (H2Bub), which showed that preventing this modification lead to an overall decrease in DSBs (Yamashita et al., 2004). H2Bub promotes Set1/COMPASS activity and thus the appearance of H3K4m3 (Dover et al., 2002; Sun and Allis, 2002). Results of Borde et al. (2009) may thus indicate that H2Bub promotes DSB formation indirectly through trimethylation of H3K4 (Figure 1). It will be interesting to determine whether other instances of known cross-talk among histone modifications also influence DSB formation. For example, presence of H3K4m3 is mutually exclusive with dimethylation of arginine-2 of histone H3 (H3R2me2), which is a modification primarily found in heterochromatin and inactive genes (Kirmizis et al., 2007). Investigation of whole-genome localization of H2Bub and H3R2me2 during meiotic prophase, and tests of whether H3R2me2 status influences DSB formation are thus of interest. Also, it should be noted that Borde et al. (2009) limited their analysis of H3K4m3 distribution to only the ‘hottest’ hotspots in the genome, that is, those which were five-fold or greater over background. Thus, it will also be interesting to examine to what extent the observed patterns also apply to the large number of much weaker DSB sites in the genome.

The study of Borde et al. (2009) joins a growing body of work revealing how chromatin modifications influence DSB formation in several organisms. Other recent examples include the demonstration of connections between DSB formation and proteins that control H3 lysine-9 methylation and H4 lysine-16 acetylation (which are marks associated with heterochromatin) and H3 lysine-36 methylation (which is found within the body of actively transcribed genes) (Reddy and Villeneuve, 2004; Mieczkowski et al., 2007; Merker et al., 2008). Interestingly, deletion of the histone acetyltransferase gene gcn5 in Schizosaccharomyces pombe leads to a decrease in recombination at a synthetic DSB hotspot that is heavily acetylated in wild-type cells (Yamada et al., 2004). The synthesis of these findings supports a framework for meiotic DSB formation that is heavily influenced by the overall ‘openness’ of the chromatin. Interestingly, a direct binding partner of Spo11 is the WD-repeat protein Ski8, which has effects on the ‘openness’ of the chromatin. Interestingly, a direct binding partner of Spo11 is the WD-repeat protein Ski8, which has recently been reported to interact physically with the pol II subunit Rpo26 (Tarassov et al., 2008).

Elucidating the factors that control where meiotic DSBs occur is important for understanding the mechanism of DSB formation itself as well as the impact of meiotic recombination on genome structure and evolution. The findings of Borde et al. (2009) emphasize the importance of histone modifications in facilitating DSB formation and open doors into an exploration of the complex and combinatorial modifications that function in this process.

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