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# On Your MARK, Get SET(D2), Go! H3K36me3 Primes DNA Mismatch Repair

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**Trimethylation of histone H3 on Lys36 (H3K36me3) by SETD2 is linked to actively transcribed regions. Li et al. identify a novel role for H3K36me3 that facilitates DNA mismatch repair (MMR) in cells by targeting the MMR machinery to chromatin during the cell cycle, thereby explaining certain cases of MMR-defective cancers.**

Cells are constantly under attack by DNA damaging agents of both exogenous and endogenous origin. These assaults cause various DNA lesions that are normally corrected by specially adapted cellular DNA repair machineries. Loss or deregulation of DNA repair factors can therefore foster the accumulation of DNA errors and genome instability, a hallmark of cancer that is also implicated in aging, immune deficiencies, and neurodegenerative disorders (Jackson and Bartek, 2009).

One specialized and highly evolutionarily conserved DNA repair pathway is DNA mismatch repair (MMR) that corrects base-base mismatches and insertion/deletion loops (IDLs) of simple repeated sequences arising as occasional errors during DNA replication. These lesions are recognized by two protein complexes, belonging to the MutS homolog (MSH) family, that are crucial for successful MMR. In human cells, one of these, the most abundant MMR recognition complex that is termed hMutSα, comprises a heterodimer of hMSH2 and hMSH6 proteins that binds to base-base mismatches and small IDLs. Although partially overlapping in function with hMutSα, the less abundant hMSH2/hMSH3 heterodimer complex (hMutSβ) mainly deals with larger IDLs. Importantly, MMR loss leads to a specific mutator phenotype characterized by microsatellite instability (MSI), a change in the number of simple sequence repeats that causes predisposition to various cancers, especially colorectal cancers (Jiricny, 2006). Nevertheless, a striking and puzzling finding in the MMR field has been that a significant number of colorectal and several other MSI-positive cancers do not display genetic or epigenetic defects in any known MMR genes. What could the molecular basis for MSI in these cancers be?

Although the biochemical properties of MMR factors on naked DNA substrates have been well characterized (Jiricny, 2006), previous work has shown that DNA mismatches in the context of tightly associated nucleosomes, in contrast to naked DNA, represent poor MMR substrates in such cell-free systems (Li et al., 2009) (Figure 1A). In light of this and because chromatin is becoming increasingly recognized for its impact on various DNA repair pathways (Miller and Jackson, 2012), Li et al. (2013), as reported in this issue of *Cell*, explored whether chromatin organization is key to effective MMR. Specifically, they began their investigations by focusing on the observation that the hMSH6 subunit of hMutSα contains a Pro-Trp-Trp-Pro (PWWP) domain, a proline and tryptophan-rich region that is found in several chromatin factors, some of which were recently shown to use their PWWP domain as a reader for H3K36 methylated histone marks (reviewed in Wagner and Carpenter, 2012). Through a series of biochemical and cellular assays, Li et al. firmly establish that hMSH6 indeed binds to tri- and, to a lesser extent, dimethylated H3K36 marks in vitro in a PWWP-dependent manner and show that this mediates hMutSα association with chromatin in cells. These data thereby define what is conceivably the first direct connection between an MMR repair factor and a histone mark (Figure 1B, left). What then is the functional significance of these findings?

H3K36me3 is tightly linked to actively transcribed genome regions, where it prevents transcription initiation from cryptic gene promoters in the wake of elongating RNA polymerase II and can also direct alternative splicing events (Luco et al., 2010; Wagner and Carpenter, 2012). What is much less appreciated is that this mark is cell-cycle regulated, with it peaking in late G1/early S—where it likely concentrates at boundaries of early replication domains (Ryba et al., 2010)—and being largely depleted in late S/G2 (Li et al., 2013). By linking it to MMR, Li et al. now provide a likely functional relevance for this regulation: because DNA mismatches usually arise through occasional proofreading errors by DNA polymerases during DNA replication, the enrichment of H3K36me3 during S phase may facilitate recruitment of the MMR machinery to where (chromatin) and when (during DNA replication) it is most needed. Consistent with this idea, Li et al. establish that abolishing this mark by shRNA-mediated depletion of the corresponding histone methyltransferase SETD2 (the sole enzyme that can catalyze the transition from H3K36me2 to H3K36me3 in mammalian cells) (Wagner and Carpenter, 2012) impaired hMutSα chromatin binding, led to MSI, and increased mutation rates in the HPRT gene in these cells. Even more impressive is the fact that Li et al. found a renal cell

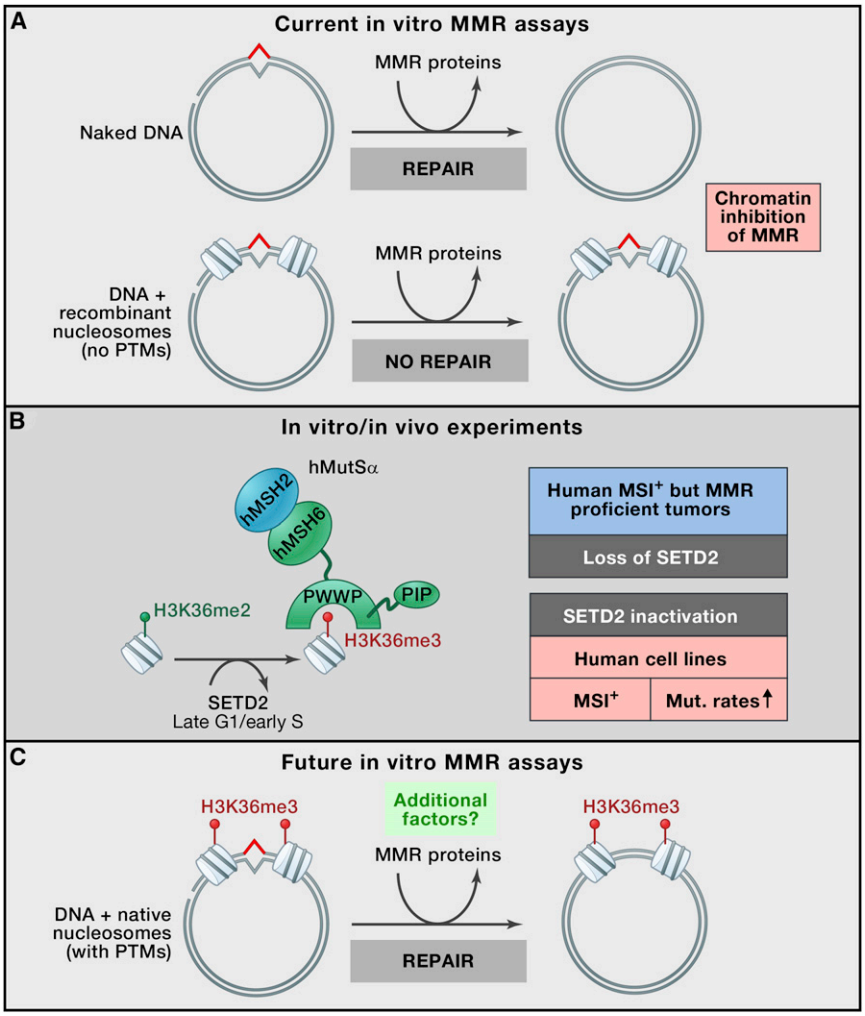


**Figure 1. A Novel Role of Chromatin in MMR**

(A) Although MMR of naked DNA (top) has been fully reconstituted in vitro, when the DNA substrate contains tightly associated nucleosomes (cylinders, bottom), MMR is inhibited, suggesting that additional chromatin factors are needed to repair mismatches in mammalian cells.

(B) Left: Li et al. identify SETD2 and the histone mark it catalyzes, H3K36me3, as MMR regulators. They find that the hMutSα subunit, hMSH6, binds H3K36me3 via its PWWP domain, especially when H3K36me3 is enriched in late G1/early S phase. Note that hMSH6α targeting also appears to be potentiated by a PIP box that appears to interact with PCNA associated with the replication complex. Right: Li et al.'s findings help explain the MSI phenotype of human cancer cell lines with no known defects in classical MMR factors. Conversely, SETD2 inactivation in human cell lines triggers MSI and increases mutation rates.

(C) Despite the impact of H3K36me3 on MMR, this mark is not sufficient to allow effective MMR, as biochemical assays containing native nucleosomes (that will contain H3K36me3) do not reconstitute MMR, suggesting the need in vivo for additional chromatin regulators and/or the presence of replication machinery components or the replication process itself.

Mut., mutation; PIP, PCNA interacting protein box; PTMs, posttranslational modifications.

carcinoma and a Burkitt's lymphoma cell line, both MSI positive but without defects in known MMR genes, to be mutated in SETD2, thus leading to impaired hMutSα recruitment to chromatin in a manner that could be corrected by restoring the H3K36me3 mark to these cells. These data thereby offer a satisfying explanation for the long-standing discrepancy between the genotypes and phenotypes of such cancers (Figure 1B, right).

Collectively, the study of Li et al. paves the way for a multitude of future investigations. For instance, because H3K36me3 marks active transcription units, it will be intriguing to test whether this chromatin-induced pathway of hMutSα recruitment displays parallels with the transcription-coupled repair branch of nucleotide excision repair that corrects various DNA lesions, including UV-induced damage, in actively transcribed genome regions (Tornaletti, 2009). Does hMutSα analogously provide a genome safety mechanism primarily dedicated toward active transcription units, and does hMutSβ, which does not contain a PWWP domain, mediate more generally distributed MMR throughout the genome? Employing new DNA sequencing technologies could shed light on such spatial preferences of different MMR pathways by investigating not only the mutation frequencies arising upon loss of certain MMR factors but also the distributions of ensuing mutations in relation to transcription units, chromatin domains, and/or other genomic features. Further data mining of existing MSI-positive cancer genome data sets for other gene mutations could also identify additional chromatin marks and regulators that influence MMR. These studies seem particularly worthwhile because MMR in biochemical assays is still inhibited by nucleosomes even in the presence of H3K36me3 (Li et al., 2013), suggesting the requirement for additional chromatin regulators and/or replication machinery components or the replication process itself for successful MMR to ensue in vivo (Figure 1C). In this regard, it will be of interest to assess whether the PWWP domain of hMSH6 could bind to additional histone marks and, if so, how such marks might influence MMR. Because hMSH6 contains not only a PWWP domain but also a PCNA-interacting protein (PIP) box (Figure 1B) that targets hMutSα to the replication fork, it will be interesting to study how these two domains function together to promote optimal chromatin/DNA targeting of hMutSα. Furthermore, Li et al.'s findings raise the question how exactly hMutSα is released from H3K36me3 to reach its final destination on nucleosome-free replicating DNA. Because H3K36me3 levels are not only determined by histone methyltransferases, such as SETD2, but also by histone demethylases, for instance of the KDM4 family (Kooistra and Helin, 2012), it seems likely that a tightly regulated interplay between the activities of these two protein families

may fine-tune H3K36 methylation during the cell cycle and may thus also regulate MMR in vivo. In this regard, it will be interesting to see whether misregulated activity of KDM4 family proteins is associated with tumors, particularly those of the MSI variety. Further studies into relationships between DNA repair pathways and chromatin regulators therefore seem poised to not only provide new insights into DNA repair processes but may also suggest new opportunities for better diagnosing and treating cancer and perhaps other age-related human diseases.

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