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Regulation of Mismatch Repair by Histone Code and Posttranslational Modifications in Eukaryotic Cells

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Abstract

DNA mismatch repair (MMR) protects genome integrity by correcting DNA replication-associated mispairs, modulating DNA damage-induced cell cycle checkpoints and regulating homeologous recombination. Loss of MMR function leads to cancer development. This review describes progress in understanding how MMR is carried out in the context of chromatin and how chromatin organization/compaction, epigenetic mechanisms and posttranslational modifications of MMR proteins influence and regulate MMR in eukaryotic cells.

Keywords

H3K36me3; histone methylation; MSH2; PCNA; phosphorylation; deacetylase; ubiquitination

Introduction

DNA mismatch repair (MMR) ensures genome stability by correcting DNA replication-associated mispairs (see Kolodner, this issue), modulating DNA damage response (see Li, Z. et al., this issue) and regulating homeologous recombination (see Tlam and Lebbink, this issue). By coupling with DNA replication [1–3], MMR preserves replication fidelity by removing misincorporated bases and insertion-deletion mispairs from newly synthesized daughter DNA strands. Loss-of-function mutations or hypermethylation of MMR genes can increase the mutation frequency, and in mammalian cells, this can increase susceptibility to certain cancers, including hereditary non-polyposis colorectal cancer (HNPCC; also called Lynch syndrome) [4–7], also see Kolodner, this issue, and Heinen, this issue). The

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eukaryotic protein components that are sufficient to reconstitute MMR *in vitro* on naked heteroduplex DNA include MutS α (MSH2-MSH6) and MutS β (MSH2-hMSH3), MutL α (MLH1-PMS2 in humans and Mlh1-Pms1 in yeast), proliferating cell nuclear antigen (PCNA), exonuclease 1 (EXO1), replication protein A (RPA), replication factor C (RFC), DNA polymerase δ , and DNA ligase I [8–11].

In the past two decades, the biochemical characteristics of the MMR pathway has been extensively studied, primarily using a well-established *in vitro* assay and a model nucleosome-free heteroduplex DNA substrate. Those studies demonstrate that MMR is targeted specifically to the nicked (newly synthesized) DNA strand [12, 13], also see Kadyrova, this issue). It is generally accepted that MMR is initiated by the binding of MutS α or MutS β to a mispair (either a base-base mismatch or a small insertion-deletion mispair), which triggers concerted interactions between MutS α , MutL α , PCNA and RPA, and facilitates communication between the mismatch and a strand break. Subsequently, EXO1 is recruited to a pre-existing nick or a nick generated by MutL α [14], typically lying 5' to the mismatch on the daughter DNA strand. EXO1 then excises nascent DNA from the nick toward and beyond the mismatch to generate a single-strand gap, which is filled by polymerase δ using the parental DNA strand as template. Finally, the nick in the daughter DNA strand is ligated by DNA ligase I [15–17].

It came as a surprise that neither purified MMR proteins nor nuclear extracts of human cells could repair DNA mismatches in the context of chromatin *in vitro* [18, 19]. One possible explanation for this result is that chromatin structure itself inhibits communication between the mismatch and nick site. Alternatively, MutS may not efficiently recognize a DNA mispair when it is bound by a histone octamer and/or condensed in compact chromatin bound to other non-histone chromosomal proteins [20]. These observations suggest that additional factors are required for efficient MMR in human cells. Consistent with this, emerging evidence suggests that chromatin remodeling/modification factors interact with both MMR proteins and the DNA replication machinery, and that epigenetic marks on histones play a role during initiation of MMR *in vivo* [1, 18, 21–24]. Recent studies also strongly implicate posttranslational modifications of MMR proteins and crosstalk between epigenetic and non-epigenetic mechanisms in regulating MMR in human cells. This review describes progress in understanding how MMR is carried out in the context of chromatin and how posttranslational modifications of MMR proteins influence and regulate MMR in eukaryotic cells.

Role of chromatin remodeling and assembly factors in MMR

The idea that chromatin structure modulates MMR [20] and the local or regional mutation rate is not new [25]. For example, a heterotrimeric remodeling complex called RFX that regulates transcription by facilitating histone acetylation [26] also stimulates MMR *in vitro* [27], although a similar role *in vivo* has not been verified. In addition, it has been reported that human MutS α (hMutS α) can disassemble nucleosomes on heteroduplex DNA [28]. Nevertheless, fully-modified nucleosomes from HeLa cells, which presumably carry an intact HeLa cell histone code, including H3 acetylation, inhibit MMR *in vitro* [18, 24]. Therefore, the hMutS α nucleosome disassembly activity, if present, is insufficient to support

MMR on chromatin, and additional factors that allow MMR to proceed in the context of chromatin have yet to be identified.

Kadyrova et al. [21] recently showed that chromatin assembly factor 1 (CAF-I), also thought to be a histone chaperone, is required during cell-free MMR to facilitate nick-dependent nucleosome assembly. Furthermore, hMutS α suppresses CAF-1-catalyzed nucleosome assembly in a mismatch-dependent manner, and nucleosome deposition by CAF-1 following mismatch removal protects the nascent DNA strand from excessive degradation by the MMR machinery. Schopf et al. [24] also demonstrated that CAF-1-catalyzed chromatin assembly occurs more slowly on heteroduplex than on homoduplex DNA. Although the detailed mechanism is not known, PCNA is thought to coordinate MMR with nucleosome loading [24] by interacting with both the hMSH6 subunit of hMutS α [29–31] and CAF-1 [24]. Interestingly, hMutS α and CAF-1 also interact with each other [24]. It is possible that in the presence of a mispair, PCNA recruits hMutS α to the mismatch to promote MMR [32]; and after mismatches are removed, PCNA interacts with CAF-1, triggering nucleosome assembly in nascent DNA, limiting the extent of DNA excision by the MMR machinery [21, 24]. Although evidence is lacking to support the idea, ubiquitylation, phosphorylation, or acetylation of PCNA might control the balance between its two roles, as reported for DNA polymerases during translesion DNA synthesis [33].

Role of histone modifications in MMR in vivo

Many chromatin modifying/remodeling factors contain a Pro-Trp-Trp-Pro (PWWP) domain, a member of the ‘Royal Family’ that also consists of Tudor, chromodomain and MBT [34]. PWWP domain-containing proteins are often involved in chromatin-associated DNA metabolisms [35]. The common feature of the “Royal Family” members is their ability to interact with methylated lysine/arginine residues in histones or other proteins through an aromatic cage [35–38]. The hMSH6 subunit of hMutS α possesses a PWWP domain [36, 39], suggesting that it interacts with histone(s). Recent studies provide evidence to support this idea, showing that hMSH6 is a ‘reader’ for trimethylated Lys36 of histone H3 (H3K36me3) [40, 41]. Surprisingly, hMutS α without the hMSH6 PWWP domain is active in MMR in vitro and forms a “normal” DNA-protein co-crystal [42] as observed for other MutS family proteins lacking a PWWP motif [43–45]. The physiological function of the hMSH6 PWWP domain and its interaction with H3K36me3 were only recently discovered [18].

Using a biochemical and cellular approach, Li et al. provided evidence that the H3K36me3-hMSH6 PWWP interaction, although dispensable in vitro, is required for MMR in vivo [18]. Both H3K36me3 and the hMSH6 PWWP domain are essential for localization of hMutS α to chromatin, a process that varies through the cell cycle according to the abundance of H3K36me3. This is because H3K36me3 peaks in late G1/early S and dips in late S/G2, effectively increasing the efficiency of MMR when MMR is needed during the cell cycle to repair replication-associated misincorporation. Cells defective in H3K36 trimethyltransferase SETD2, despite being MMR-proficient in vitro, display a mutator phenotype, as if they were functionally MMR-deficient. Recent studies have confirmed the importance of H3K36me3 in MMR and genome stability. Down-regulation of SETD2 by

long non-coding RNA (LncRNA) HOTAIR leads to MSI and MMR deficiency [46]. Similarly, depletion of H3K36me3 by overexpressing H3K36me2/3 demethylases, KDM4A-C, disrupts MSH6 chromatin localization and induces a mutator phenotype [47]. Taken together, these observations strongly suggest that the H3K36me3 histone mark plays a critical role in MMR in vivo. We now understand that H3K36me3 effectively recruits hMutS α to chromatin through its interaction with the hMSH6 PWWP domain, immediately before DNA replication initiates.

A working model for the role of H3K36me3 in MMR is presented in Figure 1. First, before cells enter S phase, SETD2 converts H3K36me2 to H3K36me3. Then, trimethylated H3K36 recruits hMutS α onto chromatin through its interaction with the hMSH6 PWWP domain. DNA replication initiates and nucleosomes are disassembled ahead of the replication fork, which also disrupts the H3K36me3-hMSH6 PWWP interaction, leading to release of hMutS α from histone octamers. hMutS α then readily binds to temporarily histone-free nascent DNA through its strong DNA binding activity and/or by interacting with PCNA via the hMSH6 PCNA-interaction protein (PIP) box. hMutS α , which possesses an ATP-dependent sliding activity [48–51], then slides along the nucleosome-free DNA [50] to locate mispairs generated during DNA replication. When hMutS α binds a mismatch, downstream MMR events ensue, such that mispaired bases are removed before mismatch-containing nascent DNA is wrapped into a nucleosome. The precise timing and sequence of events are critical, as nucleosomes inhibit MMR [19, 21, 24]. The discovery of the relationship between H3K36me3 histone and the precise kinetics of MMR has been an important step in understanding how the histone code contributes to high replication fidelity in eukaryotic cells, by enhancing MMR efficiency when cells need it the most.

However, there are many unanswered questions. For example, is SETD2/H3K36me3 a useful biomarker for cancer susceptibility, and might its absence correlate with microsatellite instability (MSI) in MMR-proficient cells (i.e., cells that lack mutations in MMR genes)? Could errors in the histone code explain the MSI-positive tumors, including some in HNPCC families, that do not have detectable mutations in nor hypermethylation of MMR genes [52, 53]? Further studies are needed to answer these questions.

Although SETD2 has been reported to be responsible for most of the conversion from H3K36me2 to H3K36me3 [54], it is also worth noting that the level of H3K36me3 is tightly regulated by several other histone methyltransferases (e.g., SETD3, SETMAR, NSD1, NSD2, NSD3, ASH1L, and SMYD2) and histone demethylases (e.g., KDM2A, KDM2B, KDM4A, KDM4B, KDM4C and NO66) [55, 56]. Defects in any one of these histone methyltransferases or histone demethylases could alter the balance of H3K36 metabolism, the effects of which remain unknown. In addition, a recent study showed that point mutation at G34 of histone H3 interferes with formation of H3K36me3 [57, 58]. To understand the significance, production and biological roles of H3K36me3, future studies should investigate correlations between: 1) MSI-positive cancers, MMR status and global or local abundance of H3K36me3; and 2) defects in H3K36me3 metabolism, Histone H3 mutation, MMR status and genome instability.

Another important question is whether the local abundance of H3K36me3 influences local mutation rate? It is well recognized that there are mutation “hot spots” in the genome that exhibit unusually high mutation frequencies [59, 60], but the exact mechanism(s) contributing to local mutation frequency are unknown. Are “hot spots” related to low abundance of H3K36me3? In other words, if the local concentration of hMutS α , which may be determined by H3K36me3 abundance, influences MMR efficiency, a low level of H3K36me3 could potentially lead to an increase in local mutation rate. A recent study reveals that the density of somatic mutations in cancer genomes correlates inversely with abundance of H3K36me3 and open chromatin [25]. A thorough understanding of the relationship between H3K36me3 abundance/distribution in individual nucleosomes and mutation frequency in the corresponding DNA sequences will provide significant insight into cancer etiology.

It is well documented that the incorporation of histone variants is particularly important for regulating a variety of biological process [61], suggesting that H3K36me3 may not be the only histone mark that influences genome stability and cell growth. Direct defective or mutational evidence from human cancers suggest that histone variants are involved in the tumorigenesis [57, 58, 61]. Recent studies indicated that the tumor suppressor protein ZMYND11 recognizes K36me3 in histone variant H3.3 (H3.3K36me3) through its bromo-PWWP domain, but does not recognize the same mark in other H3 variants; this interaction was linked to cancer cell growth in vitro and tumor formation in vivo [62]. It is worth considering the possibility that the hMSH6 PWWP domain also recognizes the K36me3 mark in a non-canonical histone H3 variant. Thus, it would be interesting to determine the genome-wide overlap of hMSH6 and H3 variants, which could provide more mechanistic insight into how the local abundance of H3K36me3 influence local mutation rate. It was also shown recently that incorporation of the H2A.Z variant by SWR-C may stimulate EXO1 activity, as well as enhance the delity of replication by Pol δ and the MMR efficiency [63].

Interestingly, the abundant acetylation on H3K56 in yeast are involved in mutation avoidance mechanisms that cooperate with mismatch repair and the proofreading activities of replicative DNA polymerases in suppressing spontaneous mutagenesis [22]. Although H3K56ac abundance is very low in human cells, we cannot rule out the possibility that H3K56ac and other histone modifications regulate human MMR and influence cancer risk. Therefore, the possible roles of histone variants as well as histone modifications in modulating MMR should be vigorously explored in future studies.

Differential recruitment of hMutS α and hMutS β

Human cells possess at least two mismatch recognition proteins, i.e., hMutS α (hMSH2-hMSH6) and hMutS β (hMSH2-hMSH3). hMutS α represents 80–90% of the cellular MSH2, preferentially recognize base-base mismatches and 1–2 nucleotide insertion/deletion(ID). hMutS β recognizes ID mismatches of 2 to about 10 nucleotides, weakly recognizes single-nucleotide ID mispairs and barely bind to base-base mismatches [64]. The cellular stoichiometric ratio of MutS α to MutS β is approximately 10:1 and overexpression of MSH3 results in a strong mutator phenotype, presumably because the excess MSH3 saturates the pool of MSH2, essentially depleting MutS α in cells [65, 66]. Both genetic and biochemical

studies suggest that MutS α and MutS β are partially redundant because MSI levels of MSH6 defective cell lines was lower than that observed for MSH2 defective cell lines [67, 68]. However, the emerging evidence from large-scale cancer genome sequencing data revealed that local MSI frequency is inversely correlated with single-nucleotide variations (SNV) mutation rate [69], implicating possible distinct role of MSH3 and MSH6 in maintaining the DNA replication fidelity that is caused by different recruitment mechanisms.

It is worth noting that eukaryotic MSH3 and MSH6 contain a PCNA interacting protein (PIP) box [29–31], which until now, was thought to play a major role in recruiting MutS α and MutS β to DNA [1, 31]. However, deletion of the PIP box only moderately (~10–15%) reduces MMR activity in yeast [1, 70] and does not abolish formation of hMSH6 foci in human cells [31]; therefore, it is likely that eukaryotic MutS proteins are initially recruited to chromatin through a histone mark, after which the PIP box helps localize the chromatin-bound proteins to newly-formed mispairs via interactions with PCNA. Further investigations are needed to explore this and other possibilities, including whether MutS proteins are independently recruited by histone marks and PCNA.

Unlike hMSH6, hMSH3 does not contain a PWWP domain for association with histone mark H3K36me3. Is hMutS β also recruited by a different histone modification? Recent studies suggest this may be the case. For example, although yeast MutS α (yMutS α) does not contain a PWWP domain, it is localized to replicating chromatin in a mismatch-independent manner [1], possibly before DNA replication initiates. If this indicates that yMutS α recognizes and is recruited by a histone mark, then, by analogy, it is possible that hMutS β also interacts specifically with a histone mark other than H3K36me3, which may explain the discrepancy between local MSI frequency and SNV mutation rate in cancer cells.

MMR and transcription-associated genome instability

H3K36me3 promotes transcription elongation in actively transcribed genes by preventing RNA polymerase II from initiating transcription at cryptic promoters [71]. This raises the following questions. First, there is compelling evidence that transcription associated genome instability requires clashes between RNAPII transcription and DNA replication [72, 73], and specifically RNAP arrest and backtracking, can give rise to replication-derived genome instability [74]. Because MMR is closely associated with both DNA replication and H3K36me3, it is possible that the transcriptional machinery and MMR components could compete and collide in the same genomic region. If this occurs, one or both processes will be disrupted, leading to transcription-associated genome instability. Recent study showed that RecQL5 controls transcript elongation and suppresses genome instability associated with transcription stress [74], although so far no DNA helicase has been identified to play a role in eukaryotic MMR as in *E. coli*, the exploration of participation of helicases such as BLM, WRN, RecQL4 or RecQL5 in the avoidance of genome instability caused by collision/competition between RNAPII transcription and DNA mismatch repair may be intriguing. Secondly, does H3K36me3 recruit hMutS α to directly or indirectly participate in the transcription-coupled nucleotide excision repair (TC-NER) [75] that preferentially repairs bulky DNA lesions in the transcribed strand of actively transcribed genome regions [76]? hMutS α is likely recruited to transcriptional domains for TC-NER through

H3.3K36me3. However, the molecular mechanism by which hMutS α participates in the repair process remains to be investigated.

PCNA tyrosine phosphorylation in cancer cells alters its function in MMR

Recent studies revealed that higher expression of EGFR is associated with microsatellite instability [77], the hallmark of MMR deficiency, and that EGFR phosphorylates PCNA at tyrosine 211 (PCNA-Y211p) in human cancer cells [78]. In EGFR-positive human cancer cells with a known MMR defect, the genetic MMR defect was only partially complemented by the appropriate recombinant wild type MMR protein [79]. One possible explanation is that EGFR phosphorylates PCNA on Y211 and PCNA-Y211p directly or indirectly inhibits MMR. Ortega et al. [79] present multiple lines of evidence to confirm this hypothesis, including direct stimulation of MMR in vitro by unphosphorylated PCNA but not by PCNA-Y211p. The study also characterized the effect of recombinant PCNA isoforms Y211D and Y211E, which mimic constitutively phosphorylated PCNA, and PCNA-Y211F, which mimics constitutively unphosphorylated PCNA, on efficiency of MMR. Consistent with the hypothesis, PCNA-Y211F neither stimulates nor inhibits MMR in vitro, while PCNA-Y211D/E inhibits MMR in vitro. Additional experiments provide evidence that PCNA-Y211p inhibits MMR at the initiation step, possibly because the phosphorylated tyrosine in PCNA-Y211p is poorly accommodated in the PCNA binding pockets of MutS α and MutS β . In summary, this novel study provides evidence that PCNA-Y211p may be more abundant in EGFR-expressing cancer cells, and this may lower the efficiency of MMR, compounding other factors that deregulate genome maintenance and/or cell growth control in EGFR-positive human cancer cells. This study suggests a novel mechanism by which post-translational modification of replicative factor PCNA could lead to increased mutation rate and/or cancer progression [79, 80]. Additional in vivo and clinical studies are needed to explore the implications of this result.

It has recently been shown that exposure to arsenic stimulates expression of EGFR and induces tyrosine-phosphorylation of PCNA [81]. In this context, the putative carcinogenic effect of arsenic in humans may be mediated by its ability to promote EGFR-dependent PCNA phosphorylation, thereby inhibiting mismatch repair. If it were true, this would also represent a novel post-translational pro-carcinogenic mechanism that involves a PCNA-dependent effect on MMR-efficiency, and a novel mechanism of action for a non-genotoxic carcinogen.

Regulation of MMR by MSH2 acetylation and ubiquitination

The importance of histone deacetylase (HDAC) enzymes in regulating chromatin compaction and gene expression is well recognized [82]. Although HDACs were initially found to remove acetyl group from lysine residues on histone proteins, some of these enzymes, e.g., HDAC6, can catalyze the reaction on cytosolic proteins. HDAC6 contains two functional tandem deacetylase domains (designated DAC1 and DAC2) and a ZnF-UBP domain, a zinc finger-containing region that is homologous with the noncatalytic domain of several ubiquitin-specific proteases (USPs) [83]. HDAC6 is now regarded as an important regulator of the cellular response to cytotoxic and genotoxic stresses [84–86].

In a recent study, Zhang and colleagues show that HDAC6 is a major MSH2 deacetylase in human cells [87]. They provide strong evidence that HDAC6 sequentially deacetylates and, via its intrinsic ubiquitin E3 ligase activity, ubiquitinates MSH2 in the MutS α heterodimer, leading to MSH2 degradation. This study suggests that HDAC6 plays a major role in regulating MSH2 protein stability through acetylating and ubiquitinating MSH2, and thereby also plays a major role in regulating MMR function and genome stability. However, much work remains to fully understand the regulation of MSH2 and other MMR proteins by post-translational modifications, such as acetylation, phosphorylation and ubiquitination.

Perspectives

MMR is an important mutation avoidance system found in all organisms from bacteria to man. In eukaryotic cells, chromatin structure modulates all DNA metabolic processes, including DNA replication and transcription; thus, the recent discovery that chromatin structure and the histone code play a role in coordinating MMR, DNA replication and transcription in eukaryotic cells is neither surprising nor unexpected. It is also not surprising that post-translational phosphorylation, acetylation and ubiquitination of MMR proteins, such as PCNA and MSH2, regulate critical aspects of MMR, including its efficiency in normal and transformed cells with or without DNA damage. Looking to the future, many more protein post-translational modifications that regulate or modulate MMR are likely to be discovered, and our appreciation of the complexity of these regulatory processes will only increase. Possibly, additional components or factors that influence MMR efficiency and/or timing remain yet to be identified, and that new roles for MMR or novel interactions or crosstalk between MMR, DNA replication, DNA transcription, cell cycle control and other cell signaling pathways will emerge in the near future.

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Abbreviations

MMR	mismatch repair
MSI	microsatellite instability
PCNA	proliferating cellular nuclear antigen
RPA	replication protein A
RFC	replication factor C
EXO1	exonuclease 1
H3K36me3	histone H3 lysine 36 trimethylation

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Highlights

- Histone mark H3K36me3 is required for mismatch repair in vivo.
- PCNA phosphorylation promotes genome instability by inhibiting mismatch repair.
- MSH2 deacetylation/ubiquitination by HDAC6 downregulates mismatch repair activity.

Figure 1. Recruitment of hMutSα to replicating chromatin

SETD2 converts H3K36me2 to H3K36me3, which interacts with the hMSH6 PWWP domain to localize hMutSα to chromatin before DNA replication initiates. During DNA replication, nucleosomes are disassembled and the H3K36me3-PWWP interaction is disrupted, releasing hMutSα from nucleosomes. hMutSα readily binds to nascent DNA independent of PCNA, and recognizes newly-formed mismatches to initiate MMR. This image was modified from Ref. [18].