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Mismatch recognition factor MutS binds hemi-methylated DNA substrates

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Abstract

Mismatch repair and DNA methylation are extensively-studied cellular processes that play key roles in a number of biological pathways. Their importance to cellular homeostasis is apparent from findings linking their loss or deregulation to pathologies ranging from neurodegeneration to cancer. The interplay between these pathways in bacteria as well as in higher eukaryotes has been amply documented, but many questions regarding its role are yet to be answered. Here, we show that the human mismatch recognition factor MutS (heterodimer of MSH2 and MSH6) binds to hemi-methylated DNA substrates. Given the involvement of DNA methylation in chromatin remodeling and transcriptional control, and the link between mismatch repair defects and DNA hypermethylation, we hope that our serendipitous finding will inspire future studies aiming to elucidate its significance.

Introduction

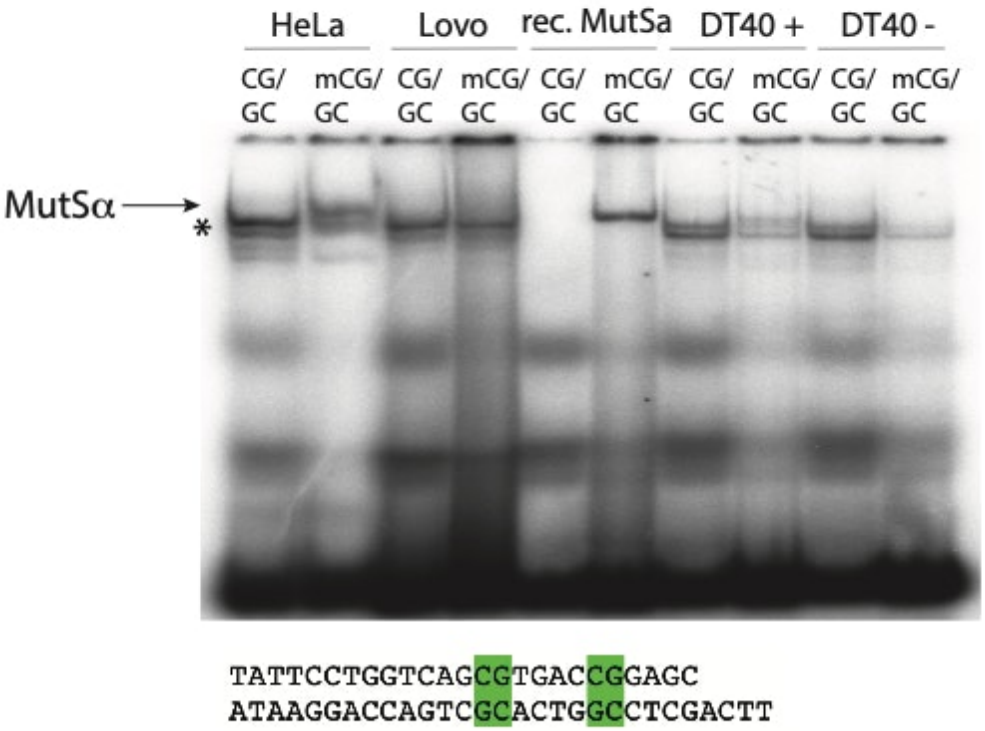
DNA methylation at the 5' position of cytosine in vertebrates plays a crucial role in imprinting, X chromosome inactivation, and gene regulation [1] [2]. It occurs primarily at CpG sites, 70–90% of which are symmetrically methylated in higher eukaryotes [3]. An exception represents the so-called CpG islands, stretches of DNA rich in CpG sites that remain generally unmethylated. CpG islands have been the subject of intense study, due to the impact their aberrant methylation has on ageing and cancer [4] [5] [6] [7]. In contrast, the importance of the methylation states of single CpG sites is still not very well understood, partly because it is harder to detect and functionally assess than high-density CpG sites in defined promoters. To learn more about the biological roles of single CpGs, it is crucial to examine how the methylation patterns at defined loci are altered and how they affect protein binding and gene expression.

In somatic cells, methylation patterns are usually strictly maintained with the help of DNA methylation maintenance machinery comprising DNA methyltransferase 1 (DNMT1) and Ubiquitin Like with PHD and Ring Finger Domains 1 (UHRF1), which travel with the replication fork and ensure that the newly-synthesized DNA strand is immediately methylated. Hemi-methylated CpG sites are therefore considered to exist only very transiently, primarily during DNA replication, long-patch repair, or in the course of an active DNA demethylation process. However, occasional persistent hemi-methylated CpG sites have been described [8], even though their function remains enigmatic. It is also unknown how they are protected from the maintenance methylation machinery.

In the course of our study concerned with the role of DNA methylation in the transcriptional control of the hormonally-regulated vitellogenin II gene, we carried out a series of electrophoretic mobility shift assays (EMSAs), using defined unmethylated, hemi-methylated and fully-methylated DNA oligonucleotide duplexes, together with extracts of human and chicken DT40 cells. As anticipated, the sequences were efficiently bound by their cognate transcription factors present in the extracts. Surprisingly, we also detected a protein/DNA complex of lower electrophoretic mobility found this protein complex to be the well-characterized mismatch repair (MMR) factor MutS (a heterodimer of MSH2 and MSH6). The possible significance of this unexpected finding is discussed below.

Objective

We aimed to characterize a specific mobility shift on hemi-methylated DNA substrates.



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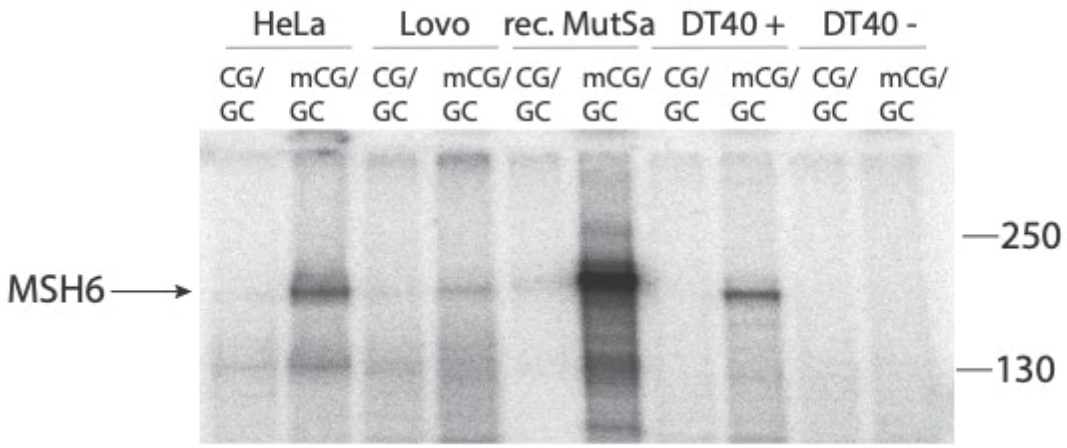

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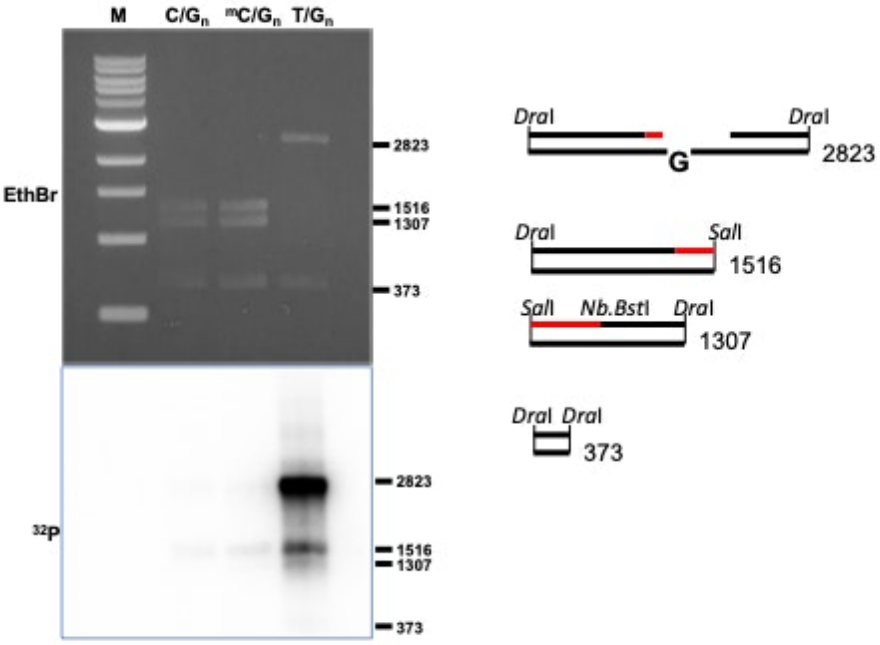
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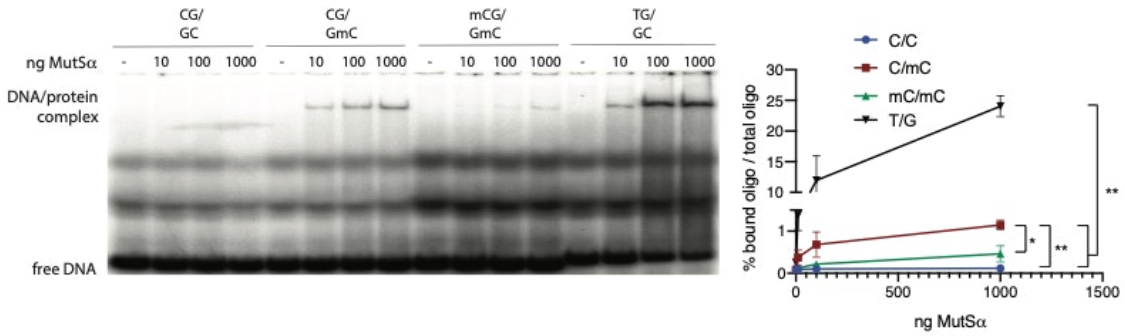
c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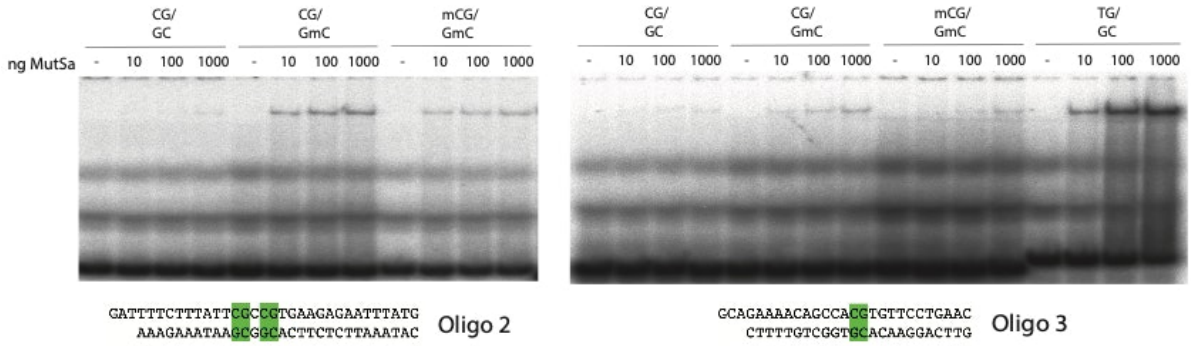
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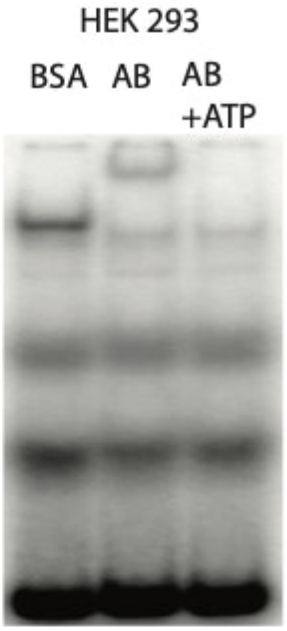

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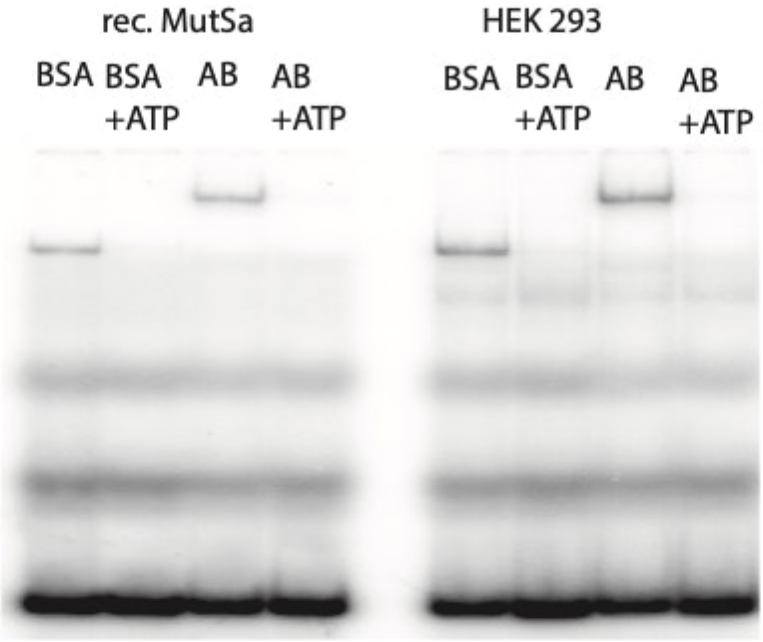

g

Figure Legend

Figure 1.

(A) EMSA with 10 µg of the indicated nuclear extracts or 100 ng recombinant MutS and oligo 1 (lower panel). Binding on unmethylated oligo (CG/GC) and hemi-methylated oligo (mCG/GC) was tested. The two CpG sites (green) were equally modified. DT40-, extracts of DT40 MSH2-/- cells; DT40+ wild type DT40 cell extracts. The asterisk indicates bands relevant for another study.

(B) The UV-crosslinked binding reaction using 60 µg of the indicated nuclear extracts or recombinant MutS (same as in **A**) and oligo 1 containing one BrdU on each strand (see Methods). Crosslinked complexes were resolved on denaturing polyacrylamide gels and visualized on phosphor screens.

(C) *In vitro* MMR assay. The substrates (see M&M) were incubated with MMR-proficient nuclear extracts of HEK293 cells, recovered and digested with *Dra*I and *Sal*I, yielding a repair-dependent restriction pattern (right panel). Mismatch recognition by MutS leads to the activation of EXOI, which degrades several hundred nucleotides from the nick until the mispair is removed. In a subsequent step, pol-delta refills the gap, which should restore the C/G pair, and thereby the *Sal*I site, at position 42. The upper left panel, an image of an agarose gel, shows that the C/G and mC/G substrates were-fully digested by the restriction enzymes, whereas the T/G substrate was largely undigested, indicative of incomplete repair. However, autoradiograph of the same gel shows heavy 32P incorporation into the 2823 bp band in the T/G reaction, indicative of substantial mismatch-dependent exonucleolytic degradation, with a subset of the substrate molecules having been repaired to C/G, as indicated by the presence of radiolabel in the 1516 bp band (left lower panel). In contrast, only very small amounts of radiolabel were incorporated into the C/G and mC/G substrates, suggesting that no appreciable repair synthesis took place on these nicked plasmids.

(D) Left panel: Representative EMSA with increasing concentrations of recombinant MutS (0, 10, 100, and 1000 ng) and oligo 1 with the indicated combinations of methylation. An oligo with a T/G mismatch was included as a positive control (last 4 lanes). Right panel: Quantification of percentage bound oligo/total oligo in three independent experiments. Data are represented as mean ± SD. Significance was assessed by an ANOVA test, followed by the Tukey's multiple comparison test, comparing each concentration separately. The asterisk corresponds to the 1000 ng concentration, whereas the other concentrations were not significant using the described test. $^{*}p \leq 0.05$; $^{**}p \leq 0.01$.

(E) Same as **D**, but on oligos depicted below the gels (CpG sites in green).

(F) Supershift experiments using either BSA as a control or an antibody against MutS (AB, Anti-MSH6 BD-610919) without or with ATP (1 mM) on oligo 1 with nuclear extracts of HEK 293 cells.

(G) Same as **F**, but with recombinant MutS (left panel) or nuclear extracts of HEK 293 (right panel) on oligo 2.

Results & Discussion

The fact that DNA methylation changes the topology of DNA and its interaction with proteins is well established, but questions concerning the control and dynamic regulation, tissue- and locus-specificity of DNA methylation, as well as the molecular determinants affecting protein/DNA recognition, remain to be elucidated.

We were investigating the methylation dynamics of single CpG sites in the chicken vitellogenin II promoter and the effect these modifications have on transcription factor binding and gene expression. Using electrophoretic mobility shift assays (EMSAs) and UV crosslinking coupled with SDS polyacrylamide gel electrophoresis (PAGEs), we detected the expected protein/DNA complexes formed by the tested DNA sequences and their cognate proteins. However, we also observed a band of slightly lower electrophoretic mobility that was specific for the hemi-methylated substrates. Its migration

characteristics in the EMSA assays, coupled with mass spectrometric analysis of the shifted complex, suggested that the bound protein might be the MMR factor MutS.

To gain more support for the above finding, we repeated the EMSAs with nuclear extracts (NE) of cell lines with different MutS expression levels as well as purified recombinant MutS, using unmethylated and hemi-methylated DNA substrates (Fig. 1A). The low mobility band specific for the hemi-methylated substrate could be detected in NEs of the endometrial cell line HeLa and the chicken B cell line DT40 (DT40+). The fact that the band migrated with similar mobility to that seen with recombinant MutS and that this band was absent in EMSAs with extracts of the colon adenocarcinoma cell line LoVo that lacks MutS, as well as of DT40 MSH2-/- (DT40-), cells provided strong evidence that the hemi-methylated DNA-specific binding protein was indeed MutS. The shift created by MutS ran slightly slower than the shift we were investigating as part of another study (marked with an asterisk).

An aliquot of the same binding reactions was irradiated with UV light and resolved on denaturing SDS PAGE (Fig. 1B). Because the oligo substrate was radiolabelled but also contained BrdU (see Methods), the protein(s) and the bound DNA substrate were covalently crosslinked by UV to generate a protein/DNA complex that was radiolabeled and that had a molecular mass equal to the sum of its two constituents. The hemi-methylated DNA-specific band was significantly stronger in HeLa and DT40 extracts and substantially so when recombinant MutS was used, while this band was not detected in NE of DT40 cells lacking MutS (DT40-). The weak band visible with the LoVo extracts could be explained by trace amounts of MSH6 present in the extracts despite the deletion of *MSH2* [9]. We were curious to see whether MutS binding to hemi-methylated CpG sites would trigger nick-dependent strand degradation and DNA repair synthesis. We performed an *in vitro* mismatch repair assays using MMR-proficient HEK293 nuclear extracts and pre-nicked plasmids containing an unmethylated (negative control) or hemi-methylated CpG site. A substrate containing a T/G mismatch was used as a control. We detected substantial repair synthesis (visualized as the incorporation of a radioactively-labeled nucleotide) in the T/G-containing control, but not in the hemi-methylated or the unmethylated substrate (1C).

Next set out to investigate the binding affinity of recombinant MutS to 3 different oligo substrates. We first compared its binding in EMSA assays to unmethylated (CG/GC), hemi-methylated (CG/GmC), and fully-methylated (mCG/GmC) substrate, using increasing amounts of MutS (Fig. 1D & 1E). A substrate harboring a T/G mismatch (TG/GC), the preferred substrate for MutS, was used as a control with oligos 1 and 3. The most significant differences in binding were seen with oligo 1 (Fig. 1D). The differences for oligos 2 and 3 were not statistically significant upon quantification, but clearly visible in all three experiments (Fig. 1E, left panel: oligo 2, right panel: oligo 3 including T/G control).

Additional evidence for the specificity of the observed shift was gained through supershift experiments, using an antibody against human MSH6 with HEK293 NEs and oligo 1 (Fig. 1F), or recombinant MutS and HEK293 NEs on oligo 2 (Fig. 1G, left panel: recombinant MutS, right panel: HEK293 NEs). Binding of the antibody caused retardation of the complex in the EMSA assay and the addition of 1 mM ATP completely abolished binding of MutS to the DNA, as previously shown [10] [11] [12], further confirming the specificity of the mobility shift. Since we showed clear binding of MutS to hemi-methylated oligos but did not observe any detectable levels of repair activity upon incubation of plasmids containing hemi-methylated sequences in repair-proficient nuclear extracts, we can only speculate about the biological significance of the observed binding behavior of MutS. However, we cannot exclude the possibility that hemi-methylated CpGs might provoke MutS-dependent repair *in vivo*.

The connection between MMR and DNMT1 has been reported before. DNMT1 deficiency has been connected with microsatellite instability and resistance to 6-thioguanine, assigning DNMT1 to the group of MMR proteins [13] [14] [15] [16] [17]. Other studies established a connection between MMR deficiency and hypermethylation in colorectal cancer [18] [19] [20], due to the frequent hypermethylation and therefore

inactivation of the MMR gene *MLH1*. It is, however, difficult to establish the cause-consequence relationship of these events. Moreover, the authors provide different explanations concerning the mechanistic interaction. A more recent report shows that the catalytic activity of DNMT1 is not required for its MMR-enhancing effect and shows an interaction of DNMT1 and MMR that does not depend on hemi-methylated DNA [21]. Our observation brings the possibility of a direct interaction back into the spotlight and it will be very interesting to find out more about the meaning and consequences of this finding.

Efficient MMR depends on two main criteria: (i) mismatch recognition, and (ii) direction of the repair reaction to the newly-synthesized DNA strand, which – by definition – carries the misincorporated nucleotide. It is interesting to note that, in Gramm-negative bacteria, the nascent strand is transiently-undermethylated at GATC sequences and that this time window is utilized by the MutH endonuclease to incise it and thus provide an entry site for the exonucleolytic degradation that will remove the error [22]. However, Gramm-positive bacteria do not use adenine methylation for strand discrimination and their genomes do not encode MutH homologues. The latter protein is absent also from all eukaryotes, as is *N*6-methyl-2'-deoxyadenine. Moreover, most eukaryotes possess highly-efficient MMR despite the fact that they do not methylate their DNA and available evidence indicates that higher eukaryotes the genomes of which do carry 5-methylcytosine do not deploy it for strand discrimination during MMR [23] [24]. Instead, MMR in the latter organisms is tightly-coupled to replication and makes use of strand discontinuities, such as nicks and single-stranded gaps, to distinguish between the parental and the daughter strands [25] [26]. (See [27] [28] [29] for reviews.) However, the possibility remains that there might exist special circumstances when the recognition of hemi-methylated CpGs, possibly followed by incision of one of the stands might aid an as yet unidentified process of DNA metabolism. The purpose of this report is to bring this possibility to the attention of those with access to experimental systems that might be able to throw more light on this interesting phenomenon.

Limitations

More different sequence contexts and varying densities of CpGs have to be examined to gain more insights into the role and mechanism of the observed phenomenon and to unravel which kind of promoters or other genomic elements might be affected.

The relatively low sensitivity of the *in vitro* MMR assay makes it hard to completely exclude the activation of MMR by the hemi-methylated site.

Alternative Explanations

Conjectures

It will be very interesting to further address this observation and decipher whether it is a physiologically irrelevant observation or whether it serves a bigger purpose either connected to mismatch repair itself or possibly to the regulation and dynamic metabolism of 5-methylcytosine in DNA, another highly-active research field, especially since the discovery of the ten-eleven translocation (TET) enzymes.

Additional Information

Methods

Preparation of nuclear extracts

The nuclear extracts were prepared according to Dignam et al. [30].

Electrophoretic Mobility Shift Assay (EMSA)

All EMSA reactions were carried out in binding buffer (10 mM Tris-HCl, pH 7.6, 50 mM NaCl, 1 mM DTT, 1 mg/ml BSA, 5% glycerol) in a volume of 5 µl using the unspecific competitor poly(dI-dC) (Sigma-Aldrich). 20 ng of poly(dI-dC) was used for 100 ng of recombinant MutS (purified as described in [12]) and 1 µg for 10 µg of nuclear extracts. For all EMSAs, 10 fmol of [α-32P]-dNTP labeled, or [γ-32P]-ATP phosphorylated oligos were used. For the supershift assays, 200 ng of antibody (Anti-MSH6; BD Biosciences 610919) and 1 mM ATP were added, together with the proteins where indicated. Proteins, and antibodies where indicated, were incubated for 20 min on ice in binding buffer. Labeled DNA was added and the mixture was left at RT for another 20 min before loading on a 5% polyacrylamide gel (Acryl/ Bis 29:1, Amresco) eluted with 1x TAE (40 mM Tris, 20 mM acetate, 1 mM EDTA). The gels were run at 200 V for 1 h in 1x TAE and dried in a gel dryer for 1 h. The gels were exposed to phosphor screens and the autoradiographs were developed in a Typhoon FLA 9500 (GE Healthcare Life Sciences).

Oligo 1 upper 5' TATTCC**T**GGTCAGCGTGACCGGAGC 3'

and lower 5'TTCAGCTCCGGTCACGC**T**GACCAGGAATA 3',

oligo 2 upper 5'GATTTTCTTTATTCGCCGTGAAGAGAATTTATG3'

and lower 5'CATAAATTCTCTTCACGGCGAATAAAGAAA3',

oligo 3 upper 5'GCAGAAAACAGCCACGTGTTCCTGAAC3'

and lower 5' GTTCAGGAACACGTGGCTGTTTTC3',

T/G oligo upper 5' CCAGACGTCTGTTGACGTTGGGAAGCTTGAG 3'

and lower 5'CTCAAGCTTCCCAACGTCGACAGACGTCTGG 3'. The bold, underlined Ts in oligo 1 were replaced by BrdU to enable crosslinking.

SDS-PAGE of cross-linked binding reactions

EMSAs were performed as described and cross-linked for 5 min (~720 mJ) in a UV Stratalinker 1800 (Stratagene). Samples were taken up in 2x SDS loading buffer and boiled for 5 min before loading on a 7.5 % denaturing polyacrylamide gel and run for 1–2 h in 10 % SDS-running buffer at 130 V. Gels were dried for 1 h, exposed to phosphor screens and developed in a Typhoon FLA 9500 (GE Healthcare).

In vitro mismatch repair assay

The substrate was a circular heteroduplex of 3196 bp, which contained either a C, a mC, or a T opposite a G within the *Sal*I site at position 42, as well as a single Nt.*Bst*NBI nick in the upper (C, mC, or T) strand at position 308. It was incubated with nuclear extracts of HEK293 cells supplemented with dGTP, dCTP, dTTP and [a-32P]dATP in a buffer containing 20 mM Tris HCl pH 7.6, 110 mM KCl, 5 mM $MgCl_2$, 1 mM glutathione, 1.5 mM ATP, 50 µg/ml BSA. Reactions were incubated at 30°C for 25 min, then incubated in a stop solution (0.5 mmol/l EDTA, 1.5% SDS, 2.5 mg/ml proteinase K) for 1 h. The DNA was purified using Qiagen MinElute Reaction Cleanup Kit, followed by digestion with *Dra*I and *Sal*I. The repair synthesis step can be visualized by the incorporation of the radiolabeled nucleotide. In the case of 100% efficient repair, all radioactivity should be found predominantly in the 1516 bp band, with some also in the 1307 bp band.

The restriction fragments were visualized under UV and incorporation of the radioactive nucleotide was detected on the dried gel using the Typhoon FLA 9500 PhosphorImager.

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Ethics Statement

Not Applicable.

Citations

[1] Julie A. Law and Steven E. Jacobsen. "Establishing, maintaining and modifying DNA methylation patterns in plants and animals". In: *Nature Reviews Genetics* 11.3 (2010), pp. 204–220. URL: https://www.ncbi.nlm.nih.gov/pubmed/20142834.

[2] Hongbo Liu et al. "DNA methylation dynamics: identification and functional annotation". In: *Briefings in Functional Genomics* 15.6 (2016), pp. 470–484. URL: https://www.ncbi.nlm.nih.gov/pubmed/27515490.

[3] T Naveh-Many and H Cedar. "Active gene sequences are undermethylated". In: *Proceedings of the National Academy of Sciences* 78.7 (1981), pp. 4246–4250. URL: http://www.ncbi.nlm.nih.gov/pubmed/6945582.

[4] A P Bird. "The relationship of DNA methylation to cancer". In: *Cancer Surveys* 28 (1996), pp. 87–101. URL: http://www.ncbi.nlm.nih.gov/pubmed/8977030.

[5] Adam E. Field et al. "DNA Methylation Clocks in Aging: Categories, Causes, and Consequences". In: *Molecular Cell* 71.6 (2018), pp. 882–895. URL: https://www.ncbi.nlm.nih.gov/pubmed/30241605.

[6] Peter A. Jones and Stephen B. Baylin. "The fundamental role of epigenetic events in cancer". In: *Nature Reviews Genetics* 3.6 (2002), pp. 415–428. URL: https://www.ncbi.nlm.nih.gov/pubmed/12042769.

[7] Peter A. Jones and Stephen B. Baylin. "The Epigenomics of Cancer". In: *Cell* 128.4 (2007), pp. 683–692. URL: https://www.ncbi.nlm.nih.gov/pubmed/17320506.

[8] H P Saluz, J Jiricny, and J P Jost. "Genomic sequencing reveals a positive correlation between the kinetics of strand-specific DNA demethylation of the overlapping estradiol/glucocorticoid-receptor binding sites and the rate of avian vitellogenin mRNA synthesis". In: *Proceedings of the National Academy of Sciences* 83.19 (1986), pp. 7167–7171. DOI: 10.1073/pnas.83.19.7167. URL: https://doi.org/10.1073/pnas.83.19.7167.

[9] James T. Drummond et al. "DHFR/MSH3 amplification in methotrexate-resistant cells alters the hMutSα/hMutSβ ratio and reduces the efficiency of base–base mismatch repair". In: *Proceedings of the National Academy of Sciences* 94.19 (1997), pp. 10144–10149. URL: https://www.ncbi.nlm.nih.gov/pubmed/9294177.

[10] Scott Gradia, Samir Acharya, and Richard Fishel. "The Human Mismatch Recognition Complex hMSH2-hMSH6 Functions as a Novel Molecular Switch". In: *Cell* 91.7 (1997), pp. 995–1005. URL: https://www.ncbi.nlm.nih.gov/pubmed/9428522.

[11] Ingram Iaccarino et al. "Mutation in the Magnesium Binding Site of hMSH6 Disables the hMutSα Sliding Clamp from Translocating along DNA". In: *The Journal of Biological Chemistry* 275.3 (2000), pp. 2080–2086. URL: https://www.ncbi.nlm.nih.gov/pubmed/10636912.

[12] Ingram Iaccarino et al. "hMSH2 and hMSH6 play distinct roles in mismatch binding and contribute differently to the ATPase activity of hMutSα". In: *EMBO J* 17.9 (1998), pp. 2677–2686. URL: https://www.ncbi.nlm.nih.gov/pubmed/9564049.

[13] Vincent Dion et al. "Dnmt1 deficiency promotes CAG repeat expansion in the mouse germline". In: *Human Molecular Genetics* 17.9 (2008), pp. 1306–1317. URL: https://www.ncbi.nlm.nih.gov/pubmed/18252747.

[14] Ge Guo, Wei Wang, and Allan Bradley. "Mismatch repair genes identified using genetic screens in Blm-deficient embryonic stem cells". In: *Nature* 429.6994 (2004), pp. 891–895. URL: https://www.ncbi.nlm.nih.gov/pubmed/15215866.

[15] Myungjin Kim et al. "Dnmt1 deficiency leads to enhanced microsatellite instability in mouse embryonic stem cells". In: *Nucleic Acids Research* 32.19 (2004), pp. 5742–5749. URL: https://www.ncbi.nlm.nih.gov/pubmed/15509869.

[16] Jayne E.P. Loughery et al. "DNMT1 deficiency triggers mismatch repair defects in human cells through depletion of repair protein levels in a process involving the DNA damage response". In: *Human Molecular Genetics* 20.16 (2011), pp. 3241–3255. URL: https://www.ncbi.nlm.nih.gov/pubmed/21636528.

[17] Keh-Yang Wang and C-K James Shen. "DNA methyltransferase Dnmt1 and mismatch repair". In: *Oncogene* 23.47 (2004), pp. 7898–7902. URL: https://www.ncbi.nlm.nih.gov/pubmed/15378011.

[18] Nita Ahuja et al. "Association between CpG Island Methylation and Microsatellite Instability in Colorectal Cancer". In: *Cancer Research* 57.16 (1997), pp. 3370–3374. URL: https://www.ncbi.nlm.nih.gov/pubmed/9269998.

[19] Minoru Toyota et al. "CpG island methylator phenotype in colorectal cancer". In: *Proceedings of the National Academy of Sciences* 96.15 (1999), pp. 8681–8686. URL: https://www.ncbi.nlm.nih.gov/pubmed/10411935.

[20] Binh N. Trinh et al. "DNA Methyltransferase Deficiency Modifies Cancer Susceptibility in Mice Lacking DNA Mismatch Repair". In: *Molecular and Cellular Biology* 22.9 (2002), pp. 2906–2917. URL: https://www.ncbi.nlm.nih.gov/pubmed/11940649.

[21] Keh-Yang Wang et al. "Epigenetic Enhancement of the Post-replicative DNA Mismatch Repair of Mammalian Genomes by a Hemi-mCpG-Np95-Dnmt1 Axis". In: *Scientific Reports* 6.1 (2016), p. 37490. DOI: 10.1038/srep37490. URL: https://doi.org/10.1038/srep37490.

[22] Robert S. Lahue and Paul Modrich. "Methyl-directed DNA mismatch repair in Escherichia coli". In: *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* 198.1 (1988), pp. 37–43. URL: https://www.ncbi.nlm.nih.gov/pubmed/3280983.

[23] J T Hare and J H Taylor. "One role for DNA methylation in vertebrate cells is strand discrimination in mismatch repair". In: *Proceedings of the National Academy of Sciences* 82.21 (1985), pp. 7350–7354. URL: https://www.ncbi.nlm.nih.gov/pubmed/2997788.

[24] S L Schlagman, S Hattman, and M G Marinus. "Direct role of the Escherichia coli Dam DNA methyltransferase in methylation-directed mismatch repair." In: *Journal of Bacteriology* 165.3 (1986), pp. 896–900. URL: https://www.ncbi.nlm.nih.gov/pubmed/3512529.

[25] Medini Manohar Ghodgaonkar et al. "Ribonucleotides Misincorporated into DNA Act as Strand-Discrimination Signals in Eukaryotic Mismatch Repair". In: *Molecular Cell* 50.3 (2013), pp. 323–332. URL: https://www.ncbi.nlm.nih.gov/pubmed/23603115.

[26] J Holmes Jr, S Clark, and P Modrich. "Strand-specific mismatch correction in nuclear extracts of human and Drosophila melanogaster cell lines." In: *Proceedings of the National Academy of Sciences* 87.15 (1990), pp. 5837–5841. URL: https://www.ncbi.nlm.nih.gov/pubmed/2116007.

[27] Mark J Schofield and Peggy Hsieh. "DNA mismatch repair: Molecular mechanisms and biology function". In: *Annual Review of Microbiology* 57 (2003), pp. 579–608. URL: http://www.ncbi.nlm.nih.gov/pubmed/14527292.

[28] Thomas A. Kunkel and Dorothy A. Erie. "DNA MISMATCH REPAIR". In: *Annual Review of Biochemistry* 74 (2005), pp. 681–710. URL: http://www.ncbi.nlm.nih.gov/pubmed/15952900.

[29] Josef Jiricny. "The multifaceted mismatch-repair system". In: *Nature Reviews Molecular Cell Biology* 7.5 (2006), pp. 335–346. DOI: 10.1038/nrm1907. URL: https://doi.org/10.1038/nrm1907.

[30] John David Dignam, Russell M. Lebovitz, and Robert G. Roeder. "Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei". In: *Nucleic Acids Research* 11.5 (1983), pp. 1475–1489. URL: http://www.ncbi.nlm.nih.gov/pubmed/6828386.