

# Acceleration of 5-Methylcytosine Deamination in Cyclobutane Dimers by G and Its Implications for UV-Induced C-to-T Mutation Hotspots

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Sunlight-induced C→T mutation hotspots occur most frequently at methylated CpG sites in tumor suppressor genes and are thought to arise from translesion synthesis past deaminated cyclobutane pyrimidine dimers (CPDs). While it is known that methylation enhances CPD formation in sunlight, little is known about the effect of methylation and sequence context on the deamination of 5-methylcytosine (<sup>m</sup>C) and its contribution to mutagenesis at these hotspots. Using an enzymatic method, we have determined the yields and deamination rates of C and <sup>m</sup>C in CPDs and find that the frequency of UVB-induced CPDs correlates with the oxidation potential of the flanking bases. We also found that the deamination of T<sup>m</sup>C and <sup>m</sup>CT CPDs is about 25-fold faster when flanked by G's than by A's, C's or T's in duplex DNA and appears to involve catalysis by the O6 group of guanine. In contrast, the first deamination of either C or <sup>m</sup>C in AC<sup>m</sup>CG with a flanking G was much slower ( $t_{1/2} > 250$  h) and rate limiting, while the second deamination was much faster. The observation that C<sup>m</sup>CG dimers deaminate very slowly but at the same time correlate with C→T mutation hotspots suggests that their repair must be slow enough to allow sufficient time for deamination. There are, however, a greater number of single C→T mutations than CC→TT mutations at C<sup>m</sup>CG sites even though the second deamination is very fast, which could reflect faster repair of doubly deaminated dimers.

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## Introduction

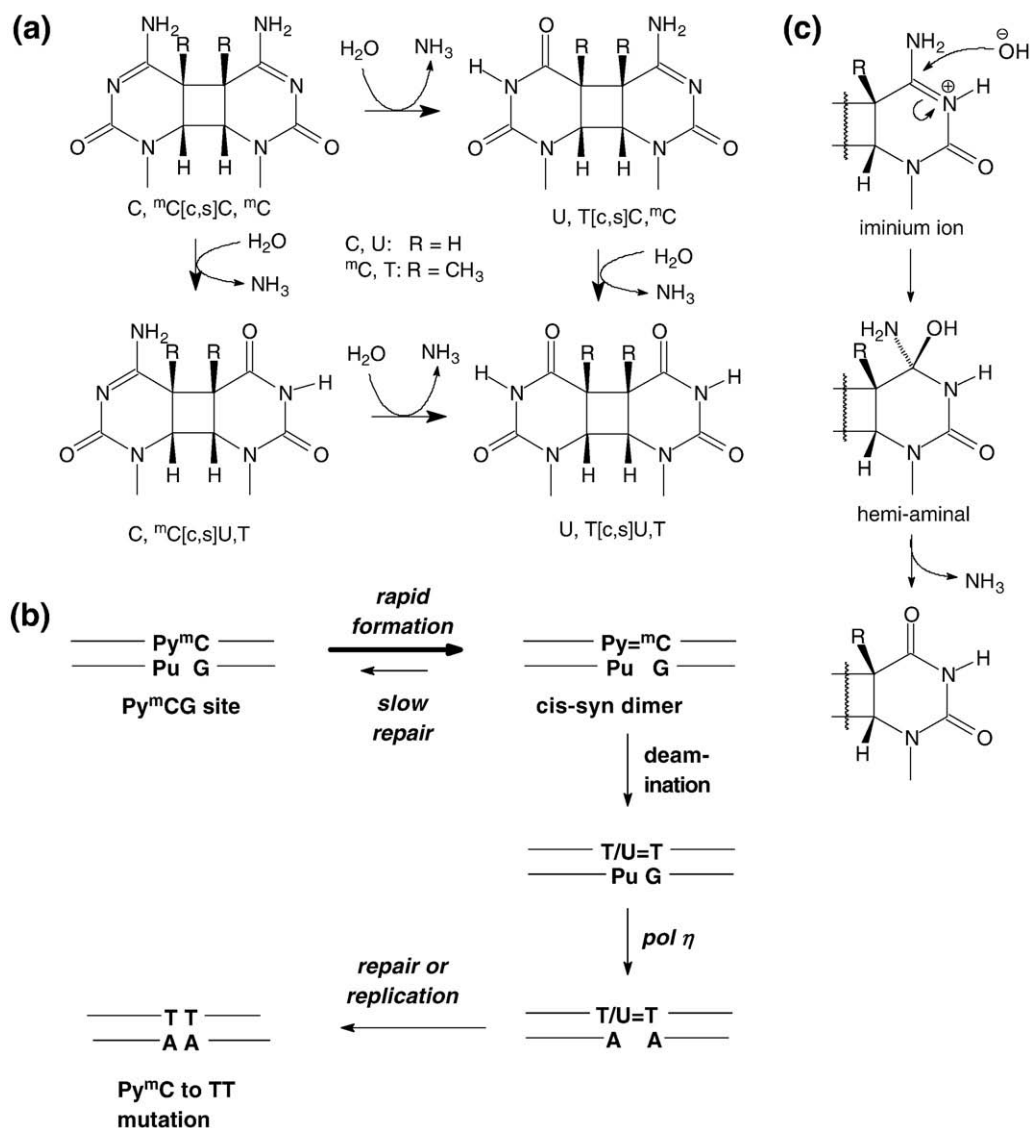
In basal and squamous cell carcinomas, the p53 tumor suppressor gene exhibits a high percentage of C-to-T transition mutations at dipyrimidine sites.<sup>1–5</sup> Previous studies on sunlight-induced mouse skin tumors indicate that 30–50% of these mutations are due to the formation of cyclobutane pyrimidine dimer (CPD) photoproducts (Fig. 1a).<sup>6,7</sup> A comparison of sunlight-induced mutation spectra of cII and

lacI transgenes as well as that of the p53 gene in human skin tumors reveal that 5-methylcytosine (<sup>m</sup>C) is involved in 25–40% of the mutations in all three systems.<sup>8</sup> Most significantly, methylation at the C of PyCG increases CPD formation in sunlight 15-fold.<sup>9</sup>

The principal polymerase involved in synthesizing past CPDs is polymerase η (pol η). Both *in vitro* and *in vivo* studies have found, however, that T-, U-, C-, and <sup>m</sup>C-containing CPDs are not measurably mutagenic when replicated by pol η.<sup>10–14</sup> Indeed, loss of functional pol η in xeroderma pigmentosum variant (XPV) results in an increased risk of skin cancer due to the replication of photodimers by more error-prone polymerases.<sup>15–17</sup> While C and <sup>m</sup>C are very stable with a deamination half-life of 30,000 years in duplex DNA,<sup>18</sup> C and <sup>m</sup>C within CPDs are more than a millionfold less stable and deaminate to U or T, respectively (Fig. 1a), in hours to days due to loss of aromatic stabilization.<sup>14,19–27</sup> The replication

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Abbreviations used: <sup>a</sup>P, 2-aminopurine; CPD, cyclobutane pyrimidine dimer; <sup>m</sup>C, 5-methylcytosine; ODN, oligodeoxynucleotide; pol η, polymerase η; Pu, purine; Py, pyrimidine; TBE, Tris-borate EDTA; XPV, xeroderma pigmentosum variant.



**Fig. 1.** Deamination of C-containing cis-syn CPDs and their proposed role in UV-induced C-to-T mutations. (a) Structures and deamination pathways. (b) Deamination bypass mechanism for the origin of UV-induced C-to-T and CC-to-TT mutations. (c) Deamination mechanism involving attack of hydroxide on the iminium ion to give the hemi-aminal intermediate.

(bypass) of these deaminated products by a pol Y family polymerase is thought to be the origin of most UV-induced C-to-T mutations, a process otherwise known as the deamination-bypass mechanism (Fig. 1b).<sup>24–26,28–31</sup> Little is known, however, about the deamination of C and <sup>m</sup>C within Py<sup>m</sup>CG mutational hotspots.

Deamination of C and <sup>m</sup>C in CPDs has been studied in dinucleotides and in single-stranded DNA by chromatographic, mass spectrometry, and enzymatic analysis.<sup>14,19,20,22,23,27</sup> Deamination has also been studied *in vivo* by genetic assays and by ligation-mediated PCR.<sup>21,24–26</sup> The reported deamination rates vary from hours to weeks depending on the substrate and organism with no clear explanation for the differences. In this article, we describe a sensitive method for determining *in vitro* deamination rates of C- and <sup>m</sup>C-containing cyclobutane

dimers in duplex DNA using site-specifically radiolabeled nucleotides. While methylation only occurs at CG sites in vertebrates, such sequence restrictions do not apply in plants and fungi<sup>32</sup> where <sup>m</sup>C-containing dimers could be formed in any sequence context. We therefore determined the deamination rates of both N<sup>m</sup>C=<sup>m</sup>CN pyrimidine dimers, where N is any base to better understand the structure–activity relationships in deamination of <sup>m</sup>C and its biological implications. We found that deamination of T=<sup>m</sup>C or <sup>m</sup>C=T cyclobutane dimers is at least 10-fold faster when flanked by G relative to A, C, or T and appears to involve the O6 carbonyl group of guanine. In contrast, deamination of C or <sup>m</sup>C in C=<sup>m</sup>CG, a known hotspot for C-to-T mutations in humans, is very slow and suggests the rate of repair of these dimers must also be slow to allow sufficient time for deamination.

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