Cell Cycle 11:18, 3345-3346; September 15, 2012; © 2012 Landes Bioscience

How a mismatch repair enzyme balances the needs for efficient lesion processing and minimal action on undamaged DNA

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The DNA base excision repair (BER) pathway is required for maintaining genomic integrity and has recently been implicated in active DNA demethylation, revealing a key role in epigenetic gene regulation.[1] To initiate BER, DNA glycosylases remove damaged or enzymatically modified bases by cleaving the base-sugar or *N*-glycosylic bond, and the resulting abasic site is replaced by the appropriate nucleotide via downstream BER proteins. Thymine DNA glycosylase (TDG) plays an essential role in DNA repair and transcriptional regulation, by excising oxidized and/or deaminated forms of 5-methylcytosine (mC) that arise at CpG sites. TDG was discovered as an enzyme that excises thymine from G·T mispairs, mutagenic lesions that can arise by deamination of mC,[2] and subsequent studies showed that TDG can excise a broad range of pyrimidine bases from DNA in vitro.[3] It was recently discovered that TDG removes 5-formylcytosine (fC)[4] and 5-carboxylcytosine (caC),[4,5] oxidized forms of mC generated by Tet (ten-eleven translocation) enzymes. Recent studies also show that TDG is essential for active DNA demethylation and for embryonic development,[6,7] due likely to TDG excision of oxidized and/or deaminated mC generated by a deaminase or Tet enzyme.[1]

Like other glycosylases, TDG does not act upon normal A·T or G·C, or G·mC base pairs, which is important, because such activity can be mutagenic and cytotoxic. Our understanding of how glycosylases avoid acting on the huge excess of undamaged DNA remains incomplete, particularly for mismatch glycosylases such as TDG and MBD4 (methyl binding domain IV), which excise a normal base (T) from a mismatched pair (G·T). We recently addressed this and other questions regarding the specificity and catalytic mechanism of TDG using a combination of structural, biochemical and computational approaches.[8]

We determined a crystal structure of human TDG (catalytic domain) bound to DNA with a target nucleotide (dU analog) flipped productively into its active site but not cleaved, providing a glimpse of the lesion recognition complex for a G·U mismatch. TDG forms interactions with the mismatched guanine (N1H, $N2H_2$) that are not compatible with adenine, which may account in part for the high specificity of TDG for G·T vs. A·T pairs (maximal glycosylase activity, k_{max}, is $10^{4.3}$-fold greater for G·T vs. A·T). The structure also reveals key interactions with the flipped uracil base and informs the unique ability of TDG to excise 5-substituted uracil and cytosine analogs (i.e., T, fC, caC).[3,8]

The structure suggested a catalytic role for His151, which is highly conserved in TDGs. Remarkably, H151A-TDG displays greatly enhanced activity for G·U and G·T mispairs. The suppression of activity by His151 is likely due to destabilization of the chemical transition state in the enzymatic reaction via repulsive electrostatic interactions between the neutral imidazole of His151 and O4 of T (or U).[8]

Previous studies of TDG show that substrate binding and base excision is weak for G·T mispairs compared with other substrates (G·U, etc.), even though G·T mispairs are an important biological substrate.[3,7,9] These studies indicated that steric hindrance limits the active-site access of thymine and other bases with a bulky C5 substituent (5-bromouracil).[3,9] Our recent study indicates that thymine flipping is suppressed by a steric clash between the C5-methyl of thymine and the methyl of Ala145, which is strictly conserved in mammalian TDG. Accordingly, G·T glycosylase activity is much greater for A145G-TDG relative to TDG, while the G·U activity remains unchanged. Molecular dynamics (MD) simulations support the finding that Ala145 suppresses flipping for dT but not dU nucleotides, and that steric repulsion for dT is relieved for A145G-TDG. Remarkably, G·T activity is 56-fold greater for the A145G-H151Q double mutant compared with native TDG. To our knowledge, observation of such large increases in repair activity upon mutation of active-site residues has not previously been reported for a repair enzyme.

One explanation for the strict conservation of active-site residues that dramatically suppress G·T repair activity is that the residues are needed to minimize aberrant excision of T from A·T base pairs. Indeed, aberrant A·T activity is 38-fold greater for A145G-TDG compared with native TDG, and 34-fold greater for H151-TDG.[8] Thus, mutations that confer large increases in lesion repair activity cause even greater increases in aberrant action on undamaged DNA,[8] an unprecedented observation for a repair enzyme. Moreover, the results suggest that the specificity for G·T vs. A·T pairs may already be optimal wild-type TDG.[8]

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Submitted: 06/26/12; Accepted: 07/20/12
http://dx.doi.org/10.4161/cc.21843
Comment on: Maiti A, et al. Proc Natl Acad Sci USA 2012; 109:8091-6; PMID:22573813; http://dx.doi.org/10.1073/pnas.1201010109.

The findings offer an explanation for the weak G·T glycosylase activity of TDG, a trait which could potentially contribute to the high mutational frequency at CpG sites in cancer and genetic disease.[10] Although enhanced G·T activity could potentially be beneficial for genetic and epigenetic integrity, our results suggest that the cost may be an intolerable increase in aberrant A·T activity. Given a limited capacity for discrimination between G·T and A·T pairs, a mechanism for suppressing thymine base flipping (A145) or base excision (H151) may be needed to maintain a sufficiently low level of activity against the million-fold excess of A·T pairs. Thus, the strict conservation of residues that severely curtail G·T activity may be driven by the need to minimize aberrant excision of T from A·T pairs.

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