RNA REPAIR

Mending the message

Repair of alkylation damage in DNA is clearly important, as at least three repair mechanisms have been described. On the other hand, little is known about what happens to alkylated RNA. However, Hans Krokan and colleagues now report in *Nature* that damaged RNA can be repaired *in vivo* — by some of the same enzymes used in DNA repair.

One of the DNA-repair systems involves the oxidative demethylation of 1-methyladenine and 3-methylcytosine. This is catalysed in *Escherichia coli* by the enzyme AlkB. Krokan and co-workers identified two human *AlkB* homologues — *ABH2* and *ABH3*. To test the enzymatic activity of these new enzymes, they did high-performance liquid chromatography (HPLC) analysis of [^{3}H]methylated DNA incubated with recombinant ABH2 and ABH3. And indeed, like AlkB, these enzymes were oxidative DNA demethylases capable of removing 1-methyladenine and 3-methylcytosine from DNA by demethylation.

When determining the substrate specificity of ABH2 and ABH3, Krokan and colleagues found that ABH2 was more active on double-stranded (ds)DNA, whereas ABH3 — like AlkB — had a preference for single-stranded (ss)DNA. Remarkably, AlkB and ABH3, but not ABH2, also repaired RNA oligonucleotides efficiently.

Next, the authors tested whether this newly discovered RNA-repair activity was biologically relevant by measuring the survival of chemically methylated (by methyl methanesulphonate; MMS) bacteriophage in the presence of plasmid-expressed AlkB, ABH2 or ABH3 in an AlkB-deficient *E. coli* strain. Consistent with the *in vitro* data, AlkB and ABH3, but not ABH2, were able to reactivate MMS-treated ssRNA phage MS2. Methylated ssDNA phage M13 could be reactivated by ABH2 and AlkB, and only partially by ABH3, whereas AlkB and ABH2, but not ABH3, reactivated dsDNA phage λ.

By studying the subcellular localization of ABH2 and ABH3, Krokan and co-workers noticed that ABH2 colocalizes with replication foci during S phase, which is consistent with its higher activity on dsDNA. By contrast, the dispersed nuclear localization of ABH3 throughout the cell cycle, and its preference for ssDNA and RNA, indicates that it might have a maintenance role in actively transcribing genes.

So, given that about 20 of the 62 known naturally occurring RNA modifications are sequence-specific methylation events, Krokan and colleagues conclude "…it is not surprising that cells have evolved RNA repair mechanisms that operate to prevent dysfunction of RNA as a result of alkylation damage".

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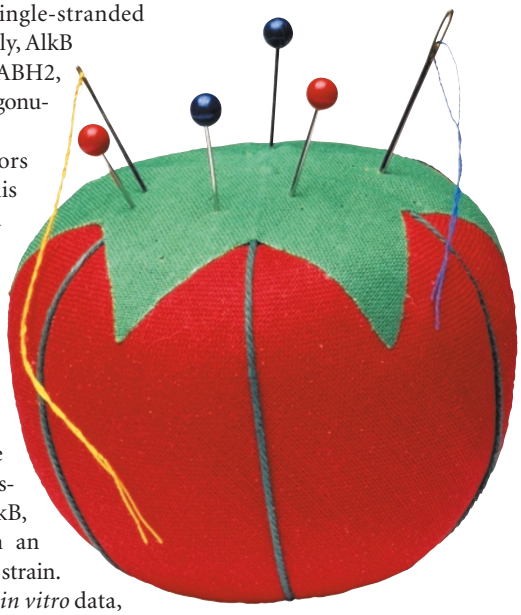
References and links

ORIGINAL RESEARCH PAPER Aas, P. A. *et al.* Human and bacterial oxidative demethylases repair alkylation damage in both RNA and DNA. *Nature* **421**, 859–863 (2003)



STRUCTURE WATCH

It takes two

Apoptosis is crucial for metazoan development and homeostasis, and it is executed by cascades of caspase activation. The catalytic activity of these caspases can, however, be inhibited by the inhibitor of apoptosis (IAP) proteins. Although we now have a good understanding of effector-caspase inhibition by IAPs, the mechanism of inhibiting initiator caspases — such as caspase-9 — has remained unclear. Now, though, in *Molecular Cell*, Shi and colleagues provide new insights by describing the 2.4-Å-resolution crystal structure of the catalytic domain of caspase-9 in complex with the third baculoviral IAP repeat (BIR3) of X-linked IAP (XIAP).

It has been shown, at least for caspase-3 and -7, that caspases must form homodimers to be catalytically active, because a crucial supporting loop (L2′) for the active site is provided by the adjacent monomer. In their structure, Shi and co-workers found that XIAP-BIR3 heterodimerizes with caspase-9 using the surface of caspase-9 that would otherwise mediate its homodimerization. XIAP-BIR3 therefore sequesters caspase-9 in its monomeric, inactive state and, in addition, it traps the caspase-9 active-site loops in an inactive conformation. The authors further showed that caspase-9 mainly exists as a monomer in solution, and that these monomers are catalytically inactive. This inactivity results primarily from the absence of the supporting L2′ loop, which is needed to stabilize the other active-site loops of caspase-9. These data, in conjunction with other studies, have therefore defined "…a unified mechanism for the activation of all caspases".

REFERENCE Shiozaki, E. N. *et al.* Mechanism of XIAP-mediated inhibition of caspase-9. *Mol. Cell* **11**, 519–527 (2003)

Not a SET pattern?

Methylation is an important type of histone modification, which has been associated with various processes including transcriptional control. Although a large family of histone methyltransferases that all contain a SET domain has been identified, the *Saccharomyces cerevisiae* Dot1 protein was recently found to be the first histone lysine methyltransferase that lacks a SET domain. Dot1 mono-, di- and trimethylates lysine 79 in the core domain of histone H3. So, to further understand lysine methylation of histone core domains, as well as the nucleosome specificity of the unique Dot1 protein family, Xu and colleagues determined the 2.5-Å-resolution crystal structure of the catalytic domain of human Dot1 (hDOT1L) bound to *S*-adenosyl-L-methionine (SAM).

The structure revealed that hDOT1L has a unique organization of a mostly α-helical amino-terminal domain together with an open α/β structure carboxy-terminal domain that resembles several SAM-dependent methyltransferases. The active site consists of SAM, which binds between the loop that connects the two domains and the open α/β structure, and a narrow, potential lysine-binding channel that leads to the SAM methyl group. The narrowest part of this channel is ~4 Å, which could accommodate a mono-, di- or trimethylated lysine. Furthermore, Xu and co-workers showed that a disordered, positively charged region at the carboxyl terminus of the hDOT1L catalytic domain is essential for nucleosome binding and enzymatic activity.

REFERENCE Min, J. *et al.* Structure of the catalytic domain of human DOT1L, a non-SET domain nucleosomal histone methyltransferase. *Cell* **112**, 711–723 (2003)