Locking mechanism found for 'scissors' that cut DNA

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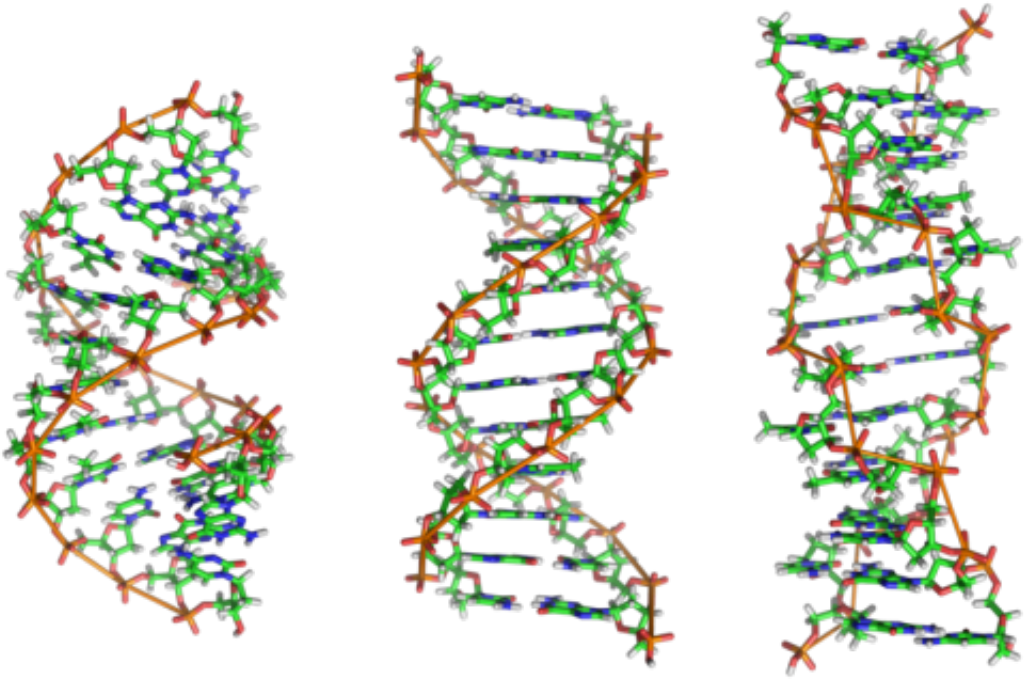

From left to right, the structures of A-, B- and Z-DNA. Credit: Wikipedia

Researchers at Johns Hopkins have discovered what keeps an enzyme from becoming overzealous in its clipping of DNA. Since controlled clipping is required for the production of specialized immune system proteins, an understanding of what keeps the enzyme in check should help explain why its mutant forms can lead to immunodeficiency and cancer. A summary of the results will be published online in the journal *Cell Reports* on Dec. 24.

The immune system relies on the formation of specialized proteins (antibodies) that can recognize and immobilize foreign invaders like viruses and bacteria. Since storing individual blueprints for each of these proteins would require huge amounts of DNA, the body instead mixes and matches different chunks of sequence to produce roughly 300 trillion possibilities. This mixing and matching, called recombination, requires that DNA be clipped by the enzyme RAG.

"Recombination is essential for the immune system's ability to recognize and fight new enemies, but too much clipping can cause harmful chromosome rearrangements," says Stephen Desiderio, M.D., Ph.D., director of the Institute for Basic Biomedical Sciences and the senior researcher for the study. "We now know that RAG has a built-in lock that prevents it from getting out of hand as it clips DNA."

To keep the system efficient, each immune cell makes only a single antibody and only does so after being activated. Several years ago, Desiderio's group found that this level of control is enforced by a segment of RAG called the PHD. The PHD binds to a chemical tag called H3K4me3, which is only found on DNA that is actively being rewritten as RNA. This prevents RAG from recombining DNA that is not active.

When the PHD segment was mutated and nonfunctional, RAG couldn't cut, suggesting that the binding of the PHD to H3K4me3 was required for RAG's function. But when the PHD was deleted entirely, RAG was just fine. To understand what was happening, Desiderio's team looked for mutations that would bring function back to the mutant PHD. They found that when 13 amino acids were deleted in front of the mutant PHD segment, RAG cut even better than it normally does.

Alyssa Ward, a graduate student in Desiderio's laboratory, says that the system works like the bolt on a door. The PHD piece is the lock,

H3K4me3 is the key and the deleted piece is the actual bolt. When all of the pieces are normal, H3K4me3 unlocks the PHD segment, which moves the bolt so that the door can open— i.e., so that RAG can cut. If there is a mutation in the PHD, the key won't fit the lock, so the door remains bolted. But, if the lock or bolt is removed entirely, the door can open and close freely.

Desiderio says that these results have implications for many other proteins that interact with DNA. "It was previously thought that H3K4me3 was simply a docking site for proteins," he says. "This study shows that it is also a key that activates them."

The team is now making a line of mice with the overactive RAG so they can see what effects it has in an animal. They hope that the overactive RAG will give them clues to how the enzyme is normally controlled, and to what goes wrong in those immunodeficiencies and cancers linked to mutations in RAG.

More information: *Cell Reports*, dx.doi.org/10.1016/j.celrep.2014.12.001

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