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## Biotechnology's Newest Chemical Tool

Exploiting biology's own chemical toolbox, researchers have developed a new technique that will allow them to modify specific sequences within a DNA molecule. The approach will not only help reveal the impact of biochemical alterations to DNA, but could have far-reaching implications for DNA-based medical diagnosis and nanobiotechnology.

Combining chemistry with biotechnology, Saulius Klimasauskas, a Howard Hughes Medical Institute (HHMI) international research scholar at the Institute of Biotechnology in Vilnius, Lithuania, and chemists at the Institute of Organic Chemistry in Aachen, Germany, have harnessed a group of essential enzymes to add various chemical groups to DNA, thereby altering its function. The work was published in an early online publication on November 27, 2005 in *Nature Chemical Biology*.

The enzymes at the heart of the study, known as DNA methyltransferases, are one of the tools cells use to turn genes on and off. By adding a simple cluster of four atoms — a carbon atom attached to three hydrogens, known to chemists as a methyl group — to specific bases within a DNA sequence, methyltransferases can effectively shut a gene off. Methylation plays an important role in embryonic development, genomic imprinting, and carcinogenesis because it regulates gene expression.

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- Saulius Klimasauskas

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Methyltransferases require a source for the methyl groups that they attach to DNA, and most often that source is a molecule called S-Adenosyl-L-methionine (AdoMet), sometimes known as SAM or SAMe. Methyltransferases grab the methyl group from AdoMet and transfer it directly to DNA, positioning it with enviable specificity within the sequence.

This specificity suggests that the enzymes can be a useful tool in the laboratory. But Klimasauskas and colleagues wanted the flexibility to attach more than just a simple methyl group.

In this study, the scientists demonstrated that methyltransferases can indeed be used to transfer larger chemical groups to large DNA molecules, in the same sequence-specific manner.

To try out their technique, the scientists synthesized molecules that mimicked AdoMet, but had chemical groups with longer carbon chains in the position where the methyl group was usually located. The enzymes were able to grab the bulkier group and transfer it to DNA. Since the family of DNA methyltransferases includes enzymes capable of recognizing more than 200 distinct sequences, this new approach provided an unprecedented ability to manipulate DNA experimentally.

To demonstrate the technique's potential to alter DNA function, the researchers modified DNA in a position that blocked another enzyme's ability to snip the molecule at its target site. "No one has really thought about possible applications [of this] before because no one thought it was possible," said Klimasauskas. He predicts that DNA methyltransferases will become a standard laboratory tool like restriction endonucleases.

Earlier studies had suggested that the transfer of chemical groups larger than a methyl group would not be possible, in part because replacing AdoMet's methyl group lowered the chemical reactivity of the compound. To overcome this problem, the authors took some tips from organic chemistry textbooks and stabilized the transfer with a multiple carbon bond.

"It turned out that our first bet, a double or triple carbon-carbon bond, placed next to the transferable carbon unit, helped to alleviate the problems that had plagued the reaction in previous studies," Klimasauskas said. He likened the chemical reaction to a mechanical spring, explaining that the chemical energy trapped in AdoMet is sufficient to deliver a small methyl group to its target compound. But delivering a larger compound required an auxiliary "spring" to ensure it would reach the target. So, he said, "chemical thinking" helped resolve the problematic enzymatic reaction.

"By demonstrating the transfer of carbon chains as long as 4 to 5 units, we provide proof of principle that further extensions should also be tolerated," Klimasauskas said.

Due to their sequence-specific nature, the scientists found that methyltransferases have a distinct advantage over other commonly used labeling techniques for DNA and other biopolymers. "Our approach allows labeling of large native DNA molecules at specific internal or terminal loci," Klimasauskas explained.

While potential applications are many, the researchers next plan to synthesize new AdoMet analogs to expand the collection of chemical groups that can be transferred to DNA by methyltransferases. Klimasauskas's group is currently working to append useful functional groups to extended chains. For example, researchers often label cellular components with a molecule called biotin, because it binds tightly to another molecule, streptavidin, and thus streptavidin can be used to retrieve the molecule of interest. If biotin were built into an AdoMet analog, Klimasauskas said, it could then be used as a molecular hook to fish out all molecules that would naturally be methylated in the cell. "There is no comparable way for global analysis of the methylation targets in the cell," Klimasauskas observed.

DNA is not the only molecule that is naturally methylated in the cell — RNA and proteins also undergo methylation, and the enzymes that carry out these reactions also rely on AdoMet as their methyl source. Since the chemistry is the same, this technique is likely to be applicable to those biomolecules as well, further expanding its utility. Klimasauskas said that one potential application might be to label various sites in the ribosome — the RNA-based site of protein production — with bright fluorophores using appropriate RNA methyltransferases, enabling real-time dynamic studies of the complicated mechanism of protein translation.