Mouse Work: New Insights on a Fundamental DNA Repair Mechanism

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(PhysOrg.com) -- Adding a new link to our understanding of the complex chain of chemistry that keeps living cells alive, a team of researchers from the University of Vermont (UVM), the University of

Utah, Vanderbilt University and the National Institute of Standards and Technology has demonstrated for the first time the specific activity of the protein NEIL3, one of a group responsible for maintaining the integrity of DNA in humans and other mammals. Their work reported last week sheds new light on a potentially important source of harmful DNA mutations.

Since it first was identified about eight years ago, NEIL3 has been believed to be a basic DNA-maintenance enzyme of a type called a glycosylase. These proteins patrol the long, twisted strands of DNA looking for lesions—places where one of the four DNA bases has been damaged by radiation or chemical activity. They cut the damaged bases free from the DNA backbone, kicking off follow-on mechanisms that link in the proper undamaged base. The process is critical to cell health, says NIST biochemist and Senior Research Fellow Miral Dizdaroglu, "DNA is damaged all the time. About one to two percent of oxygen in the body becomes toxic in cells, for example, creating free radicals that damage DNA. Without these DNA repair mechanisms there wouldn't be any life on this planet, really."

The glycosylases seem to be highly specific; each responds to only a few unique cases of the many potential DNA base lesions. Figuring out exactly which ones can be challenging. NEIL3 and its kin NEIL1 and NEIL2 are mammalian versions of an enzyme found in the bacterium E. coli, which first was identified in work at UVM. The lesion targets of NEIL1 and NEIL2 have been known for some time, but NEIL3, a much more complicated protein twice the size of the others, had resisted several attempts to purify it and determine just what it does. In a significant advance, a research team at UVM managed to clone the house mouse version of NEIL3 (99 percent identical to the human variant), and then prepare a truncated version of it that was small enough to dissolve in solution for analysis but large enough to retain the portion of the protein that recognizes and excises DNA lesions.

Using a technique they developed for rapidly analyzing such enzymes, NIST researchers Dizdaroglu and Pawel Jaruga mixed the modified protein with sample DNA that had been irradiated to produce large numbers of random base lesions. Because glycosylases work by snipping off damaged bases, a highly sensitive analysis of the solution after the DNA has been removed can reveal just which lesions are attacked by the enzyme, and with what efficiency. The NIST results closely matched independent tests by others in the team that match the enzyme against short lengths of DNA-like strands with a single specific target lesion.

In addition to finally confirming the glycosylase nature of NEIL3, says UVM team leader Susan Wallace, tests of the enzyme in a living organism—a tailored form of E. coli designed to have a very high mutation rate—had an unexpected bonus. Measurements at NIST showed that NEIL3 is extremely effective at snipping out a particular type of lesion called FapyGua (2,6-diamino-4-hydroxy-5-formamidopyrimidine) and seems to dramatically reduce mutations in the bacterium, a result that points both to the effectiveness of NEIL3 and the potentially important role of FapyGua in causing dangerous mutations in DNA.

More information: M. Liu, V. Bandaru, J.P. Bond, P. Jaruga, X. Zhao, P.P. Christov, C.J. Burrows, C.J. Rizzo, M. Dizdaroglu and S.S. Wallace. The mouse ortholog of NEIL3 is a functional DNA glycosylase in vitro and in vivo. Proc. Natl. Acad. Sci. USA, Early Edition, Published online before print Feb. 25, 2010, doi:10.1073/pnas.0908307107

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