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Scientists discover role of enzyme in DNA repair

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Scientists from the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), National Cancer Institute (NCI), and Integrative Bioinformatics Inc. have made an important discovery about the role of an enzyme called ataxia telangiectasia mutated protein (ATM) in the body's ability to repair damaged DNA. NIAMS and NCI are part of the National Institutes of Health (NIH).

When DNA within a cell is damaged, the cell's protective mechanism must do one of two things: repair the defect or "commit suicide," says Rafael Casellas, Ph.D., an investigator in NIAMS' Molecular Immunology and Inflammation Branch and leading author of a new paper describing the discovery. But the way in which the cell performs these protective functions has been largely a mystery, says Casellas, whose research is beginning to unravel this mystery.

Casellas' research focuses largely on certain genes that are deliberately broken and repaired as part of the immune response. Through a tightly controlled process of breaking and rejoining DNA segments, immune system cells called B lymphocytes are able to produce tens of millions of different types of antibodies to fight almost limitless types of invaders. This process of genetic recombination requires the activity of repair enzymes, which must be able to recognize and repair breaks in tightly wrapped and inaccessible DNA. During immunoglobulin gene recombination, DNA is rendered accessible by the process of transcription, which unzips double-stranded DNA as part of the conversion of genetic information into functional proteins.

While transcription ensures accessibility to DNA lesions, Casellas wondered how it was possible for repair enzymes to do their job if transcription continued once DNA had been damaged. “Imagine a piece of DNA as a zipper,” he says. “The head of the zipper (the transcription complex) will repeatedly go through the two interlocked sides, coming to the broken part, and eventually falling off. One could imagine that this unzipping activity might interfere with the mechanism that is trying to repair the damaged DNA.”

Casellas hypothesized that once DNA lesions were generated, a regulatory activity would shut down transcription until repair enzymes corrected the damage. But because B lymphocyte cells are relatively scarce, Casellas and his colleagues chose to focus their investigation on a more abundant family of genes, known as ribosomal genes, as a substitute. They attached a green fluorescent protein to Polymerase I, a key component in the machinery that transcribes these genes, and were able to visualize the activity of this enzyme using microscopy. They then used a particular laser attached to the microscope to introduce DNA breaks at sites where the polymerase was active. This microscopy approach was developed by NCI’s Michael Kruhlak, Ph.D., first author in the report. Using the ProcessDB software developed by Integrative Bioinformatics Inc, Robert Phair, Ph.D. developed a computer model that allowed the authors to test their hypothesis and show that while transcription continued in the cells with uninjured DNA, it came to a halt within 5 minutes at sites where the DNA had been damaged.

While it was possible that the DNA lesions themselves physically interfered with transcription, the authors hypothesized that repair enzymes recruited by the damage could shut down the transcription machinery polymerase. To test this hypothesis, they repeated the experiment in cells that were deficient in a variety of repair proteins. Most deficiencies did not appear to affect the arrest; however, in cells that were missing one of three repair proteins factors — ATM, Nbs1 or

MDC1 — transcription continued even after damage was induced.

“What these results told us was that these proteins were responsible for shutting down the transcription machinery near sites of DNA damage. This activity perhaps ensures repair in an undisturbed environment. If this is indeed the case, one could suspect that in the absence of these factors, repair is compromised, leading to genetic aberrations,” Casellas says. Indeed, scientists already know that people deficient in ATM develop such genetic abnormalities, cell transformation and tumor development. Although it’s too soon to say whether these laboratory discoveries will translate into clinical use, Casellas is enthused about the work. “With this new technology we can visualize for the first time the interplay between complex mechanisms such as DNA repair and gene transcription, not in a test tube, but in living cells and in real time. This approach will help us unravel the inner molecular pathways of our cells in health and disease, such as cancer.”

Source: National Institute of Arthritis and Musculoskeletal and Skin Diseases