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Paired enzyme action in yeast reveals backup system for DNA repair

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The combined action of two enzymes, Srs2 and Exo1, prevents and repairs common genetic mutations in growing yeast cells, according to a new study led by scientists at NYU Langone Medical Center.

Because such mechanisms are generally conserved throughout evolution, at least in part, researchers say the findings suggest that a similar DNA repair kit may exist in humans and could serve as a target for controlling some cancers and treating a rare, enzyme-linked genetic disorder called Aicardi-Goutieres syndrome. The syndrome, an often fatal neurological condition, is found in only a few families in small towns in Italy, Algeria and Japan, and among North American Cree Indians.

In a report to be published in the journal *Nature* online June 1, NYU Langone researchers, aided by colleagues at Yale University, found that the paired enzyme action prevents and repairs mistakes made during DNA replication, when molecular subunits known as rNMPs get inserted into DNA. The rNMPs are building blocks of DNA's chemical cousin RNA, which is the key intermediary involved in making all proteins from DNA.

Researchers say while some misplaced rNMPs naturally occur—and are repaired—as DNA is replicated during cell growth, enzymes quickly recognize such foreign intruders as lesions. If not removed, such lesions raise the likelihood of mutations in the DNA code, which if allowed to accumulate, create genomic instability in yeast and human cells, and can lead to cell death and cancer-promoting immune reactions.

"Taking our cue from yeast, which shares a third of its genetic make-up with humans, our study shows for the first time that a very robust backup DNA repair mechanism is in place to deal with common rNMP-induced mutations," says senior study investigator and NYU Langone yeast geneticist Hannah Klein, PhD. "Without a robust backup system for DNA repair, cells will die."

Among the study's key findings was that one of the enzymes, Srs2, helps open up the tightly bound, ladder-like yeast DNA structure so that the other enzyme, Exo1, can cleave out any misplaced rNMPs. Such rNMP misinsertions during replication, scientists say, contaminate DNA and are often lethal structural alterations. Both enzymes were previously known to play a role in DNA replication and repair, but the scientists say this is the first evidence of their role in preventing and correcting rNMP-derived mutations.

Moreover, the research team found that the Srs2-Exo1cell-repair mechanism prevents mutations from accelerating in yeast already deficient in a third enzyme coded by the gene RNaseH2. That enzyme serves as the primary removal mechanism for rNMPs during cell growth, a major role in DNA repair. But in yeast deficient in both the RNaseH2 enzyme and Srs2, the number of mutations, chromosome losses, and chromosome breakages rise 10-fold.

According to Dr. Klein, interim chair of biochemistry and molecular pharmacology at NYU Langone, her team's study is also the first to show how Srs2 and Exo1 backs up the routine rNMP maintenance function of the RNaseH2 enzyme, highlighting nature's constant need to balance cell growth, genetic mutation and DNA repair in preventing disease and cell death.

Dr. Klein cautions that while no known human Srs2 counterpart exists, Exo1 is found in human cells, so it is likely that a similar backup DNA

repair mechanism exists in people. And if further testing shows that its repair function can be manipulated in humans, the enzyme mechanism could be used as a basis to stall or reverse cancers derived from RNaseH2 mutations. Dr. Klein says breaking down how tumors develop in RNaseH2-deficient yeast cells is critical to formulating and testing potential treatments for people.

Other research has implicated overproduction of RNase H2 as one of several genetic features of many cancers, including cancers of the bladder, brain, breast, head, and neck squamous cell carcinomas, as well as leukemias (T- and B-cell acute lymphoblastic leukemia and acute myeloid leukemia), melanomas, and seminomas.

Even more specifically, she says, the enzyme repair mechanism could potentially be used to decipher and counteract the root causes of RNaseH2 enzyme deficiency, which in humans is known to be one of the main hereditary signatures behind Aicardi -Goutieres syndrome. The syndrome causes spinal inflammation and brain shrinkage, fatally stalling physical and mental development in early childhood. Although rare and currently untreatable, the disease afflicts hundreds in isolated communities where inbreeding among families has occurred and when both parents have RNaseH2 or other Aicardi -Goutieres-related mutations.

For the study, lead investigator and fellow yeast geneticist Catherine Potenski, PhD, monitored how various mutant yeast strains grew in the laboratory, including those deficient in the RNaseH2 enzyme and Srs2. (Dr. Klein's lab in the late 1980s was the first to isolate RNaseH2 mutations in yeast.)

Dr. Potenski, a postdoctoral fellow at NYU Langone, says yeast strains deficient in both enzymes accumulated mutations and did not grow well, while those depleted of only the RNaseH2 enzyme, were able to

minimize mutations and continue growing. However, in experiments with Exo1, its removal spiked mutations in RNaseH2-deficient strains, while depletion of Srs2 had no worsening effect. This evidence confirmed to researchers that Srs2 and Exo1 acted together to prevent mutations in RNaseH2-deficient cells.

Analysis by colleagues at Yale later confirmed the linked action between Srs2 and Exo1, showing how Srs2 stimulated Exo1 to act on yeast DNA, allowing for the cleaving and repair of rNMP lesions.

Dr. Potenski says her latest studies of Srs2, Exo1, and RNaseH2 enzymes should also serve as a reminder to other researchers that known enzymes may have many roles in the cell life cycle, some of which are not yet known, and that even more backup roles could be found.

Dr. Potenski says the team next plans to investigate what other biological factors may act on Exo1, as a possible third backup repair mechanism, and to investigate what factors might trigger RNaseH2 mutations more prone to lead to cancer.

More information: Avoidance of ribonucleotide-induced mutations by RNase H2 and Srs2-Exo1 mechanisms, DOI: 10.1038/nature13292

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