Researchers explore how genomic integrity is preserved in double-strand breaks

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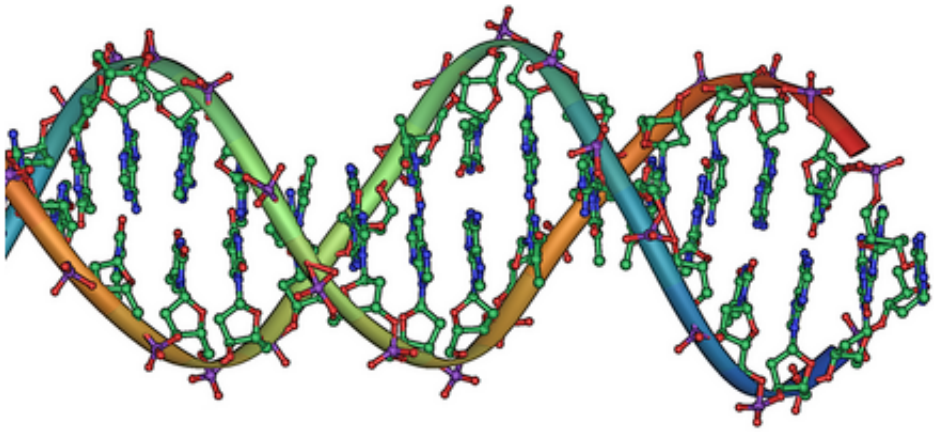

DNA double helix. Credit: public domain

(Phys.org)—Genome editing for purposes of replication and repair is handled by enzymes called endonucleases, which cleave DNA strands at very specific sites. These are called "restriction sites," composed of palendromic sequences of nucleotides. The repair of broken DNA forks is not fully understood; among other things, scientists would like to determine how break-induced replication (BIR), a genome rearrangement-repair mechanism, avoids destabilization of the genome that leads to rapid evolution, adaptation and tumorigenesis.

Double-strand breaks are particularly hazardous to the cell because they can result in genome rearrangement. Researchers studying eukaryotic DNA reporting this week in *Science* have found strong experimental evidence that an endonuclease called Mus81 operating at double strand breaks suppresses the template switches associated with genome instability. The research is pertinent to human genetic studies as human DNA has a similar pathway.

BIR is a highly mutagenic process, and is considered to be particularly prone to template switches for a number of reasons. To determine the role of the Mus81 endonuclease in suppressing mutations during BIR events, the researchers bred a Mus81 knockout variant of a particular eukaryotic cell and induced double strand DNA breaks.

It is known that outside the context of replication, Mus81 is not necessary for strand repair. Its job is structure-specific, converting a DNA structure called a displacement loop, or D-loop, into a replication strand. However, consistent with previous observations in yeast, the researchers found that the elimination of Mus81 resulted in dramatic defects in broken fork repair.

The authors note that more than half of the human genome is composed of interspersed repeating sequences, and that *Alu* repetitive elements are the most abundant. *Alu*s are often found at break points and in complex genomic rearrangements that are associated with disease. The researchers inserted a pair of *Alu*s known to mediate deletions associated with disease. In the Mus81 knockout cells, the rate of mutations was observed to be 83 times higher than in wild-type controls, "underscoring the role of Mus81 in suppressing switches between highly diverged templates."

Additionally, mutagenicity is constrained by converging replication forks. During replication, a fork structure is created when helicases

break the bonds that hold the two DNA strands together. The two resulting branches become the template for the resulting replicated strands as polymerases match up nucleotides with their compliments. In the case of a broken fork, Mus81 endonuclease suppresses template switches that can occur. "We demonstrate that fidelity of repair at broken replication forks depends on two partially compensatory mechanisms," the authors write, "cleavage by Mus81 and arrival of a converging fork. Converging forks limit the need to reestablish fully functional forks, illustrating an advantage of the multi-origin nature of eukaryotic chromosomes."

The authors conclude that deficiencies in Mus81 or in the timeliness of converging DNA forks may cause reliance on other recombinant DNA and repair mechanisms that give rise to mutations that make cancer cells more adaptive, thus promoting tumor progression.

More information: "Mus81 and converging forks limit the mutagenicity of replication fork breakage." *Science* 14 August 2015: Vol. 349 no. 6249 pp. 742-747. DOI: 10.1126/science.aaa8391

ABSTRACT
Most spontaneous DNA double-strand breaks (DSBs) result from replication-fork breakage. Break-induced replication (BIR), a genome rearrangement–prone repair mechanism that requires the Pol32/POLD3 subunit of eukaryotic DNA Polδ, was proposed to repair broken forks, but how genome destabilization is avoided was unknown. We show that broken fork repair initially uses error-prone Pol32-dependent synthesis, but that mutagenic synthesis is limited to within a few kilobases from the break by Mus81 endonuclease and a converging fork. Mus81 suppresses template switches between both homologous sequences and diverged human Alu repetitive elements, highlighting its importance for stability of highly repetitive genomes. We propose that lack of a timely converging fork or Mus81 may propel genome instability observed in

cancer.