

Researchers at NIEHS discover role for a unique complex in DNA repair

By Heather Franco

Mutations in genes encoding proteins in the MRN complex and Ctp1 have been implicated in the origin of human diseases, characterized by markedly greater sensitivity to DNA damage and increased susceptibility to cancer. These proteins are involved in homology-driven recombinational repair (HDRR), a mechanism of DNA double-strand break (DSB) repair.

In a [study](http://www.plosgenetics.org/article/info%3Adoi%2F10.1371%2Fjournal.pgen.1003420) (<http://www.plosgenetics.org/article/info%3Adoi%2F10.1371%2Fjournal.pgen.1003420>) published March 28 in PLOS Genetics, NIEHS [Chromosome Stability Group](http://www.niehs.nih.gov/research/atniehs/labs/lmg/cs/index.cfm) (<http://www.niehs.nih.gov/research/atniehs/labs/lmg/cs/index.cfm>) biologist James Westmoreland and lead researcher Michael Resnick, Ph.D., provide the first direct evidence that the yeast homologs of these proteins — the MRX complex and Sae2 — coordinate the initiation of HDRR at both ends of the DSB. Since exposure to environmental agents can cause DSBs, these results highlight the importance of this complex in yeast and humans.

The ability to investigate coincident resection during HDRR was aided by two factors — the development of a new technology and the use of yeast as a model system. Prior to this study, it was not possible to directly distinguish resection at one versus two ends of a DSB. Therefore, Westmoreland developed a new technology called two-dimensional pulsed-field gel electrophoresis (2D-PFGE) to observe this phenomenon (see [text box](#)).

Resnick described why they used yeast, rather than another organism, for these experiments. “Yeast has often served as a test tube for human function. There is strong conservation in many basic cellular processes, including DSB repair, from yeast to humans.” He added that yeast can also maintain circular chromosomes, which provide unique advantages to the study of DSBs and coincident resection.

Resolving DSBs in DNA

Westmoreland said DSBs can be caused by many environmental agents and can lead to genomic instability, including gross chromosomal rearrangements and carcinogenic mutations, if left unrepaired. Two mechanisms have evolved to resolve DSBs — nonhomologous end-joining and HDRR. “HDRR is unique because it provides for accurate repair of broken or gapped regions of the DNA,” Westmoreland said.

In HDRR, DNA base pairs at the ends of the break need to be cut away to generate a single stranded DNA, in a process known as resection. The single-stranded DNA can then insert itself into the homologous region of its sister chromosome and act as a template for repair.

Resection occurs in two stages. First, a few base pairs are removed, making a small single strand gap in the DNA. Second, resection is greatly extended so that kilobases of base pairs are excised, exposing a large region of single-stranded DNA. Until now, it hasn't been clear if resection occurs at one or both ends of the break. Resection at only one end of the break is postulated to increase the instability of the DNA. Therefore, resection at both ends of the break, known as coincident resection, appears to be the favored mechanism.

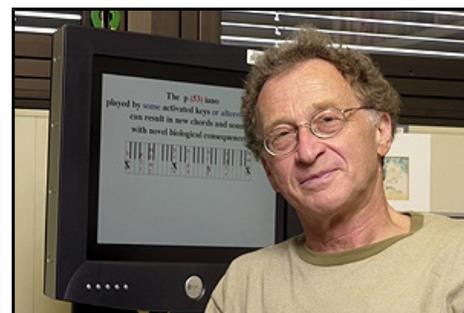
Extrapolating results from the yeast model to humans

Through analysis of mutant yeast strains, Westmoreland and Resnick demonstrated that the MRX complex and Sae2 are essential for the initiation of coincident resection with differing degrees of necessity, depending on the type of break and a more stringent requirement in repair of “dirty” breaks. A dirty break occurs when there is additional damage to the region of the DSB end that prevents a simple rejoining or ligation of the ends. The finding of additional requirements for repair is important, since DSBs induced by environmental agents are often likely to have dirty ends.

The results of this study have broad implications for human health. In fact, a mutation in the NBS1 gene, which is a component of the MRN complex, causes Nijmegen breakage syndrome, an inherited disease in which the body's DNA is prone to breakages resulting in a number of issues, including frequent infections and an increased risk of developing cancer.



First author Westmoreland
(Photo courtesy of Steve
McCaw)



Resnick is head of the Chromosome Stability
Group in the NIEHS Laboratory of Molecular
Genetics. (Photo courtesy of Steve McCaw)

By understanding the mechanism of coincident resection during DSB repair in yeast, as well as describing new roles for the MRX, complex and Sae2, researchers have gained insight into the etiology of, and potential therapeutic targets for, human diseases.

Citation: Westmoreland JW, Resnick MA. (<http://www.plosgenetics.org/article/info%3Adoi%2F10.1371%2Fjournal.pgen.1003420>) 2013. Coincident resection at both ends of random, gamma-induced double-strand breaks requires MRX (MRN), Sae2 (Ctp1) and Mre11-nuclease. PLoS Genet 9(3):e1003420.

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New technology allows for novel discovery

In pulsed-field gel electrophoresis (PFGE), electrical currents change direction along the length of an agarose gel in a zigzag pattern, which allows for the separation of larger DNA molecules than is possible with a standard agarose gel. Through rigorous analysis, the researchers were able to identify DNA molecules that had undergone resection at 0, 1, or 2 ends — m , m^* , and m^{**} , respectively — based on their migration pattern. They discovered that molecules resected at both ends migrated less than those that had not undergone resection or undergone one end resection. The migration in the gel was opposite to initial expectation, in that with the reduction in mass due to resection, there was decreased migration. While the reason why this reduction occurs remains a mystery, it is somehow tied in with the conformation of single strand DNA under PFGE conditions, which on its own would be an interesting topic for future study, according to the researchers.

Westmoreland then further analyzed the molecules with presumed one-end (m^*) and two-end (m^{**}) resections identified in the round of PFGE by subsequently applying PFGE in a second dimension. From this second analysis, he was able to demonstrate that the intermediate resections were actually resections at only one end and not a reduced length of excision at each end of DSBs. As a result, with his development of 2D-PFGE, Westmoreland could observe, for the first time, resection at 0, 1, or 2 ends of a DSB.

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