NIEHS researchers determine new function for DNA repair protein

By Robin Arnette

When NIEHS scientist Scott Williams, Ph.D., was a graduate student in 1998, he began studying a protein called Ctp1, also known as CtIP. He had no idea that nearly 17 years later, a visiting fellow in his own group would unveil the first molecular structures of Ctp1 and discover new functions for the protein.

The paper that touted the findings appeared online Jan. 12 in the journal Nature Structural and Molecular Biology. Reporting the only known crystal structure of the Ctp1 DNA binding region, the article is the first to describe the architecture of Ctp1 and suggest a new function — that of a DNA binding and bridging protein.

Previously published studies have determined that mutations in the human version of Ctp1 result in two rare developmental disorders known as Jawad syndrome and Seckel syndrome.

The NIEHS team found that mutations in fission yeast, corresponding to conserved amino acids that are absent in people with the two syndromes, interfered with the ability of Ctp1 to properly attach to broken DNA and facilitate DNA repair.

Ctp1 bridges the gap

Williams, lead of the NIEHS Genome Integrity and Structural Biology Group, is interested in how cells recognize and repair DNA double-strand breaks caused by environmental exposures and normal cellular metabolism. He said DNA double-strand breaks are some of the most dangerous DNA lesions, because if left unrepaired, they can lead to genome rearrangements and contribute to carcinogenesis.

As a graduate student in biochemistry at the University of Alberta, Canada, Williams studied the protein interactions of the BRCA1 tumor suppressor. During that time, published studies revealed that the mammalian version of Ctp1 binds to BRCA1, which sparked Williams' curiosity about what roles Ctp1 plays in the cell.

While Williams worked his way through his doctoral program and postdoctoral training at the Scripps Research Institute in La Jolla, California, a large body of literature was accumulating on the role of Ctp1in the repair of DNA double-strand breaks. The underlying molecular basis for the function, however, remained unknown.

Making Editor's Choice in Environmental and Molecular Mutagenesis

Another journal has taken notice of the work coming out of the Williams group. Andres was also lead author on a literature review picked for Editor's Choice by the Environmental Mutagenesis and Genomics Society (EMGS) *(http://emgs-us.org/)*. According to Jeffrey Wickliffe, Ph.D., chair of the EMGS Public Relations and Communications Committee, selection of a review article as an Editor's Choice is rare. Most are important new observations.

"What sets this review apart is that the authors have condensed a large amount of information about a complex system into a concise, complete, and readable review," Wickliffe said. "I have no doubt that this review will become part of cell and molecular biology graduate courses and serve as a starting point for investigations into cell responses to stress, evolutionary biology, neurology, aging, and oncology."

The paper, which also received a cover highlight, reviews the biochemistry underlying the ways cells identify and repair spontaneous, radiation-induced and chemical-induced DNA damage that occurs at DNA ends.

Citation: Andres SN, Schellenberg MJ, Wallace BD, Tumbale P, Williams RS. *(http://www.ncbi.nlm.nih.gov/pubmed/25111769)* 2015. Recognition and repair of chemically heterogeneous structures at DNA ends. Environ Mol Mutagen 56(1):1-21.

Williams joined NIEHS in 2009 and combined structural methods with biophysical techniques and yeast genetics to find the answer. "We discovered that Ctp1 is made up of four flexible arms that can bind to the two broken DNA ends and act as a bridge," Williams said.

"We think this function is important in coordinating both damaged DNA and proteins involved in DNA repair. In doing so, Ctp1 protects the integrity of the genome against environmental and natural insults," he explained.

Proteins working in collaboration

Because the DNA molecule is a flexible structure, Williams said, it makes sense that the protein that grabs onto broken DNA ends should be flexible, too. He suggested imagining the entire genome as a bowl of spaghetti, in which one spaghetti strand breaks in two. "How do you ensure that the two ends don't get lost in the bowl, because you need those same two pieces to become one again?" Williams asked. "A flexible Ctp1that provides a bridging scaffold helps to solve the problem."

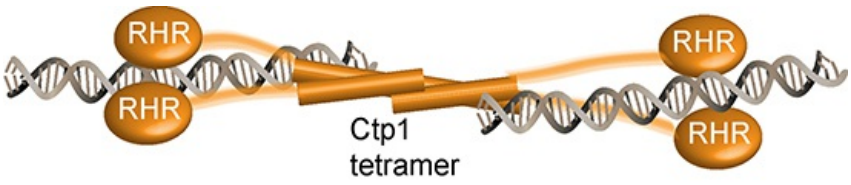
Current research shows that Ctp1 doesn't work alone. The protein acts in concert with the Mre11-Rad50-Nbs1 nuclease complex and influences its activity. Since Mre11, Rad50, and Nbs1 are also mutated in heritable, cancer predisposition syndromes, the team wants to know how Ctp1 collaborates with these complex members to recognize and repair DNA double-strand breaks. Future work should bring further clarity to Ctp1 functions.

Sara Andres, Ph.D., lead author and visiting fellow in the Williams group, said that although they have learned a lot, there's still more to understand. "Like any biological system, knowing how DNA repair functions at the molecular level provides an instruction manual of sorts, with Ctp1 being chapter 1," Andres said. "When DNA repair does go awry, causing diseases like Seckel syndrome or cancer, we have the knowledge to find a way to fix it."

Citation: Andres SN, Appel CD, Westmoreland JW, Williams JS, Nguyen Y, Robertson PD, Resnick MA, Williams RS.
(http://www.ncbi.nlm.nih.gov/pubmed/25580577)
2015. Tetrameric Ctp1 coordinates DNA binding and DNA bridging in DNA double-strand-break repair. Nat Struct Mol Biol; doi:10.1038/nsmb.2945 [Online 12 January 2015].

DNA damaging agents, such as ionizing radiation (IR), shear both strands of DNA, creating damaged DNA. When the Williams group disrupted the Ctp1 tetrameric core in fission yeast and zapped the yeast with IR, the cells died, indicating that Ctp1 binding to DNA is vital for yeast survival. (Photo courtesy of Steve McCaw)

Andres is keen to continue her research on Ctp1. "Finding that Ctp1 is like a flexible bridge linking DNA is just the first step," Andres said. "The real challenge now is to determine how that works in the context of the entire DNA repair complex." (Photo courtesy of Steve McCaw)



Using biochemistry, structural biology, and genetic approaches in fission yeast, the Williams group generated a model that may explain how Ctp1 binds DNA. Williams said the Ctp1 tetramer interlocks at the center of the four-armed protein to bind and bridge broken DNA ends. RHR represents DNA binding domains, made up of the amino acids arginine (R), histidine (H), and arginine (R), that stabilize Ctp1 at the DNA break site. (Graphic courtesy of Scott Williams)

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