Study decodes structure of protein complex active in DNA repair

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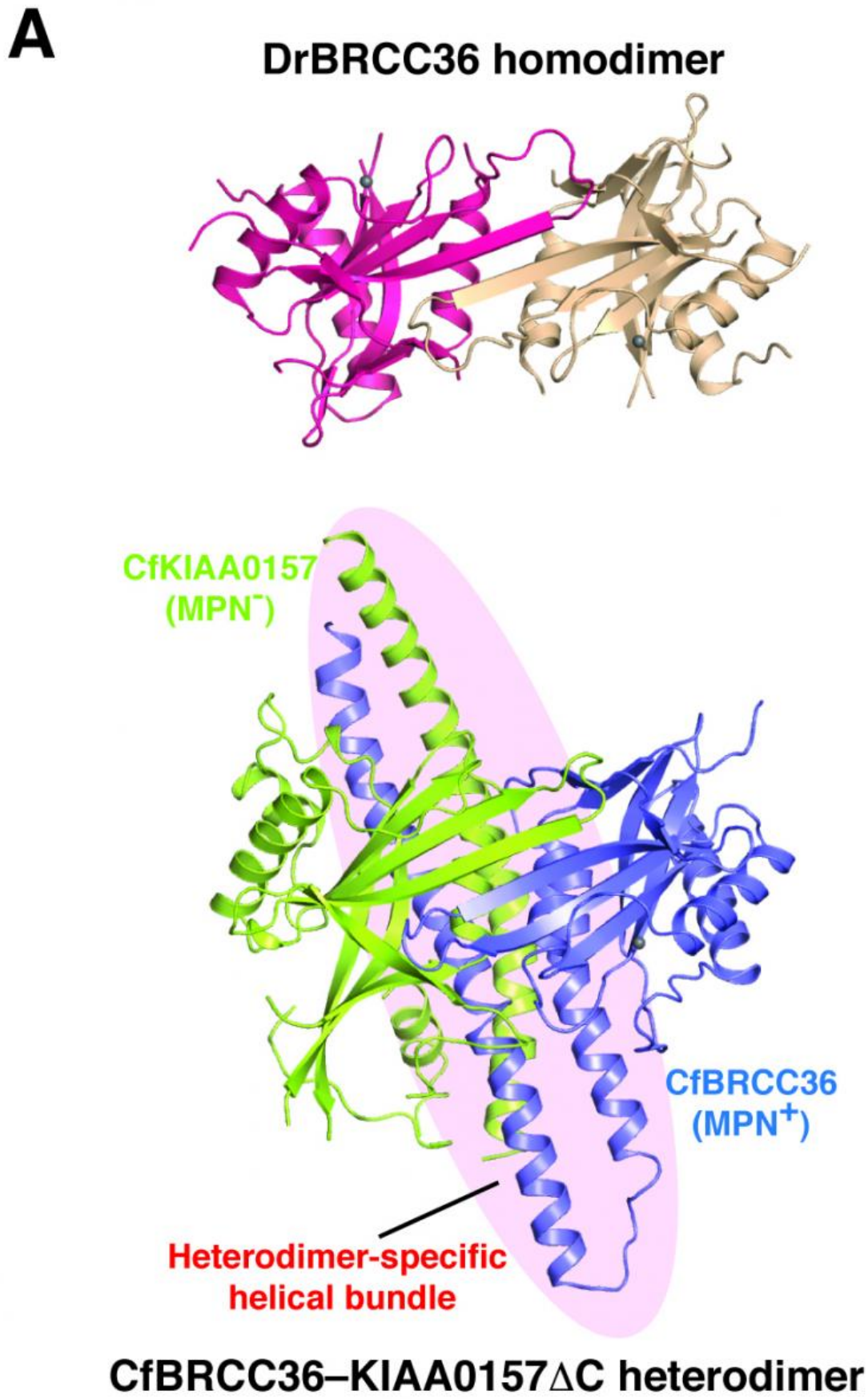
A
DrBRCC36 homodimer
CfKIAA0157
(MPN-)
CfBRCC36
(MPN+)
Heterodimer-specific
helical bundle
CfBRCC36–KIAA0157ΔC heterodimer

Comparison of the BRCC36 homodimer (top) with the BRCC36-KIAA0157DC heterodimer(bottom) reveals similarities in MPN domain association but disorder of the helical bundle region. Credit: Roger Greenberg, MD, PhD, Perelman School of Medicine, University of Pennsylvania

The human body, if anything, is economical. The fittingly named and abundant protein ubiquitin is best known for its central role in recycling misfolded proteins. But it has other functions, too - the addition or removal of ubiquitin chains can tweak the activity of newly made proteins and enzymes. Indeed, this process, called ubiquitination, influences DNA damage repair via the BRCA1 breast cancer-associated protein and anti-inflammatory, immune responses.

One enzyme in particular, BRCC36, removes a specific type of ubiquitin that is central to DNA damage repair as well as inflammatory responses. But BRCC36 doesn't act on its own—it functions as part of a complex comprised of several other proteins, one of which is KIAA0157. But how these two work together has remained unclear. Now, thanks to work performed in collaboration between researchers at the Perelman School of Medicine at the University of Pennsylvania and the Lunenfeld-Tanenbaum Research Institute in Toronto, the mechanism is coming into focus.

A team led by Roger Greenberg, MD, PhD, an associate professor of Cancer Biology at Penn, and Frank Sicheri, PhD, in Toronto, report online in *Molecular Cell* ahead of print, the atomic structures of several BRCC36-containing complexes. Greenberg is also an associate investigator at the Abramson Family Cancer Research Institute and director of Basic Science for the Basser Center for BRCA.

They found that BRCC36 and KIAA0157 are structurally related proteins, but while the BRCC36 is capable of removing ubiquitin (generally called a DUB for its deubiquitinating function), KIAA0157, is not quite up for that job since it does not bind the metal ions necessary for removing ubiquitin, so is referred to as a "pseudo-DUB."

By comparing high-resolution structures of two complexes - one containing both a DUB and a pseudo-DUB, and two copies of an inactive DUB by itself—the team determined that KIAA0157 alters the shape of the BRCC36 active site, by swinging an amino acid into position to make the complex able to catalyze chemical reactions.

They also found that the complete, functional complex contains two BRCC36-KIAA0157 molecule pairs, a symmetrical configuration the authors call a "super-dimer."

To validate these observations, the team - along with postdoctoral researchers Lei Tian, PhD, in the Greenberg lab and Elton Zeqiraj, PhD, in the Sicheri lab—made mutations to knock out key amino acids in the complex. For instance, some mutations eliminated enzymatic activity and the ability for the complex to associate with its downstream molecular partners, suggesting that the super-dimer complex is critical.

Experiments with mouse embryo support cells that express mutant DUB or pseudo-DUB proteins show an impaired immune response when infected with a virus and impaired DNA damage repair when exposed to ionizing radiation, further validating the need for complex's correct structure.

According to Greenberg, BRCC36-KIAA0157 is just one of many deubiquitinating enzyme complexes present in cells of complex organisms: "It turns out to be a general mechanism of how this class of deubiquitinating enzymes works." Given the similarity of these related

proteins to one another, knowing the structure and mechanism of BRCC36-KIAA0157 should enable researchers to better model the structures of related complexes, and also probe their mechanisms.

More importantly, says Greenberg, the BRCC36-KIAA0157 complex may represent an attractive drug development target for inflammatory diseases - an effort that should be aided immeasurably by the detailed structural information this study provides. "The structure will help guide inhibitor design," Greenberg said.

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