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Taking the stress out of replication

The ubiquitin ligase TRAIP helps cells restart stalled replication forks.

New parents aren't the only ones who can find reproduction trying. Cells that are copying their DNA in preparation for division can undergo replication stress. Hoffmann et al. have identified a protein that helps cells relieve this form of stress and continue DNA duplication (1).

Replication stress refers to the various impediments that can slow or stall replication forks. A cell may run short of nucleotides to plug into the new DNA strands, for example, or the replication machinery might run into a section of DNA that is damaged or tricky to copy. Because stalled forks can lead to DNA double-strand breaks and genome instability, cells take a series of measures to clear the obstacles and restart replication (2). The long stretches of single-stranded DNA at stalled forks attract the protein RPA (3). In turn, RPA coats single strands and draws in the ATR kinase, which halts the cell cycle and triggers other responses that protect the genome while the cell removes the blockage (4). The protein PCNA, which clamps onto DNA, also helps cells resolve replication stress by serving as a platform for repair proteins.

Researchers have discovered numerous proteins that are involved in replication stress, and Hoffmann et al. wanted to determine if the list is complete. When the researchers used mass spectrometry to identify the proteins that accumulate at damaged DNA undergoing replication in *Xenopus* egg extracts, they found one that hadn't turned up in previous studies: the E3 ubiquitin ligase TRAIP. The protein also amassed at DNA lesions that the researchers created in human cells. Although TRAIP normally remains in the nucleolus, Hoffmann et al. showed that, if DNA damage occurs, it relocates to the site of the injury.

TRAIP's previously discovered roles include stimulating cell proliferation and regulating the spindle assembly checkpoint. To find out how it gets into position at the sites of DNA damage, the researchers removed different sections of TRAIP and tested the truncated proteins' ability to home in on the lesions. Lopping off the molecule's C-terminal domain prevented it from accumulating at DNA damage sites. This end of TRAIP contains a sequence known as a PIP box that allows proteins to attach to PCNA. Using in vitro binding studies, the researchers verified that TRAIP's C-terminal domain binds to PCNA through the PIP box.

> "Absence of TRAIP from cells compromises the response to replication stress."

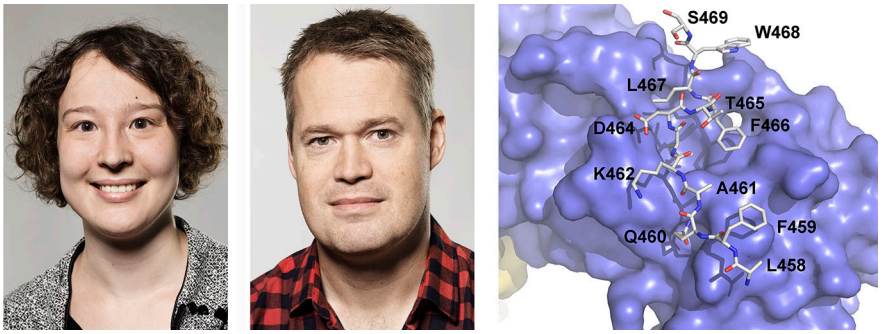
The team then determined the crystal structure of TRAIP's C terminal attached to PCNA. TRAIP's C-terminal tail slips into a groove on PCNA, and a small hydrophobic patch in this region slots into a hydrophobic pocket in PCNA.

To find out what happens once PCNA and TRAIP link up, Hoffmann et al. used siRNA to deplete TRAIP in cells exposed to agents that induce replication stress. Less RPA accumulated at DNA lesions after TRAIP depletion, the researchers found. The cells also contained less single-stranded DNA and showed fewer signs of ATR activation. "Absence of TRAIP from cells compromises the response to replication stress," says senior author Niels Mailand.

TRAIP's absence also caused long-term effects. Cells remained longer in G2 and sometimes got stuck in that stage, suggesting that they couldn't complete DNA duplication. TRAIP depletion also boosted the number of chromosome abnormalities and left cells more vulnerable to a DNA-damaging compound.

"We've identified a new factor that has an important role in the response to replication stress," says Mailand. The study suggests that TRAIP promotes the resolution of replication stress by spurring the formation of RPA-covered single-stranded DNA and ATR activation. How TRAIP triggers this effect remains a mystery. Hoffmann et al. determined that TRAIP's ubiquitylating activity is essential for resolving replication stress, whereas the PIP box isn't. They are now working to pin down which proteins TRAIP ubiquitylates.

FOCAL POINT



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Saskia Hoffmann (left), Niels Mailand (center), and colleagues discovered that the ubiquitylating enzyme TRAIP helps prevent genome instability by enabling cells to overcome replication stress. To perform its function, the enzyme interacts with the protein PCNA, which serves as a landing site for DNA repair proteins. A crystal structure (right) shows that the C-terminal domain of TRAIP nestles into a groove in PCNA (purple).

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