Crosstalk of proteases in DNA-protein crosslink repair

EB-04-4

M. Popovic[I], C. Supina[I], N. Ivić[I], I. Anticevic[I], C. Otten[I]

[I]Ruder Boškovic Institute, HR-10000 Zagreb, Croatia

DNA-protein crosslinks (DPCs) are severe DNA lesions which occur when a protein becomes irreversibly covalently linked to DNA. On a cellular level, aberrant DPC repair leads to the formation of DSBs, genomic instability and/or cell death, while on the organismal level impaired DPCR causes premature aging phenotypes and cancer. Despite the fact that DPCs are frequently occurring in the nucleus and cause severe damage, not much is known about its repair mechanisms at a molecular and organismal level. Our discovery of proteolysis-coupled DPC repair centred on SPRTN protease led to recognition of DNA-protein crosslink repair as a separate DNA damage repair pathway. We and others have shown that SPRTN removes proteinaceous part of DPC, thus initiating subsequent removal of peptide remnant crosslinked to DNA. Considering that SPRTN is a replication-specific protease, we searched for another protease which would act in lowly proliferative cells where DPCs pose a threat to transcription progression. Indeed, we have identified a SPRT-domain containing protein, ACRC (acidic repeat containing) using phylogenetic analysis. In line with the phylogenetic proximity, the 3D structure of the protease core of ACRC is very similar to that of SPRTN. The putative protease core of ACRC includes two α-helices bearing three Zn-binding histidines and a catalytic glutamate residue which together form a HEXXH motif, a characteristic of all Zn-dependent metalloproteases. Our goal is to determine if ACRC is proteolytically active, if it bears a role in DPC repair and what is its relation to SPRTN. To address functionality of ACRC, protein purification and biochemical analysis are under way. Using CRISPR gene manipulation, we created an enzymatic impaired version of ACRC through mutation knock-in in mammalian cells and in zebrafish (Danio rerio) model organism. Our study reveals the contribution of ACRC to the DPC removal on the cellular and organismal level.