STRUCTURAL AND MECHANISTIC INSIGHTS INTO PARP1 AND PARP2 FUNCTION AND PARP INHIBITOR RESPONSE

M.J. SUSKIEWICZ [1, 2]

[1]Centre de Biophysique Moléculaire, UPR4301 CNRS - Orléans (France), [2]Sir William Dunn School of Pathology, University of Oxford - Oxford (UK)

Poly(ADP-ribose) polymerase 1 (PARP1) and its close paralogue PARP2 are sensors of DNA damage in human cells that become activated upon binding to DNA breaks. Once activated, PARP1 and PARP2 use NAD^+ to modify numerous substrates, including themselves and histones, with the post-translational modification called poly(ADP-ribosyl)ation, which then recruits DNA repair factors and facilitates repair by promoting chromatin decompaction. However, as PARP1 is the most abundant nuclear protein after histones with high affinity for DNA breaks, it tends to get trapped on damaged chromatin, inhibiting DNA repair and causing other toxic events, especially in cells with mutations in DNA repair machinery. By both blocking PARP1's and PARP2's positive role in DNA repair and enhancing PARP1 trapping, PARP inhibitors promote cell death and are particularly effective against cancer cells mutated in DNA repair factors BRCA1 or BRCA2. During my post-doctoral work performed in Ivan Ahel's group at Sir William Dunn School of Pathology at Oxford University over the last three years, I have investigated fundamental mechanistic questions regarding the function of PARP1 and PARP2 in DNA repair and inhibitor-induced PARP1 trapping. In this presentation, I will first describe our discovery that both PARP1 and PARP2 are incomplete enzymes that require complementation by an accessory factor, HPF1. We demonstrated with crystal and cryo-EM structures, as well as NMR, biochemical, and cellular studies, how HPF1 completes the PARP active site by providing an additional catalytic base, Glu284, which allows PARP1 and PARP2 to modify their typical physiological targets, protein serine residues. We also showed with Cryo-EM that the PARP2-HPF1 complex is able to bridge two double-strand DNA breaks, presumably for subsequent ligation. Finally, by using cell biology approaches, we demonstrated that PARP1 counteracts its own trapping through a negative feed-back loop, whereby PARP1 activation by DNA breaks leads to its automodification on three main serine residues and subsequent release from chromatin. As this self-regulatory process requires a PARP1-HPF1 complex, HPF1-deficient cells show enhanced PARP1 trapping and are hypersensitive to PARP inhibitors. I will continue my studies of DNA repair-associated post-translational modifications as a CNRS researcher in Bertrand Castaing's laboratory at the Centre de Biophysique Moléculaire, CNRS, UPR 4301, Orléans using French integrative structural biology infrastructure.

Bibliography

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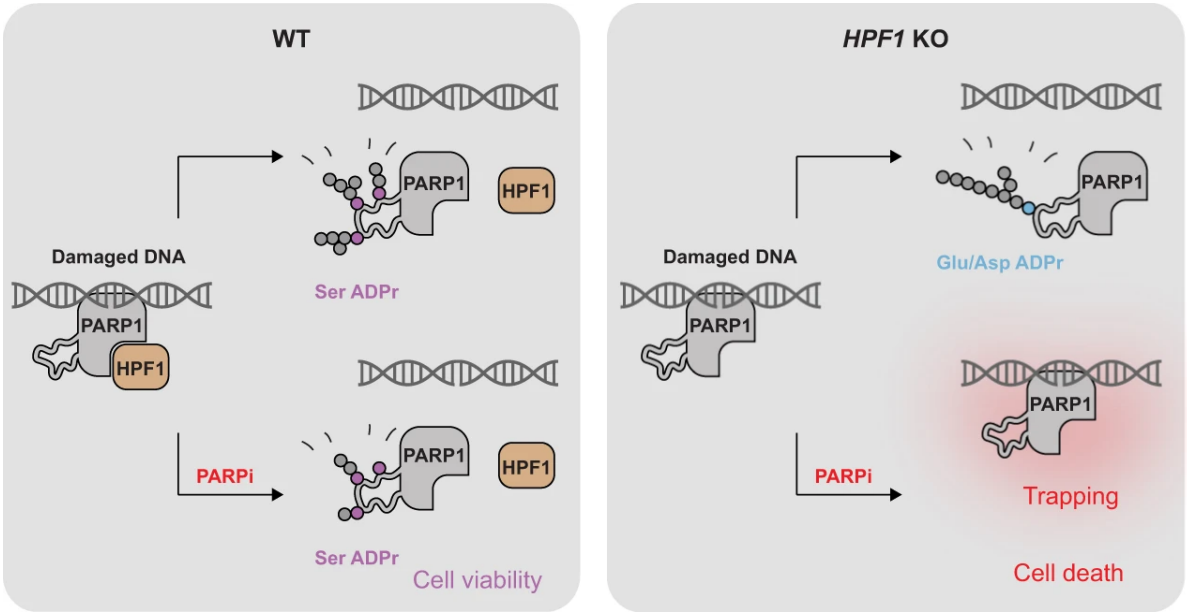
HPF1-dependent regulation of PARP1 trapping


WT
Damaged DNA
PARP1
HPF1
Ser ADPr
PARPi
PARP1
HPF1
Ser ADPr
Cell viability
HPF1 KO
Damaged DNA
PARP1
Glu/Asp ADPr
PARPi
PARP1
Trapping
Cell death