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DNA Damage Response

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DNA is highly susceptible to damage and must be repaired correctly to prevent loss of vital genetic information. Failure to correctly sense and/or repair DNA damage is the underlying cause of a number of hereditary cancer predisposition syndromes such as Fanconi anemia (FA) and Blooms. We are employing a number of complementary experimental systems, including *C. elegans*, mouse models and mammalian cell culture, to understand how DNA lesions are sensed and repaired during S-phase and in meiosis. We are particularly interested in understanding the function and regulation of the S-phase checkpoint, which senses replication stress, and Fanconi anemia (FA) and homologous recombination (HR) repair pathways.

FANCM-FAAP24 facilitate ATR signaling

The replication stress response pathway is a critical surveillance mechanism that coordinates the cellular response to replication stress. Central to this response is the ATR kinase, which is recruited to and activated by stalled replication forks. We have recently identified and partially

characterised HCLK2 (also known as Tel2), which interacts with and regulates the stability of all PI3K-related kinases (PIKKs), including ATR. Through its association with ATR, HCLK2 is required for the replication stress checkpoint and activation of the FA and HR pathways. To better understand how HCLK2 functions, we have carried out proteomic analyses of purified HCLK2 complexes. In addition to previously reported interactions (e.g. ATR, DNA-PKcs) we also identified an association between HCLK2 and FANCM-FAAP24, the chromatin-targeting component of the FA core complex. Our studies revealed that in addition to defects in FA pathway activation, down-regulation of FANCM or FAAP24 also compromises ATR/HCLK2-mediated checkpoint signalling leading to increased endogenous DNA damage and a failure to efficiently invoke cell cycle checkpoints. Previous studies have shown that FANCM possesses DNA translocase and branch migration activities, which may function to remodel stalled replication forks. Intriguingly, we were able to show that the DNA translocase activity of FANCM, whilst being dispensable for FA pathway activation, is required for its role in ATR/HCLK2 signalling. Our data suggest that DNA damage recognition and remodelling activities of FANCM-FAAP24 co-operate with ATR/HCLK2 to promote efficient activation of DNA damage checkpoints (Collis *et al.*, Mol. Cell 2008). More recently we have found that HCLK2 is subjected to various post-translational modifications. Investigations are underway to determine the functional relevance of these modifications and how they impact on the checkpoint and repair functions of HCLK2.

PBZ: a novel Poly(ADP-ribose)-Binding Zinc finger motif in DNA repair/checkpoint proteins

Poly(ADP-ribosyl)ation (PAR) is one of a large number of post-translational modifications of proteins that play an important role in mediating protein interactions and/or the recruitment of specific protein targets. PAR often involves the addition of chains of ADP-ribose units linked via glycosidic

ribose-ribose bonds, and is critical for a wide range of fundamental processes but is best known for its role in DNA repair. PAR synthesis is very rapidly induced at DNA damage sites where it is believed to promote recruitment of DNA repair factors (Figure 1). Targeting of proteins to these sites is dependent upon the efficient recognition of PAR by defined PAR-binding motifs or modules. We have recently identified a novel zinc finger motif, a Poly(ADP-ribose)-Binding Zinc finger (PBZ), in a number of eukaryotic proteins involved in the DNA damage response and checkpoint regulation. We were able to demonstrate interaction of poly(ADP-ribose) with this motif in two representative human proteins, APLF (Aprataxin PNK-Like Factor) and CHFR (CHeckpoint protein with FHA and RING domains), and show that the actions of CHFR in the antephasis checkpoint are abrogated by mutations in PBZ or by inhibition of poly(ADP-ribose) synthesis. PBZ provides the first description of a zinc finger that binds poly(ADP-ribose), which is also required for post-translational poly(ADP-ribose) modification (Ahel *et al.*, Nature 2008). Current studies are focused on characterisation of a novel PAR binding protein (PAR-BP) (Figure 1) that appears to play important roles in DNA repair and transcription.

RTEL1 is an anti-recombinase that impacts on genome stability and cancer

Unscheduled or excessive HR can lead to gross chromosomal rearrangements characteristic of cancer cells, but the mechanisms that restrain HR remain poorly understood. The Yeast Srs2 helicase suppresses aberrant

recombination by disrupting a specific step in HR, however functional homologues are not obviously conserved in higher eukaryotes. We recently described a genetic screen in *C. elegans* to identify uncharacterised helicases that are synthetic lethal in combination with *C. elegans* BLM mutants, based on the *srs2 sgs1* (BLM) synthetic lethality observed in yeast. This screen identified a novel helicase, RTEL-1 that is conserved from *C. elegans* to humans and exhibits many of the genetic and biochemical hallmarks of yeast Srs2 including hyper-recombination and exquisite sensitivity to various DNA damaging agents. Support for an anti-recombinogenic function for RTEL1 has come from biochemical studies. Purified human RTEL1 can actively disassemble D-loop recombination intermediates in an ATP-dependent manner. Previous work has shown that Rtel knockout mice die between days 10 and 11.5 due to dramatic genome instability and rapid telomere loss and Human RTEL1 is over-expressed in gastric tumours. Collectively, our data indicate that the phenotypes observed in *C. elegans*, mice and human cells are likely caused by a failure to correctly regulate HR. Promiscuous disassembly of recombination intermediates is also the likely underlying cause of genome instability in RTEL1 over-expressing cancers (Barber *et al.*, Cell 2008). Current work is exploiting proteomic approaches, *C. elegans* synthetic lethal screens and conditional Rtel knockout/over-expressing mice to investigate the role of RTEL1 during meiotic recombination, DNA replication, telomere maintenance and tumourigenesis.

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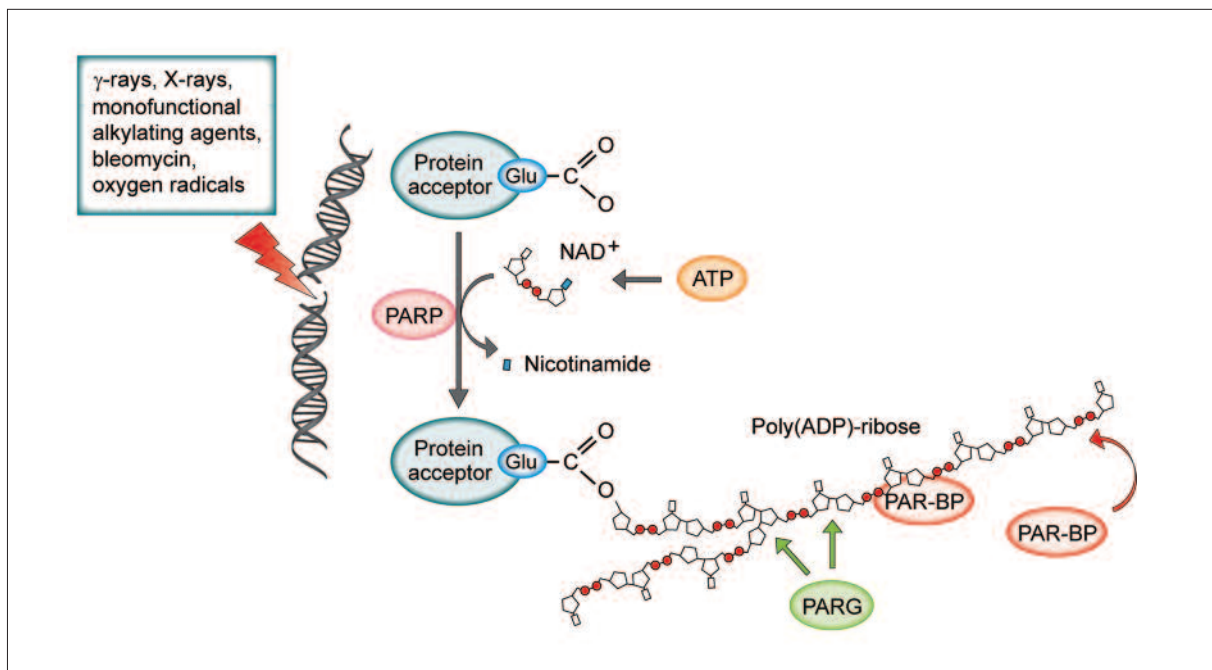


Figure 1. Poly(ADP-ribosylation) (PAR) is a post-translational modification that is rapidly induced at sites of DNA damage. Poly-ADP-ribose polymerase (PARP) utilise NAD⁺ and ATP to catalyze the formation of PAR chains on protein acceptors that act to recruit PAR-binding proteins (PAR-BP) to damage sites. PAR chains are degraded by PARG.