

Recognition and Translocation of Branched DNA Structures by FANCM Protein

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Objective: FANCM displays DNA-dependent ATPase activity and promotes the dissociation of DNA triplexes, perhaps owing to its ability to translocate along duplex DNA. The objective of this work was to test whether FANCM acts on branched DNA structures that mimic DNA replication and DNA repair intermediates.

Methods: Recombinant human FANCM protein was produced in insect cells and purified to near homogeneity. Branched DNA structures were generated by annealing of oligonucleotides and purified by native PAGE. Large recombination intermediates were produced via strand exchange between gapped circular and linear duplex DNA. Recognition and translocation of branched DNA structures was assessed *in vitro* using 32P-labelled DNA substrates.

Results: We find that purified FANCM binds to model Holliday junctions and replication forks with high specificity, and promotes migration of their junction point in an ATPase dependent manner. We have visualised FANCM-DNA complexes by electron microscopy. Using large recombination intermediates, we show that FANCM can move a four-stranded DNA branch point through more than 2.6 kb of DNA.

Model: Our current hypothesis is that FANCM recognises and moves the branch point of stalled replication forks, allowing DNA repair enzyme to gain access to the replication-blocking lesion.

Translational Applicability: Specific and reversible inhibitors of FANCM may represent a valuable adjunctive therapy for the sensitization of tumors to DNA cross-linking agents in the general population. Knowledge of the molecular pathophysiology of Fanconi anemia is essential for the design of antitumor therapeutic strategies adapted to Fanconi anemia patients.