UCF Biophysics Group Seminar

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The processing of Holliday junctions in DNA by resolving enzymes

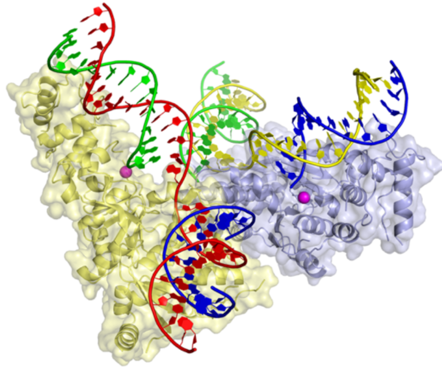
Dr. David MJ Lilley

Professor
School of Life Sciences
University of Dundee, UK

ABSTRACT

Holliday junction-resolving enzymes are critical to cell survival. A single unprocessed junction can link chromosomes and prevent cell division. Junction-resolving enzymes are dimeric nucleases that are structurally selective for four-way DNA junctions, making symmetrically-paired hydrolytic cleavages. We have solved crystal structures for junction-resolving enzymes derived from bacteriophage to eukaryotes. These offer significant insight into the recognition processes, and the chemistry of the cleavage reactions. Detailed kinetic analysis reveals that the two cleavages are effectively coordinated so as to ensure a productive cleavage reaction.

While we understand the manner of interaction with the four-way junction, and the processes by which the strands are coordinated, until recently we knew almost nothing about how the enzymes locate Holliday junctions within a long dsDNA molecule. Using a combination of optical trapping and confocal microscopy in a microfluidic cell we have studied how a single fluorescently-labeled resolving enzyme dimer locates the junction. Indeed, we can observe a complete trajectory from binding to DNA, location of the junction to its eventual resolution. These processes can then be related back to the structural data to give a good understanding of how the resolution events work in molecular terms.



Crystal structure of GEN1 bound to a DNA junction as a product complex. The two polypeptides are colored yellow and grey. Metal ions bound to the active sites are colored magenta.

Y. Liu, A. D. J. Freeman, A.-C. Déclais, T. J. Wilson, A. Gartner and D. M. J. Lilley Crystal structure of a eukaryotic GEN1 resolving enzyme bound to DNA. *Cell reports* **13**, 2565–2575 (2015).

BIO

Professor Lilley has a long-standing interest in nucleic acid structure and function (both DNA and RNA) going back more than forty years. He was the first to solve the structure of the Holliday junction in DNA, and has made extensive studies of its interaction with junction-resolving enzymes. He has solved the crystal structure of a complex with eukaryotic GEN1 resolving enzyme. Lilley has had a long interest in the structures and mechanisms of RNA catalysis. He has solved crystal structures of the twister, TS and pistol ribozymes, and defined their chemical mechanisms. Most recently he has determined the structure of a methyltransferase ribozyme, and shown that it employs a remarkably sophisticated catalytic mechanism that involves nucleobase-mediated general acid catalysis. He has solved crystal structures of six different riboswitches, the most recent being the NAD^+ riboswitch. Lilley has made a detailed study of the structure and folding k-turn motif. This is arguably now the best understood structural motif in RNA. Recently he has shown how N^6-methylation of a critical adenine blocks the key first stages of box C/D snoRNP assembly. Professor Lilley is a fellow of the Royal Society.