

Towards the structure of a bacteriophage T7 endonuclease I / Holliday junction complex

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Homologous genetic recombination is important in the repair of double-strand breaks in DNA, in the rescue of stalled replication forks, and in the creation of genetic diversity. The central intermediate in this process is the four-way (Holliday) DNA junction. This must be ultimately resolved by nucleases that are selective for the structure of the junction.

Bacteriophage T7 DNA undergoes genetic recombination during infection. The phage-encoded junction-resolving enzyme is endonuclease I. Mutants in the gene encoding this enzyme are deficient in recombination and accumulate branched DNA intermediates. We recently presented the crystal structures of a catalytically impaired mutant of endonuclease I (E65K) without metals bound (Hadden, *et al.* 2001) and the wild-type protein with metals bound (Hadden, *et al.* 2002) both in the absence of DNA. We are currently trying to crystallise an endonuclease I / Holliday junction complex. By studying the structure of this complex we hope to be able to study the mechanism of Holliday junction cleavage in more detail.

A number of different synthetic Holliday junctions have been synthesized and these have been incubated with different inactive mutants of endonuclease I. After incubation the complex was subjected to gel-filtration chromatography using a Superdex75 column. The peak corresponding to an endonuclease I Holliday junction complex was isolated and concentrated prior to setting up crystallisation trials. Each complex was subjected to a 576 condition crystallisation trial using robotically based high-throughput procedures. To date 36 potential crystallisation conditions have been identified. Some of the crystals obtained are shown in Fig. 1.

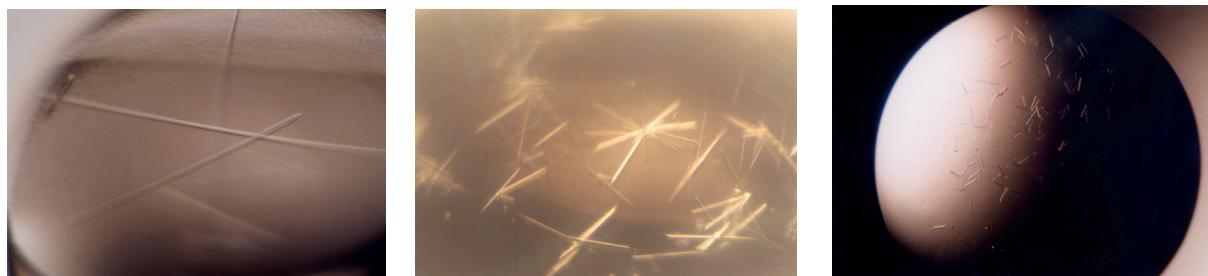


Fig. 1: Crystals of an Endonuclease I / Holliday junction complex

The best crystals obtained diffract X-rays to 5Å and we are currently investigating a number of different methods to improve upon this.

Publications

Déclais, A.-C, Fogg, J.M., Freeman, A., Coste, F., Hadden, J.M., Phillips, S.E.V. and Lilley, D.M.J. (2003) The complex between a four-way DNA junction and T7 endonuclease I. *EMBO J.*, **22**, 1398-1409.

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