

P028 Studies of the gap endonuclease activity of flap endonuclease

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Flap endonucleases (FENs) are an essential for nucleic acid metabolism playing crucial roles in DNA replication and repair. During replication FENs are responsible for processing Okazaki fragments, removing the 5'-flapped primer nucleic acid prior to ligation. Reactions catalysed by FENs include 5'-exonucleolytic (EXO) reactions of double stranded and nicked DNA substrates and structure specific endonucleolytic (FLAP-ENDO) reactions of bifurcated nucleic acids containing a 5' single stranded (ss) flap. FLAP-ENDO reactions occur around the double stranded (ds)-ss junction, predominantly 1 nt into the duplex region.

Recently, FEN activity termed GAP-ENDO (GEN) has been described. This is comparable to FLAP-ENDO activity except that the 5'-ss overhang contains a region of ds nucleic acid.

To create GEN substrate for FENs we have used a fluorescent labelled single oligomer to create gapped DNA terminating in two hairpin terms. We observe both EXO and GEN reactions on this intact substrate confirming that FENs can deal with secondary structure present in the 5'-flap region. These observed reactions take place on a similar timescale to other FEN activities suggesting a biologically significant role.

The type of fold-back secondary structure contained in our substrate is potentially created from triplet repeats thus GEN may be a mechanism to prevent triplet expansion *in vivo*.