

# DNA:DNA interactions mediate sequence specificity in the termination of plasmid replication.

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## Introduction

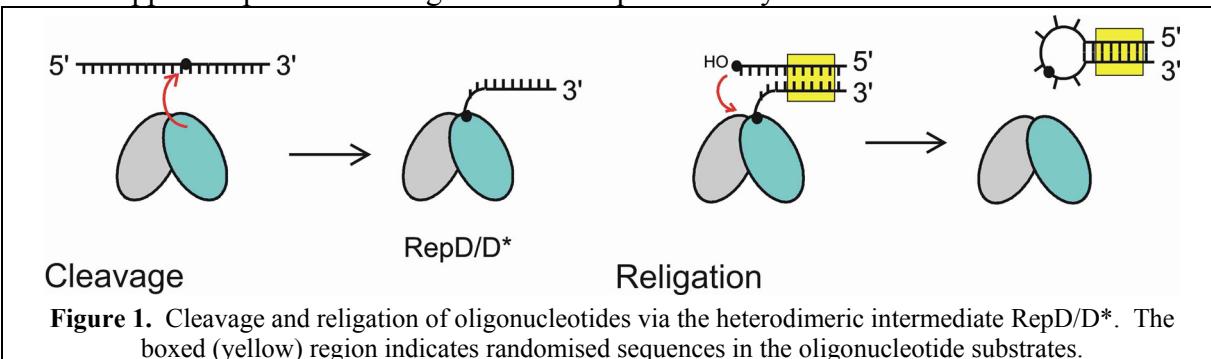
RepD is the protein required for the initiation of replication of plasmid pC221. This replication is via a rolling-circle process; after nicking the (+) strand of the replication origin to provide a primer for DNA synthesis, the protein becomes covalently attached to the 5' end of the DNA. The covalent protein-DNA complex then tracks with the replisome until the next encounter with the origin. Here, RepD mediates a concerted cleavage-religation reaction to terminate replication: this includes the religation of both the displaced single-stranded circle and the nascent DNA strand of the double-stranded daughter plasmid.

## The role of the inverted complementary repeat

The cleavage site falls between the arms of an inverted complementary repeat (ICR). Current literature is based on the presumption that nicking, and hence initiation of replication, requires the extrusion of a stem-loop structure as the functional DNA target. However, this model is challenged by the results of experiments with purified RepD protein and either double-stranded plasmids or single-stranded oligonucleotides as substrates. In both cases efficient nicking is still possible, even when the ICR is completely disrupted.

## The ICR is a consequence of, not a requirement for, the action of RepD

We have recently re-appraised the religation reaction using a model heterodimeric intermediate, RepD/D\*. After cleavage of a single-stranded oligonucleotide substrate with RepD, one of the protein subunits becomes substituted with a covalent DNA adduct at the active site (Fig. 1). This intermediate is isolated, then challenged with an acceptor oligonucleotide to reverse the reaction; religated products are amplified in a variation of the SELEX approach prior to cloning and DNA sequence analysis.



**Figure 1.** Cleavage and religation of oligonucleotides via the heterodimeric intermediate RepD/D\*. The boxed (yellow) region indicates randomised sequences in the oligonucleotide substrates.

Using randomised sequences in either the acceptor substrate, or within both acceptor and the covalent adduct of RepD\*, we have found that a significant proportion of recovered, religated products display base complementarity despite being randomised to begin with (Table 1).

**Table 1.** Sample sequences obtained following religation

Sequence	Sequence (5' → 3'; ' indicates nick)
wild-type	..AAAACCGGCTACTCT'AATAGCCGGTTAA..
I-T1	..AA <u>ACCGGT</u> CTACTCT'AATAGCCGGTTAA..
I-U1	..AAC <u>CCGG</u> CTACTCT'AATAGCCGGTTAA..
II-T2	..AAG <u>GCCG</u> CTACTCT'AATAGCC <u>GTGAA</u> ..
II-R	..AAG <u>CTCAG</u> CTACTCT'AA <u>ATAGCTGAGTC</u> ..

Bases initially randomised in the substrate are underlined

Complementarity between a randomised acceptor substrate and the wild-type RepD/D\* intermediate could be explained by DNA sequence specificity inherent within RepD. However, the recovery of products in which the complementary sequences are altered in both acceptor substrate and RepD/D\* intermediate argues

strongly in favour of a DNA-mediated base pairing between the DNA adduct of RepD/D\* and the acceptor substrate, as depicted in Figure 1.

The RepD/D\* complex may thus be using base pairing with the covalent DNA adduct in conjunction with the conserved nick site to identify the target for termination of replication. To our knowledge, this is the first example of a covalent DNA adduct being utilised by a protein for discrimination of sequence in such a way. Because base paring is involved, the product of the reaction will possess an inverted complementary repeat, even though one is not required for the initial cleavage reaction to occur. Over many generations, the extent of the base-paired region may reach an optimum length, as found *in vivo*. The SELEX approaches demonstrated in this study are currently being redesigned to investigate this.

### **Acknowledgements**

We thank Val Sergeant for technical support, and Denise Ashworth for DNA sequence analysis.

### **Funding**

This work is supported by the Wellcome Trust.