

# Understanding the recruitment and processivity of the staphylococcal helicase PcrA in the context of plasmid replication

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## Background

The helicase PcrA is not only essential for the viability of the Gram positive human pathogen, *Staphylococcus aureus*, but is also required for the rolling circle replication of staphylococcal plasmids such as pC221 – our model 4.6 kb plasmid encoding chloramphenicol resistance.

Work by our group and collaborators has previously centred on the interaction between the *Bacillus stearothermophilus* PcrA and the pC221 replication initiator protein, RepD. Advances in expression and purification of the staphylococcal helicase, resulting in greater yields of protein, have allowed us to return to a more homologous system to characterise the recruitment and processivity of the helicase by RepD.

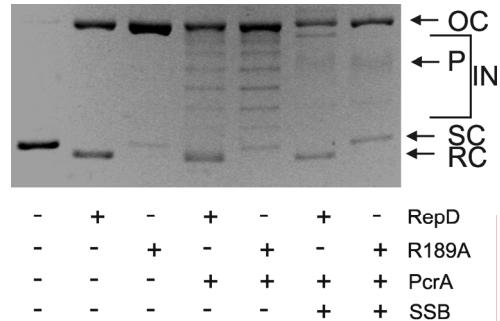
## Recent findings

RepD can nick and religate at its cognate *ori* sequence, *oriD*. In the presence of RepD the negatively supercoiled substrate pCER*oriD* is nicked and relaxed. RepD forms a covalent attachment to the nicked plasmid which is a prerequisite for unwinding by PcrA (Fig. 1). R189A is a mutant of RepD that can nick at *oriD* but cannot religate: this was used to generate a stable protein:DNA covalent adduct.

Upon the addition of PcrA and ATP, partially unwound intermediates are observed. With further addition of single-strand DNA binding protein (SSB) these intermediate bands disappear, being replaced by a single, faint band (believed to be ssDNA, resulting from complete unwinding of the DNA) suggesting that this protein is also necessary for plasmid replication *in vivo*.



**Fig. 2.** Linear double stranded DNA is uniform in appearance (right). When linearised pCER*oriD* is incubated with R189K and PcrA the displaced, single strands forms a condensed, complex on the mica (top left). The scale bar represents 200 nm.



**Fig. 1.** Agarose gel showing nicking and unwinding of pCER*oriD*. OC, nicked open circular plasmid form; IN, partial unwind intermediates; SC, supercoiled; RC, relaxed covalently closed; P, main product in the presence of SSB.

Recruitment of PcrA by RepD to a linear DNA substrate has also been visualised by AFM (Fig. 2). This approach will prove useful in future work characterising the dissociation of the helicase from the replisome at termination.

## Publications

Zhang, W., Dillingham, M.S., Thomas, C.D., Allen, S., Roberts, C.J. & Soultanas, P. (2007) Directional loading and stimulation of PcrA helicase by the replication initiator protein RepD. *J. Mol. Biol.* **371**, 336-348.

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