The staphylococcal PcrA helicase requires the action of both the replication initiator RepD and SSB proteins to unwind the small plasmid pCER*oriD*

Gerard P. Lynch, Neil H. Thomson and Christopher D. Thomas

Background

The *pcrA* gene is almost ubiquitous in Gram-positive bacteria and encodes an essential but poorly processive helicase. Although the role of PcrA in Gram-positive bacteria such as the human pathogen *Staphylococcus aureus* remains unclear, it has been shown to be important for the rolling circle replication of the pT181 family of staphylococcal plasmids.

Using our 3 kb model plasmid pCER*oriD* as substrate, we have studied the mechanism by which PcrA unwinds duplex DNA into its component single-stranded form. Work has previously focused on how PcrA is recruited by the plasmid replication initiator protein RepD to partially unwind negatively supercoiled plasmid DNA, but recent advances with linearised plasmid DNA substrates have highlighted additional roles of RepD and single-stranded DNA binding protein (SSB) in stimulating the unwinding activity of PcrA.

Recent findings

Atomic force microscopy (AFM) was used to visualise the recruitment and unwinding of linear pCER*oriD* by PcrA. RepD can nick and religate at its cognate origin of replication, *oriD,* forming a transient covalent attachment with the plasmid pCER*oriD* via the active site residue Y191. Digestion of pCER*oriD* with HindIII results in a linear DNA fragment with *oriD* located at one end. The RepD mutant R189K retains the ability to nick at *oriD* but cannot religate. This mutant was used to form a stable DNA:protein replication initiation complex (Fig. 1A). The complex is recognised by the PcrA helicase, which alters the terminal appearance of the DNA fibre once bound (Fig. 1B). On the addition of ATP, the helicase appears to translocate along the DNA (Fig. 1C). Interestingly the terminal end of the DNA fibre is still in contact with the position of the helicase, suggesting that not only is R189K important for recruitment of PcrA, but it is also essential in "clamping" the helicase onto the DNA. Under such conditions the DNA appears to remain in double-stranded form after passage of the helicase. Full unwinding of pCER*oriD* is only seen in the presence of SSB, which sequesters single-stranded DNA (Fig. 1D). The combination of RepD-R189K for formation of the initiation complex plus visualisation by AFM thus represents a powerful tool for the study of PcrA helicase activity.

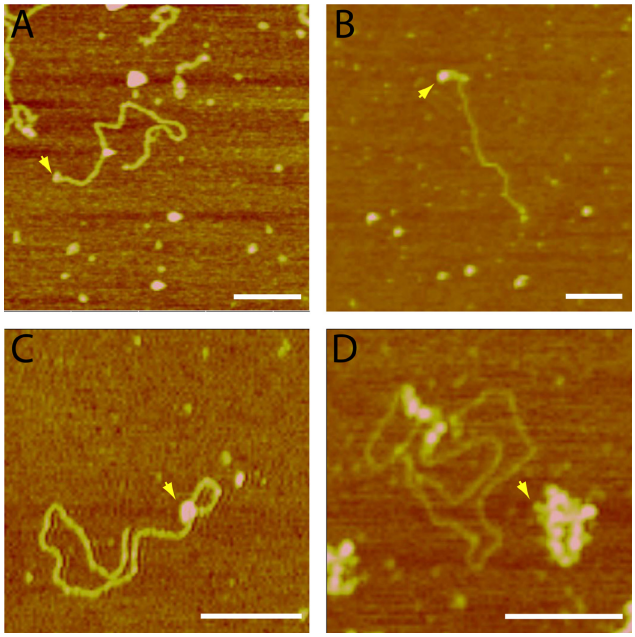

Fig. 1 AFM image in air of linearised pCER*oriD* in the presence of various components of the rolling circle replicative apparatus. **A**, R189K; **B**, R189K and PcrA; **C,** R189K, PcrA and ATP; **D**, R189K, PcrA, ATP and SSB. Yellow arrows indicate components described in the text. The white scale bar represents 200 nm.

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