Defining the specificity of the Rep:PcrA interaction

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Introduction

The *pcrA* gene is 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.

To date, our work has focussed on helicases obtained from two organisms: *Staphylococcus aureus* (*Sau*PcrA) and (*Geo*)*Bacillus stearothermophilus* (*Bst*PcrA). We have observed that the activity of both helicases is stimulated in the presence of the plasmid initiator protein RepD, encoded by the staphylococcal plasmid pC221. Furthermore, we have also observed that the *Bst*PcrA is recruited to the replication origin of pC221 by RepD [1]. However, no details of the Rep:PcrA interaction are currently known.

Recent Findings

In order to create a homologous system for the study of *Bacillus* Rep:PcrA interactions we have cloned and purified the Rep protein from the *Bacillus stearothermophilus* plasmid pSTK1. This protein, RepSTK, demonstrates sequence-specific type-I topoisomerase activity against plasmids carrying the cognate replication origin but with a temperature optimum of 65°C, considerably higher than that for the comparable activity of RepD.

RepSTK also demonstrates specificity for the cognate PcrA helicase. As indicated above, in oligonucleotide displacement assays PcrA alone is a poor helicase. Although RepD can stimulate both *Sau*PcrA and *Bst*PcrA activities, RepSTK failed to demonstrate stimulation of *Sau*PcrA (Table 1).

Table 1. Stimulation of helicase activity.

PcrA	RepD	RepSTK
Sau	✓	×
Bst	✓	✓
SBS	×	×
BSB	✓	✓

The two helicases share 59% sequence identity; the known structure of *Bst*PcrA indicates four domains (1A, 1B, 2A, 2B; see Fig. 1) plus an unstructured C-terminal tail. We have now begun the dissection of PcrA to identify the region(s) conferring specificity on the Rep:PcrA interaction. Deletion of the C-terminal domain has no effect on stimulation. We have also exchanged the 2B domain between helicases (differences being shown in red in Fig. 1). Although the *Bst*2B domain in the context of *Sau*PcrA (SBS) produced an inactive helicase, the complementary *Sau*2B in *Bst*PcrA (BSB) gave a protein which was stimulated by both Rep proteins. This argues against a specific interface between Rep and the 2B domain; current efforts are now focussed on 1A, 1B and 2A to find the site of interaction necessary for the stimulation of helicase activity.

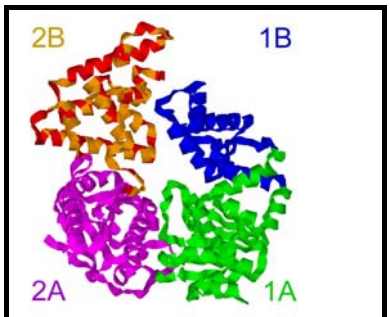

Figure 1: domains of PcrA.

Publications

Machon, C., Lynch, G., Thomson, N., Scott, D., Thomas, C. & Soultanas, P. (2010) RepD-mediated recruitment of PcrA helicase at the *Staphylococcus aureus* pC221 plasmid replication origin, *oriD*. *Nucleic Acids Res.* **38:**1874-1888.

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