

Molecular mechanism of Staphylococcal plasmid transfer

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Background

Horizontal gene transfer in bacteria results in genetic diversity with important medical consequences. Small, non-self transmissible, mobilisable, staphylococcal plasmids such as pC221 offer a simple system that embodies the initial events in plasmid mobilisation.

pC221 is a 4.6kb chloramphenicol resistance plasmid of *Staphylococcus aureus*. Although not self-transmissible, it can be mobilised by a co-resident self-transmissible plasmid such as pGO1. Typically for a small plasmid, pC221 contains only those genes required for its own DNA processing and contains four such loci: an origin of transfer (*oriT*); a DNA relaxase, MobA; and the putative accessory proteins, MobB and MobC.

Recent findings

MobC binds to *oriT* at two sites containing a 9 bp consensus sequence (Fig. 1), and additionally within the *mobC* gene at a 7 bp conserved sequence. The addition of MobA to the MobC-*oriT* complex modified the observed footprint profile at the nick site and the adjacent MobC binding site. The *oriT* has recently been functionally characterised with respect to these sites. A 78 bp region of *oriT*, encompassing the nick site and the proximal MobC binding site (MCB) has been used as a nicking substrate (Fig. 2). Mutations in both MCB and the region modified by the addition of MobA (SRA) abolish nicking, thus demonstrating that nicking by the MobA relaxase requires the binding of MobC, and the presence of an intact SRA sequence.

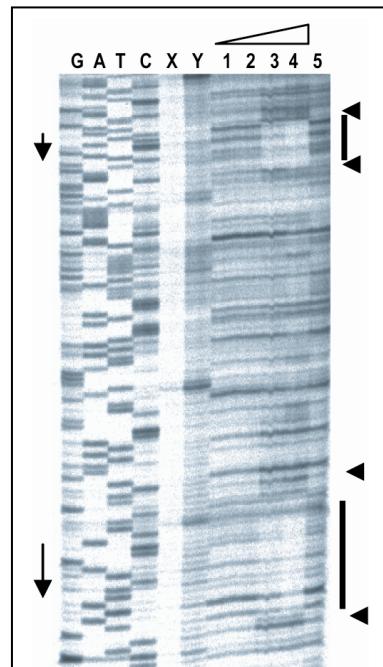


Fig. 1. DNaseI footprinting of MobC at *oriT*. Titration of MobC. Vertical bars indicate protection. Arrows indicate consensus direct repeats.

Nicking by:

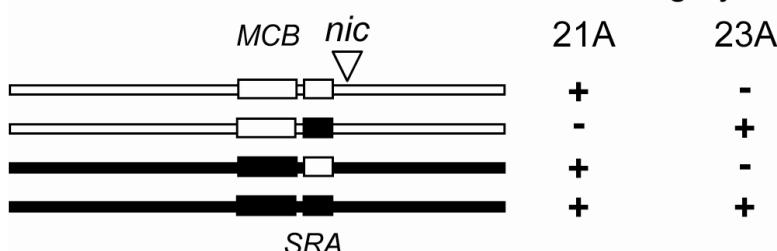


Fig. 2. Schematic detailing SRA-swap between pC221 *oriT* (white) and pC223 *oriT* (black), and the nickability of recombinant substrates by pC221 MobA 21A or pC223 MobA (23A)

The SRA sequence is not conserved between the related *oriTs* of pC221 and pC223. It was thought this sequence may therefore be functionally important in substrate recognition. The SRA regions (which differ by 4 bp) were swapped between the pC221 78 bp nick target and a comparable nick target of pC223 (Fig. 2). Exchange of this region was indeed found to swap

the substrate specificity, thus SRA represents a recognition sequence by which the relaxases recognise their specific substrate. Determination of the corresponding specificity domain in the MobA protein and the nature of any MobA-MobC interactions are currently under investigation.

Publications

Caryl, J.A., Smith, M.C.A. and Thomas, C.D. (2004). Reconstitution of a staphylococcal plasmid-protein relaxation complex *in vitro*. *J. Bacteriol.* **186**: 3374-3383.

Caryl, J.A. and Thomas, C.D. (2005) Initial events in small staphylococcal plasmid transfer. In, Thomas, C.M. *et al.* (eds). Plasmid Biology 2004: International Symposium on Molecular Biology of Bacterial Plasmids and other Mobile Genetic Elements. *Plasmid* **53**: p.47-48.

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