

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*. It is a non-self transmissible plasmid that can be mobilised by a co-resident self-transmissible plasmid such as pGO1. Being 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.

The nicking reaction has been reconstituted *in vitro* and has demonstrated the requirement for MobA and MobC proteins, in the presence of Mg^{2+} or Mn^{2+} , for site- and substrate-specific nicking.

Recent findings

DNaseI footprinting by primer extension has been performed with MobC and MobA on supercoiled pC221cop903 *oriT* DNA. MobC binds to two regions within the *oriT* (Fig. 1), and additionally to a region within the *mobC* gene. A consensus binding sequence has been identified at all three protected regions. Between the *oriT* binding sites are regions of periodic MobC induced structural perturbation.

The addition of MobA to the complex induces modified protection footprints to the left of the nick site and increased hypersensitivity immediately downstream from the nick site.

The requirement for each of the binding sites for nicking and mobilisation has been investigated by selective cloning. Only the binding site adjacent to the nick site was required for both processes. The role of these binding sites in regulation of Mob protein expression is currently being investigated.

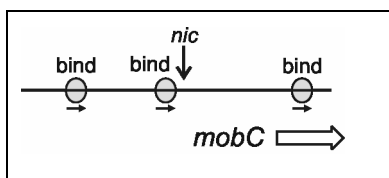


Fig. 2. Relative positions of MobC binding sites, MobA *nic* site, and *mobC* reading frame.

Publications

Smith, M.C.A. & Thomas, C.D. (2004) An accessory protein is required for relaxosome formation by small staphylococcal plasmids. *J. Bacteriol.* (in press)

Caryl, J.A., Smith, M.C.A. & Thomas, C.D. (2004) Reconstitution of a staphylococcal plasmid-protein relaxation complex *in vitro*. *J. Bacteriol.* (in press)

This work has also been presented at the UK Mobile Genetic Elements Workshop 2003, Birmingham, UK.

Acknowledgements

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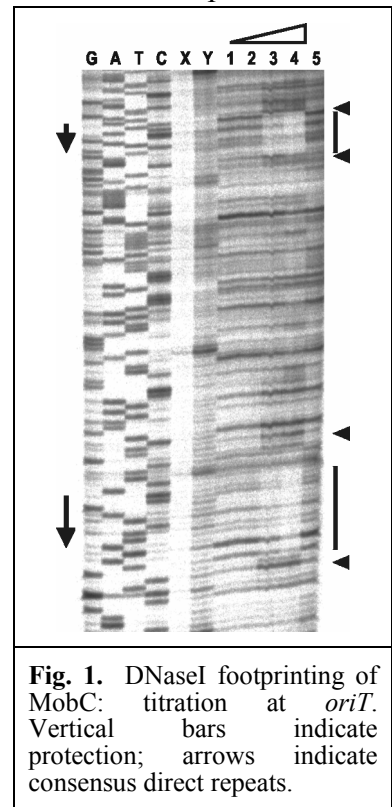


Fig. 1. DNaseI footprinting of MobC: titration at *oriT*. Vertical bars indicate protection; arrows indicate consensus direct repeats.