

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 4.6 kb 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 (*mcb*) containing a 9 bp consensus sequence, and additionally within the *mobC* gene at a 7 bp conserved sequence. The *oriT* of pC221 has been functionally characterised with respect to the sites of Mob protein-DNA interaction. A functional region, sufficient for

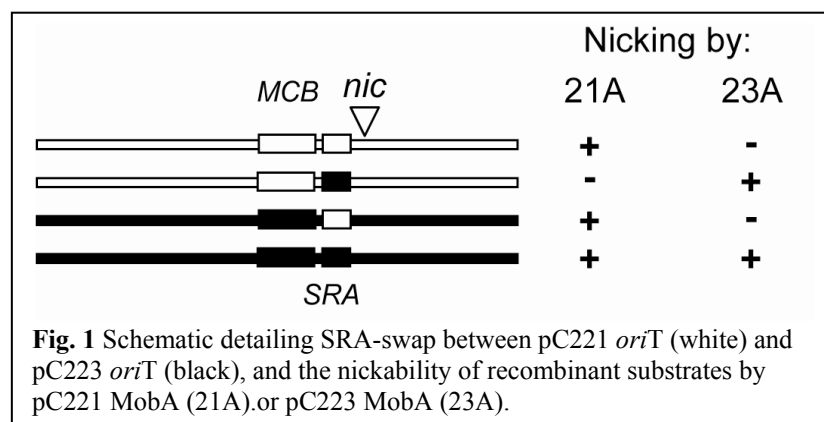
nicking, is defined by 77 bp sequence encoding a 9 bp MobC binding site (*mcb2*), a site of MobA interaction (*sra*) and the nick site (*nic*). Mutagenesis of *mcb2* has demonstrated that binding of MobC is a pre-requisite for subsequent nicking by MobA.

The MobA relaxase recognises a plasmid-specific sequence within *sra*, permitting cleavage at *nic*. The *sra* of pC221 and pC223 differ by 4 bp. Swapping of these base-pairs between the two substrates, which otherwise share identical nick sites, effectively exchanges the substrate specificity and therefore recognition by their respective cognate relaxases (Fig. 1).

Recently, each of the MobC binding sites has been mutated, in the context of pC221, to investigate the requirement of each in isolation. Four mutant plasmids that differ from the wild-type only at *mcb1*, *mcb2*, *mcb3* or *mcb1+3* were assayed for *in vivo* nicking and mobilisation activity. In each case, mutation was found to abolish mobilisation. Agarose gel electrophoresis of whole cell lysates showed that with the exception of the *mcb2*, all the mutant plasmids were susceptible to nicking. Thus MobC may function at several levels: primarily to initiate nicking by MobA, presumably via interaction at *mcb2*; but furthermore by mediating a potential high-order complex formation, additionally involving *mcb1* and *mcb3*, to yield a mobilisable substrate.

Publications

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