Rolling circle replication

Chris Thomas, Steve Carr, Jamie Caryl and Val Sergeant

Introduction

Many plasmids and bacteriophage replicate their DNA by a common mechanism - referred to as the "Rolling Circle". Our interests lie with the proteins that mediate this process, and the DNA structures at which they act. In the case of staphylococcal plasmids (such as pC221) and filamentous bacteriophage (including M13), a single protein is sufficient to recognise and cleave at the origin of replication, initiating replication. Here our efforts remain committed to the structural characterisation of these proteins, underpinned by biochemical studies of the nicking-closing process.

Since transfer of DNA by conjugation is also a rolling circle process, we have also been interested in the mechanisms by which pC221 may be mobilised from one cell to another. Unlike the maintenance replication described above, several plasmid-encoded functions are required to initiate the replicative process leading to conjugation. Our recent progress in this area is described below.

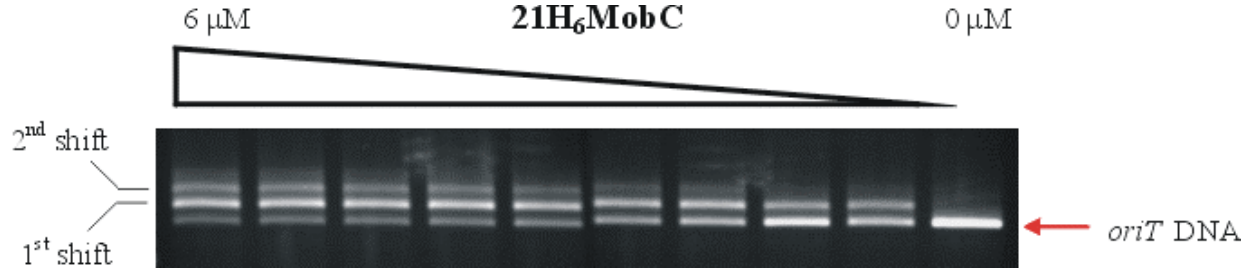
Conjugative transfer of DNA in *Staphylococcus aureus*

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.

It has been demonstrated that mutations in either the *mob*A or *mob*C ORFs of pC221 result in a loss of observable nicking *in vivo*. In addition, previous work has failed to demonstrate nicking with purified MobA protein alone. To investigate this further, the *mob*C ORF was cloned, expressed, and MobC was purified. Gel retardation experiments demonstrated a substrate-specific, cooperative interaction between MobC and its cognate *ori*T sequence (Figure 1). The *mob*A ORF has been expressed and MobA protein purified. It has now been shown that MobA and MobC are required, in the presence of Mg^{2+}, for site- and substrate-specific nicking *in vitro*. The protein combination is specific for cognate substrate pC221 as no nicking was observed when pC223 (sharing ~80% whole sequence identity) was used as a substrate; likewise with the related plasmid pT181*cop608*.

Figure 1: Gel retardation assay of isolated *ori*T DNA against increasing concentration of MobC protein.



Recent cross-specificity studies have suggested that MobA, in particular, may encode plasmid specificity, as (low efficiency) nicking of pC221 was observed in presence of cognate MobA and non-cognate MobC (from pC223). It is currently believed that MobC acts at a specific sequence in the *ori*T, present in pC221 and related plasmids such as pC223, as a targeting protein for MobA and produce a single-stranded substrate at which MobA can nick.

Further to this, the *ori*T region from pC221 has been cloned into an *E.coli* vector and used as substrate in an assay with cognate MobA and MobC. Nicking has been demonstrated using this substrate, although only at 67% efficiency of pC221 substrate. Footprinting studies of bound MobA and MobC are presently being planned. Following resolution of the *ori*T to the minimum region required for DNA-processing, mobilisation studies will be conducted to determine whether this region is sufficient for conjugal transfer.

Presentations
This work has been presented at **NACON-V** 5th International Meeting on Recognition Studies in Nucleic Acids, and the **UK Plasmid Biology Workshop 2001**, Birmingham.

Collaborators
Dr June Polak, Long Island University Visiting Researcher (2001)
Prof. Simon Phillips (Leeds);
Carl Brown and Phil Eastlake (Transgenomic)

Funding
BBSRC, The Wellcome Trust.