**Col E1 plasmid replication**

ColE1 plasmid replication is tightly controlled by an antisense RNA mechanism that is highly dynamic, tuning plasmid metabolic burden to the physiological state of the host. Replication of ColE1 is under the control of a 550 bp sequence known as the plasmid origin of replication or ori.

The plasmid ori encodes an RNA pre-primer that forms a stable hybrid with a 30-nucleotide stretch of the DNA template strand. This stable hybrid, known as R-loop, is processed by RNAseH to create a 3’ -OH end. Extension of this end by DNA polymerase I initiates leader strand DNA synthesis.

The elongating leader strand separates the double-stranded DNA template, creating a structure known as a D-loop and exposing a primosome assembly signal (n’-pas).

This signal is bound by PriA, PriB and DnaT to form a complex that initiates replication fork assembly, which includes the replicative polymerase Pol III. Pol III completes the replication of the plasmid. Right positioning of the 3’ –OH end of the pre-primer is critical for RNA processing and for Pol I extension.

Replication is regulated by transcription of a 108 nucleotide-long antisense RNA encoded by the plasmid (known as RNA I), which is transcribed from antisense promoter P1. The pre-primer (known as RNA II) is transcribed from a sense promoter, P2.

The antisense promoter is much stronger, resulting in a 100-fold excess of inhibitor relative to pre-primer. This constitutes a negative feedback loop, as the levels of inhibitor are proportional to the number of plasmids. The result is a specific copy number/per cell for a given set of conditions.

RNA I prevent a transcript (RNA II) from the ColE1 primer promoter to form a hybrid with the template DNA and thereby inhibits formation of primer for DNA replication. Binding of RNA I to RNA II is responsible for the inhibition. The formation of an RNA-RNA duplex is stabilized by a plasmid-borne protein, Rop. Rom (the gene encoding Rop) has been removed in most vectors, which raises plasmid copy number by 3 to 5-fold.

