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Antisense-RNA mediated control of plasmid replication – pIP501 revisited

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ABSTRACT

Over the past decade, a wealth of small noncoding RNAs (sRNAs) have been discovered in the genomes of almost all bacterial species, where they constitute the most abundant class of posttranscriptional regulators. These sRNAs are key-players in prokaryotic metabolism, stress response and virulence. However, the first *bona-fide* antisense RNAs had been found already in 1981 in plasmids, where they regulate replication or maintenance. Antisense RNAs involved in plasmid replication control – meanwhile investigated in depth for almost 35 years – employ a variety of mechanisms of action: They regulate primer maturation, inhibit translation of essential replication initiator proteins (Rep proteins) as well as leader peptides or the formation of activator pseudoknots required for efficient *rep* translation. Alternatively they attenuate transcription or translation of *rep* mRNAs. Some antisense RNAs collaborate with transcriptional repressors to ensure proper copy-number control. Here, I summarize our knowledge on replication control of the broad-host range plasmid pIP501 that was originally isolated from *Streptococcus agalactiae*. Plasmid pIP501 uses two copy number-control elements, RNAIII, a cis-encoded antisense RNA, and transcriptional repressor CopR. RNA III mediates transcription attenuation, a rather widespread concept that found its culmination in the recent discovery of riboswitches. A peculiarity of pIP501 is the unusual stability of RNA III, which requires a second function of CopR: CopR does not only repress transcription from the essential *repR* promoter, but also prevents convergent transcription between *rep* mRNA and RNAIII, thereby indirectly increasing the amount of RNAIII. The concerted action of these two control elements is necessary to prevent plasmid loss at dangerously low copy numbers.

1. Introduction

Plasmids are selfish genetic elements that normally constitute a burden for the bacterial host cell. This burden is expected to favor plasmid loss. Consequently, plasmids have evolved mechanisms to control their replication and ensure their stable maintenance. Replication control can be either mediated by iterons or by antisense RNAs. Whereas iteron control targets the origin directly (rev. in Chattoraj, 2000), antisense control targets the expression of the replication initiator protein. Antisense RNAs are constitutively synthesized and metabolically unstable and employ a negative control circuit: Antisense RNAs act both as a measuring device and a regulator, and regulation occurs by inhibition. Target of regulation is an essential molecule: In one case a replication primer, in all other cases the mRNA encoding the replication initiator protein (Rep protein). Upward-fluctuations of plasmid copy numbers entail increasing antisense-RNA concentrations, which, in turn, lead to inhibition of target function. By contrast, downward-fluctuations of copy numbers result in reduced antisense-RNA concentrations, yielding an increasing replication frequency. Inhibition is achieved by

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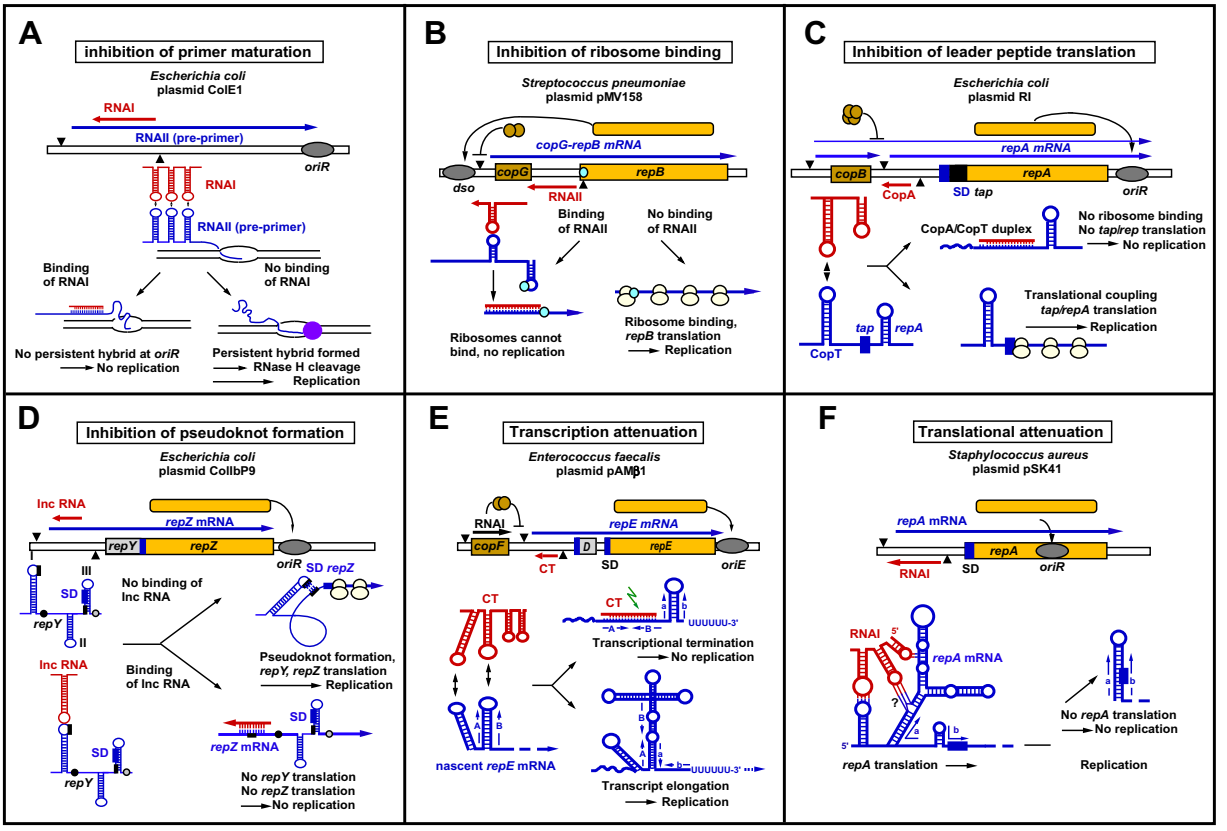

Fig. 1. Mechanisms of antisense-RNA mediated plasmid replication control. Antisense RNAs are drawn in red, sense RNAs in blue. ORFs encoding essential replication initiator proteins are shown as orange boxes, ORFs encoding transcriptional repressor proteins are shown as brown boxes. SD sequences for *rep* ORFs are blue rectangles. Promoters are symbolized by black triangles and replication origins by dark grey ovals. Arrows indicate positive interaction, black bars indicate repression. Ribosomes are in light yellow. (A) Inhibition of primer maturation: Plasmid ColE1. Upper part: Schematic representation of the minimal replicon. Lower part: Mechanism of inhibition of primer maturation. Violet circle, RNA polymerase. For details see text. Translational inhibition. (B) Translation inhibition by inhibition of ribosome binding. Upper part: Working model on regulation of plasmid pMV158 replication. Lower part: The antisense RNA binds directly upstream of the extended non-SD sequence (light blue circle) 5′ of the *repB* start codon and prevents binding of the 30S ribosomal subunit. The CopG protein represses transcription from the *copG-repB*-promoter and from the *repB* promoter. (C) Inhibition of leader peptide translation. Upper part: Working model on regulation of plasmid R1 replication. Lower part: Translation of the leader peptide (dark-grey box) *tap* is required for efficient *repA* translation. The CopB protein represses transcription from the *repA*, but not from the *copB* promoter. (D) Inhibition of pseudoknot formation: Plasmid ColIb-P9. Upper part: The minimal replicon with the leader peptide *repY* (dark grey) and *repZ* genes is shown. White: leader region of *repZ* mRNA. Lower part: Genes for *repY* and *repZ* are translationally coupled. On the mRNA, the *repY* SD sequence is exposed, whereas structure III sequesters both the *repZ* SD sequence (black rectangle) and the 5′-rCGCC-3′ sequence (thick black line) and, thereby, *repZ* translation. Inc = region complementary to the antisense-RNA; black circle, *repY* start codon; grey circle, *repY* stop codon. Unfolding of structure II by the ribosome stalling at the *repY* stop codon results in formation of a pseudoknot by base paring between the 5′-rGGCCG-3′ and 5′-CGCC-3′ (thick black lines) sequences distantly separated, and allows the ribosome to access the *repZ* RBS. Binding of Inc RNA to the loop of structure I of *repZ* RNA directly inhibits formation of the pseudoknot and the subsequent IncRNA-*repZ*-mRNA duplex formation inhibits *repY* translation. (E) Transcriptional attenuation: Plasmid pAMβ1. Upper part: Working model on regulation of pAMβ1 replication. The minimal replicon with the *copF* and *repE* genes is shown, separated by the leader region. CopF represses transcription from the *repE* promoter and – at the same time-indirectly increases transcription initiation from the antisense promoter p_{ct}. The antisense RNA (CT) causes premature termination of *repE* (sense) RNA transcription at the attenuator (*att*). The grey rectangle denotes ORF D, which comprises 300 bp and was found to be dispensable for replication (Le Chatelier et al., 1996). Lower part: Mechanism of transcriptional attenuation. For details see text. Complementary sequence elements are designated A, B, a and b. Green arrow, RNase III. (F) Translational attenuation. Upper part: Working model on regulation of plasmid pSK41 replication. Lower part: The antisense RNA interacts via three loops with the nascent *repA* mRNA resulting in a stem-loop structure that sequesters the ribosome binding site. In the absence of RNAI, the *repA* mRNA refolds into an alternative structure that exposes the ribosome binding site, allowing *repA* translation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

a variety of mechanisms summarized in Fig. 1 and briefly discussed below.

2. Antisense-RNA mediated control of plasmid replication

After nearly 35 years of research on antisense RNA-mediated plasmid replication control, six mechanisms of action employed by the antisense RNAs are known, which have been reviewed in detail before (Brantl, 2002, 2013). Some of them are discussed extensively in other contributions of this issue.

Escherichia coli plasmid ColE1 is the only plasmid that does not need a Rep protein, but uses the host DNA polymerase I to extend the essential plasmid-encoded RNA primer. RNAI, the 108 nt long ColE1 antisense RNA, inhibits maturation of pre-primer RNAII (Masukata and