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Mutations in an antisense RNA, involved in the replication control of a *repABC* plasmid, that disrupt plasmid incompatibility and mediate plasmid speciation

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ABSTRACT

The maintenance of large plasmid in a wide variety of alpha-proteobacteria depends on the *repABC* replication/segregation unit. The intergenic *repB-repC* region of these plasmids encodes a countertranscribed RNA (ctRNA) that modulates the transcription/translation rate of RepC, the initiator protein. The ctRNA acts as a strong incompatibility factor when expressed *in trans*. We followed a site directed mutagenesis approach to map those sequences of the ctRNA that are required for plasmid incompatibility and for plasmid replication control. We found that the first three nucleotides of the 5′-end of the ctRNA are essential for interactions with its target RNA. We also found that stretches of 4–5 nucleotides of non-complementarity within the first 10 nucleotides of the left arm of the ctRNA and the target RNA are sufficient to avoid plasmid incompatibility. Additionally, miniplasmid derivatives expressing ctRNAs with mutations in the 5′ end or small deletions in the ctRNA are capable of controlling their own replication and coexisting with the parental plasmid. We suggest that a mechanism that could have a crucial role in the speciation process of *repABC* plasmids is to accumulate enough changes in this small region of the ctRNA gene to disrupt heteroduplex formation between the target RNA of one plasmid and the ctRNA of the other. Plasmids carrying these changes will not have defects in their maintenance.

1. Introduction

Plasmids are extrachromosomal elements that have a fundamental role in bacterial evolution, primarily because they are efficient vectors of horizontal gene transfer. Analysis of barriers to horizontal gene transfer and the evolutionary mechanisms shaping the structure of mobile elements are complex and compelling long-term research topics (e.g., Harrison and Brockhurst, 2012; Popa and Dagan, 2011). Our current understanding suggests that plasmid mobility between cells of the same or different species depends on several factors. Some factors are intrinsic to their gene content; plasmids should carry all the genes required for conjugation or at least the DNA sequences that allow them to use the conjugative machinery of other plasmids. Other factors limiting plasmid mobility are host encoded; for example, a plasmid's recipient needs to encode elements required to sustain the replication and maintenance of the incoming plasmid. Plasmid incompatibility is another factor that restricts plasmid mobility. Two plasmids are unable to coexist in the same cell if their replication and/or segregation machineries interfere with each other (Austin and Nordström, 1990; Novick et al., 1976). In this way, the host plasmid content limits the diversity of new replicons that can be accepted and maintained in the recipient cell.

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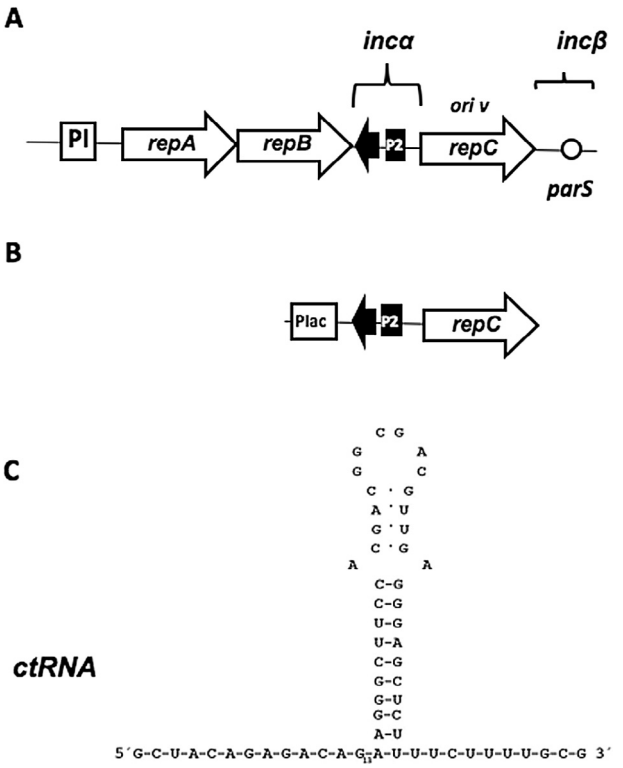

Fig. 1. (A) Genetic organization of a *repABC* replication/segregation unit. (B) Genetic structure of the replication unit of pDOPαC miniplasmid. White arrows represent genes encoding the partitioning and the initiator proteins. Circle represents the centromeric-like region *parS*. Black arrow represents the gene encoding the ctRNA that modulates *repC* expression. Boxed P1 and P2, indicate the position of the promoters found within the *repABC* replication/segregation unit; meanwhile boxed Plac indicates the position of promoter governing *repC* transcription in the miniplasmid. Brackets indicate regions involved in plasmid incompatibility. (C) Secondary structure model of the ctRNA of plasmid pRetCFN42d (p42d).

To cope with plasmid incompatibility, bacteria have developed a wide variety of plasmid replication systems to minimize potential interferences. A close examination of bacterial genomes shows that the plasmid replication systems of one strain are usually not closely related (unpublished results). An exception to this observation is alphaproteobacteria genomes. The genome architecture of this bacterial class, especially in the Rhizobiales, Rhodospirillales, and Rhodobacterales orders, commonly consists of one chromosome and several large plasmids of very low copy number that frequently belong to the same plasmid family. The most prevalent replication/segregation unit of these alphaproteobacteria plasmids is the *repABC* operon and, for this reason, they are classified as members of the *repABC* plasmid family (Cevallos et al., 2008; Pappas, 2008; Petersen et al., 2009; Pinto et al., 2011). This plasmid family includes several incompatibility groups, meaning that more than one *repABC* plasmid can coexist in the same strain (Castillo-Ramírez et al., 2009; Cevallos et al., 2002). It is extremely common within Rhizobiales that all plasmids from one strain are members of the *repABC* family. For example, the six large plasmids of *Rhizobium etli* CFN42 belong to the *repABC* plasmid family (Cevallos et al., 2008; Crossman et al., 2008; González et al., 2003).

A *repABC* operon contains all of the elements required for the replication and maintenance of a plasmid and must be considered as a complete replication/segregation unit. This operon consists of three protein-encoding genes that are always in the same order, *repA, repB* and *repC*, and of a small antisense RNA regulatory gene located in the complementary DNA strand between the *repB* and *repC* genes. RepA and RepB have dual roles. They are involved, in conjunction with the centromere-like sequence (*parS*), in plasmid segregation and in the negative regulation of their own transcription (Pappas and Winans, 2003b; Pérez-Oseguera and Cevallos, 2013). RepC is the replication initiator protein and acts on the origin of replication located within the central region of its own coding sequence (Fig. 1A) (Cervantes-Rivera et al., 2011; Pinto et al., 2011). The position and number of partitioning-site sequences, *parS*, vary widely from plasmid to plasmid (Cevallos et al., 2008; Pappas and Cevallos, 2011). The antisense RNA plays a crucial role in modulating the *repC* transcription/translation rate (Cervantes-Rivera et al., 2010; Chai and Winans, 2005).

We have shown that plasmid pRetCFN42d, also known as p42d, of *R. etli* CFN42 possesses three regions that exert plasmid incompatibility when they are introduced *in trans*. The first region contains the *repA* and *repB* genes. Coexpression of these genes represses the *repABC* operon and induces parental plasmid loss. Expression of *repA* or *repB* does not interfere with the maintenance of the parental