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ExcA proteins of IncI1 plasmid R64 and IncIγ plasmid R621a recognize different segments of their cognate TraY proteins in entry exclusion

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ABSTRACT

Entry exclusion is a process whereby plasmid transfer between donor and recipient cells harboring identical or closely related conjugative plasmids is inhibited. Exclusion proteins in the recipient cells are responsible for entry exclusion. Although IncI1 Plasmid R64 and IncIγ plasmid R621a exhibit similar genome structure in replication, transfer, and leading regions, they belong to different incompatibility and exclusion groups. The amino acid sequences of TraY and ExcA proteins are significantly different between R64 and R621a. In the present study, TraY proteins of R64 and R621a were exchanged. Transfer of R64 derivative carrying R621a TraY was inhibited by recipient R621a ExcA but not R64 ExcA and transfer of R621a derivative carrying R64 TraY was inhibited by recipient R64 ExcA but not R621a ExcA. This indicates that R64 and R621a TraY proteins in the donor cells are the targets of cognate ExcA proteins in the recipient proteins. Since two segments, an internal and a C-terminal segment, were found to vary between R64 and R621a TraY proteins, various chimera TraY proteins were constructed. Conjugation experiments suggested that the R64 internal variable segment recognizes R64 ExcA protein and the R621a C-terminal variable segment recognizes R621a ExcA protein.

1. Introduction

Bacterial conjugation is a complex process that allows plasmid DNA in a donor cell to be transferred into a recipient cell via cell-to-cell contact. During conjugation between donor and recipient cells harboring identical or closely related conjugative plasmids, plasmid transfer may be inhibited. This phenomenon is termed entry exclusion (Garcillán-Barcia and de la Cruz, 2008). All conjugation systems in plasmids are thought to carry a cognate exclusion system to inhibit redundant DNA transfer. Entry exclusion genes, such as *traS* in IncF plasmids F and R100, *trbK* in IncP plasmids RP4 and R751, *eex* in IncW plasmid R388, and *excAB* in IncI plasmids R64, CollB-P9, R144 and R621a, function in the recipient cells for entry exclusion (see Garcillán-Barcia and de la Cruz, 2008 for references). In addition, surface exclusion genes *traT* have been identified in some plasmids such as F and R100. In most cases, there is no involvement of exclusion genes from the donor cells for entry exclusion. In the case of IncH plasmid R27, however, *eexA* and *eexB* were required in both donor and recipient cells for exclusion (Gunton et al., 2008).

Usually entry exclusion is mediated by the interaction of an exclusion protein in the recipient cells with its cognate target protein in the donor cells. In IncF plasmids F and R100-1 (Anthony et al., 1999; Audette et al., 2007), and in IncF-related integrative conjugative elements SXT and R391 (Marrero and Waldor, 2005), exclusion target genes were experimentally exchanged to reveal the exclusion gene-target gene relationship. In IncF plasmids, F and R100-1 TraG was identified as the donor target protein for recipient F and R100-1 TraS proteins, respectively. Similarly, in SXT and R391, the TraG protein was identified as

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donor target protein for recipient proteins EexS and EexR, respectively. The combination of donor target protein and recipient exclusion protein determined the specificity of entry exclusion.

In IncI1 plasmids R64 and R144, belonging to the same exclusion groups, an overlapping gene, *excAB*, was found to govern entry exclusion (Furuya and Komano, 1994; Hartskeerl et al., 1983, 1985a). The *excAB* gene produced two proteins, ExcA and ExcB: the 17-kDa ExcB protein was produced by translational reinitiation of the reading frame of the 26-kDa ExcA protein (Furuya and Komano, 1994; Hartskeerl et al., 1985a, 1986). The ExcA protein was shown to be essential for entry exclusion. Cell fractionation experiments indicated that the ExcA protein exists in two forms: an inner membrane-bound form and a soluble form in the cytoplasm, while the ExcB protein is probably an inner membrane protein (Hartskeerl et al., 1985b).

Recently we have determined the entire genome sequences of IncI1 plasmid R64 and IncIγ plasmid R621a (Sampei et al., 2010; Takahashi et al., 2011). Both plasmids exhibit similar genome structure in the replication, transfer, and leading regions. Three major differences were found in *inc*, *parAB*, and *excA* regions. Seven nucleotide replacements and one nucleotide deletion were found in the putative *inc* sequences of R64 and R621a, suggesting that the sequence difference is the reason for their distinct incompatibility. Conjugation experiments indicated that R64 and R621a belong to different exclusion groups (Takahashi et al., 2011).

Among the Tra/Trb proteins of the R64 and R621a transfer region, all proteins except ExcA and TraY exhibit > 95% amino acid sequence identities (Takahashi et al., 2011). Amino acid sequences of ExcA proteins, however, are significantly different between R64 and R621a (Figs. 1B and 2A). R64 *excAB* gene encodes the ExcA and ExcB proteins, while R621a *excA* gene encodes only one protein. R64 and R621a ExcA proteins are 220- and 204-amino-acids long, respectively. Amino acid sequences of R64 and R621a ExcA exhibit 95% identity in the C-terminal 40 residues, 39% identity at the internal 120 residues, and no identity at the remaining N-terminal region. Sequence differences between R64 and R621a ExcA might, therefore, result in different exclusion specificity. R64 and R621a TraY proteins are 745-amino-acids long and carry two variable segments, the internal and C-terminal variable segments (Figs. 1A and 2A). Amino acid sequence identities between R64 and R621a of the internal and C-terminal TraY variable segments are 49% and 41%, respectively, while those of the other parts share > 86% identity.

The above results raised the possibility that the R64 and R621a TraY protein is the donor target protein for each ExcA protein, respectively. In this study we tested this hypothesis and confirmed that R64 and R621a TraY proteins are indeed the target proteins for the cognate ExcA proteins. Construction of chimera proteins from R64 and R621a TraY proteins suggested that the R64 internal variable segment and the R621a C-terminal variable segment recognize the R64 and R621a ExcA proteins, respectively.

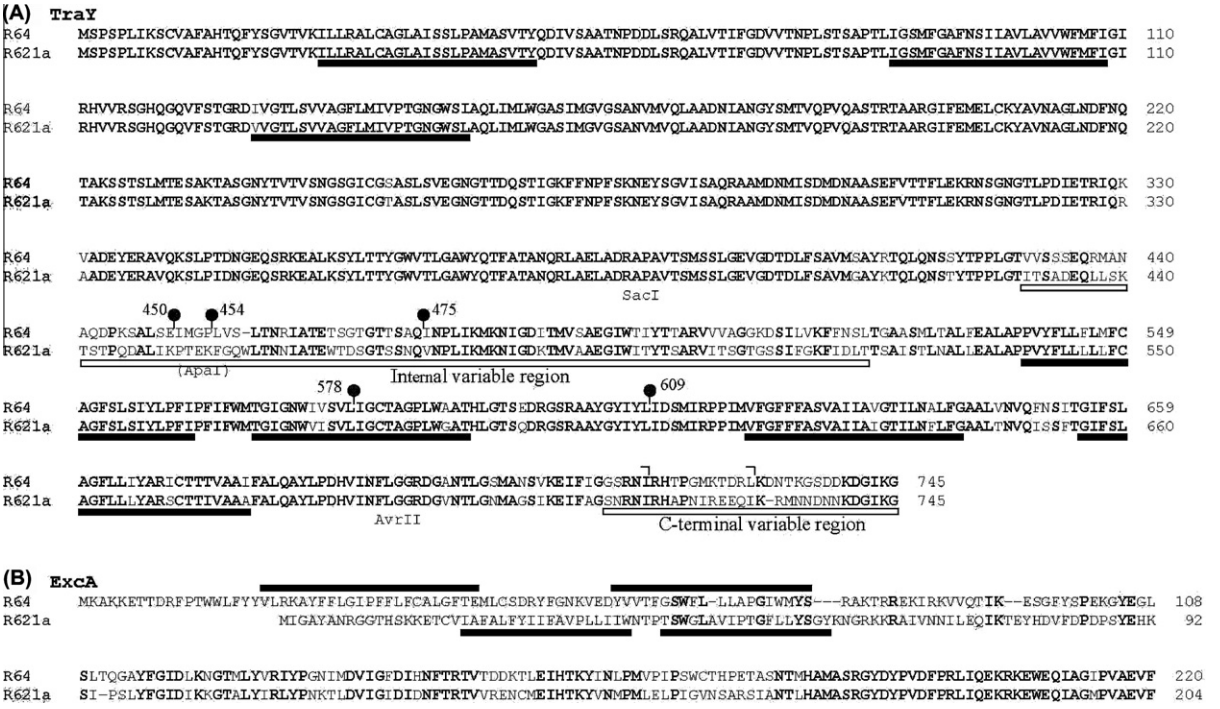

Fig. 1. Alignment of the amino acid sequences of (A) TraY and (B) ExcA proteins of plasmids R64 and R621a. Conserved amino acids are printed in boldface. Gaps, marked by dashes, are introduced to reveal maximal similarity among the sequences. Predicted transmembrane helices are indicated by gray bars. TraY internal and C-terminal variable segments are indicated by open bars. The limits of C-terminal deletions are indicated by half brackets. The locations of four-amino-acid insertions are indicated by gray circles. The locations of SacI, ApaI, AvrII restriction sites are indicated.