

# Mobility of the Native *Bacillus subtilis* Conjugative Plasmid pLS20 Is Regulated by Intercellular Signaling

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## Abstract

Horizontal gene transfer mediated by plasmid conjugation plays a significant role in the evolution of bacterial species, as well as in the dissemination of antibiotic resistance and pathogenicity determinants. Characterization of their regulation is important for gaining insights into these features. Relatively little is known about how conjugation of Gram-positive plasmids is regulated. We have characterized conjugation of the native *Bacillus subtilis* plasmid pLS20. Contrary to the enterococcal plasmids, conjugation of pLS20 is not activated by recipient-produced pheromones but by pLS20-encoded proteins that regulate expression of the conjugation genes. We show that conjugation is kept in the default “OFF” state and identified the master repressor responsible for this. Activation of the conjugation genes requires relief of repression, which is mediated by an anti-repressor that belongs to the Rap family of proteins. Using both RNA sequencing methodology and genetic approaches, we have determined the regulatory effects of the repressor and anti-repressor on expression of the pLS20 genes. We also show that the activity of the anti-repressor is in turn regulated by an intercellular signaling peptide. Ultimately, this peptide dictates the timing of conjugation. The implications of this regulatory mechanism and comparison with other mobile systems are discussed.

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## Introduction

Horizontal Gene Transfer (HGT) plays a significant role not only in bacterial evolution but also in the spread of antibiotic resistance and pathogenicity determinants. The main mechanisms responsible for HGT are transformation mediated by natural competence, transduction, phage-related chromosomal islands (PRCI) and conjugation performed by plasmids or ICEs [1–4]. Conjugation is the process by which a DNA element is transferred from a donor cell to a recipient cell. Consequently, conjugation requires direct contact between the donor and the recipient cells. Often conjugative elements are present on plasmids, but they can also be found as mobile elements that are integrated in a bacterial chromosome. These latter forms are generally named integrative and conjugative elements (ICE).

The basics of the conjugation mechanism among plasmids are conserved. For a plasmid to be conjugative it requires a set of genes encoding proteins that (i) process the plasmid DNA into the form that can be transferred, which generally is single-stranded DNA, and (ii) generate a membrane-associated mating channel, called transferosome, through which the ssDNA is transported. The intercellular transferosome is a form of type IV secretion system. Generation of the ssDNA plasmidic form involves a relaxase, which forms a nucleoprotein complex called the relaxosome that introduces a site- and strand-specific nick within

the origin of transfer (*oriT*). The relaxase remains covalently attached to the nicked DNA and the relaxosome is linked to the transferosome via the so-called coupling protein. Upon transfer of the ssDNA strand into the recipient cell through the transferosome, the attached relaxase directs recircularization of the ssDNA in the recipient cell.

Good understanding of the process of conjugation and its transcriptional regulation can provide insights into bacterial evolution. Such knowledge will also have important socio-economic, medical and biotechnological implications. For instance, it may provide valuable information to help control the explosive global spread of antibiotic resistance, and it may form the basis to construct tools to modify clinically or industrially important bacteria that are reluctant to genetic manipulation by other approaches. The process of conjugation and its transcriptional regulation has been studied in considerable detail for various plasmids present in Gram-negative (Gram<sup>-</sup>) bacteria (for review see, [5–8]). However, comparatively little is known about conjugation systems on plasmids from Gram-positive (Gram<sup>+</sup>) bacteria, many of them industrially and medically important organisms, although interest in this field is increasing (for general review see, [7,9]). The conjugation machineries of plasmids from some Gram<sup>+</sup> bacteria have been studied in more depth. Examples of these are (i) the broad host-range plasmid pIP501, originally isolated from *Streptococcus agalactiae* [10,11, and references therein],