Available online at www.sciencedirect.com
SCIENCE DIRECT®
Research in Microbiology 156 (2005) 1–6
Research in Microbiology
www.elsevier.com/locate/resmic

Mini-review

Bacterial conjugation: a potential tool for genomic engineering

Matxalen Llosa *, Fernando de la Cruz

Departamento de Biología Molecular, Universidad de Cantabria, C. Herrera Oria s/n, 39011 Santander, Spain

Received 30 July 2004; accepted 30 July 2004

Available online 2 September 2004

Abstract

Bacterial conjugation is a mechanism for horizontal DNA transfer with potential for universal DNA delivery. The conjugal machinery can be separated into three functional modules: the relaxosome, the coupling protein, and a type IV protein secretion system. Module interchangeability among different conjugative systems opens up the possibility of "à la carte" engineering of DNA delivery into virtually any cell type.

Keywords: Bacterial conjugation; Coupling proteins; Type IV secretion; DNA relaxases; Genomic manipulation

1. Introduction

To achieve an adequate level of genetic diversity, bacterial cells overcome their lack of sexuality by horizontal DNA transfer. Thus, an extended pool of genetic information is made available to the whole population. Any bacteria, in spite of carrying a limited amount of genetic information in its gene-packed chromosome(s), can access the extended DNA pool. Any specific gene in the extended gene pool is maintained in the population by just a few cells, thus consuming little energy from the population as a whole, but is ready to spread upon selective pressure. The most dramatic example of the power of this process has been illustrated by the spread of antibiotic resistance genes among prokaryotes in just a few decades, since antibiotics started to be widely used in hospitals [26].

Horizontal DNA propagation is mediated by several mechanisms; bacterial conjugation is the most widespread and the one that contributes most to the horizontal gene pool in the prokaryotic world [7]. The promiscuity of bacterial conjugation goes beyond prokaryotes. The ultimate example of this versatility is the DNA transfer system of *Agrobacterium tumefaciens* [38], mediated by a transfer machinery highly homologous to a Gram-negative conjugative apparatus [17], that efficiently transfers DNA into the nucleus of plant cells. In addition, but only under laboratory conditions, conjugative DNA transfer has been observed from bacteria into yeast, plant and animal cells [3,14,36].

2. Modular elements in the transfer machinery

The molecular mechanism of conjugative DNA transfer has been studied extensively, especially in Proteobacteria. The DNA molecule to be transferred (usually a plasmid, that encodes its own transfer apparatus) must carry an origin of transfer (*oriT*), a short DNA sequence where the process starts and ends. The rest of the conjugal machinery consists of no less than 15 proteins that carry out several functions, including the DNA processing reactions and the active transport into the recipient cell. The conjugal machinery can be thought as consisting of three functional modules:

- The substrate *selector* consists of a nucleoprotein complex (also called relaxosome) formed by an *oriT*, a relaxase and one or more accessory nicking proteins. The

* Corresponding author.
E-mail address: llosam@unican.es (M. Llosa).

0923-2508/$ – see front matter
doi:10.1016/j.resmic.2004.07.008

relaxase protein specifically cleaves *oriT* in the DNA strand to be transferred, remains covalently bound to the cleaved strand, and presumably religates it at the end of the transfer process. Conjugative relaxases are related to rolling-circle replication initiator proteins, and their target *oriT* sequences are related to rolling-circle replication *oriV* sequences [37]. The selector is so called because it is very specific for each plasmid system. Relaxases act specifically on their cognate *oriT*, and not on those of related plasmids.

- The transmembranal *conduit* is a multiprotein complex, formed by about 10 different proteins, that spans both the inner and outer membrane. Its components belong to a family of protein transporters known as type IV secretion systems (T4SS) [6,23]. Many T4SS family members are constituents of mammalian pathogens that use them to inject virulence factors into target host cells [8,31].
- The *coupling protein* (CP) brings together the selector and the conduit, thus approaching both parts of the transfer machinery. In addition, based on its atomic structure and similarity to other DNA transporters, it is proposed that it actively pumps the T-DNA strand out of the donor and into the recipient cell [20].

3. Molecular architecture of the conjugal machinery

Our research team has extensively studied the conjugative system of the IncW plasmid R388, a 34-kb self-transmissible plasmid that devotes half of its genetic information to conjugative functions. Still, it represents one of the smallest known transfer regions in Gram-negative bacteria. Its simplicity and promiscuity make it an ideal model system to study the transfer process. The R388 transfer region (TRAw) is encoded in a contiguous 15-kb DNA segment that consists of the *oriT* at one end and 15 *trw* genes (*trwA* to *trwN*) that code for the corresponding Trw proteins (Fig. 1a). Our knowledge of the individual components is outlined below. Other well-characterized bacterial conjugation systems, such as RP4 or F, contain the same minimal set of molecular components plus a number of additional components.

The *oriT* is a DNA segment of 330 bp that includes the *nic* site (the site recognized and cleaved by the relaxase) and a number of structural features, namely inverted and direct repeats for protein binding [18]. R388 proteins TrwA and TrwC, and the host protein IHF, bind *oriT*. Together, this nucleoprotein complex is called the relaxosome. The architecture and assembly of the R388 relaxosome have been studied in detail [29,30]. TrwA and IHF are involved in al-

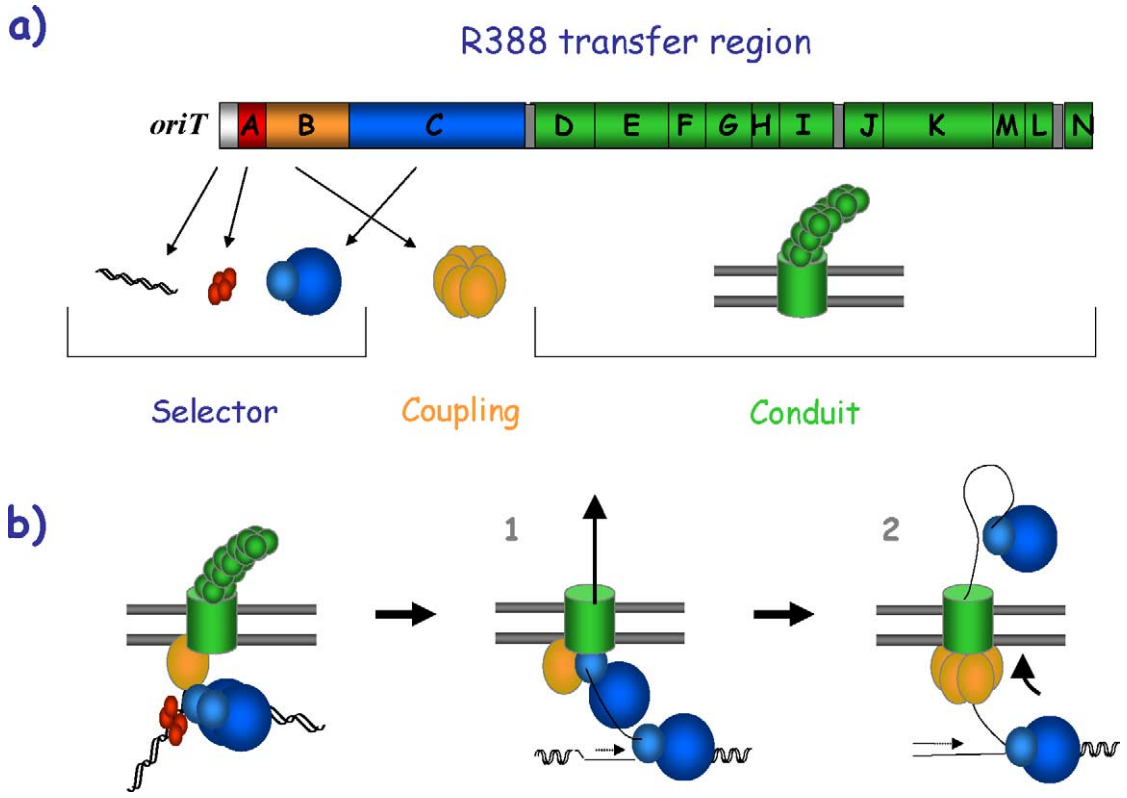

Fig. 1. Structure and function of a conjugative DNA transfer system. (a) Genetic organization of the transfer region of plasmid R388 and the resulting protein products. The *trw* gene prefix has been omitted for clarity. Proteins are arranged in the indicated functional modules. (b) Scheme of the shoot-and-pump model for conjugal DNA transfer. Step 1: the relaxase is secreted through the T4SS, with the trailing covalently bound DNA strand. Step 2: the remaining DNA is pumped out via the CP.