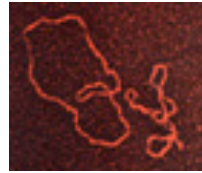


411 Outline: 25 Feb 2009



1. Plasmids in motion
2. Mechanism of conjugative transfer
3. Mobilization vs. conjugation
4. Movement of non-plasmid sequences
5. Conjugation vs. Generalized Transduction

Recap of bacterial plasmids

See Tables 4.1/4.2/4.4

Plasmid (Inc)	Size (Kb) / CN	Host range	Traits
F (IncF1)	100 / 1	Narrow (<i>E. coli</i>)	Conjugative; phage sensitivity
RP4 (IncP)	60/4-7	Broad: Gram neg.	Conjugative; drug ^R ; phage sensitivity
Ti (IncRh1)	200 / 1	<i>Agro/Rhizo</i>	Conjugative; plant tumor-inducing
ColEI	6.6/15	Narrow	Mobilizable; colicins

The next plasmid frontier: intercellular transmission

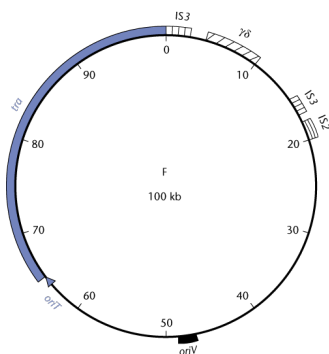


Figure 5.2

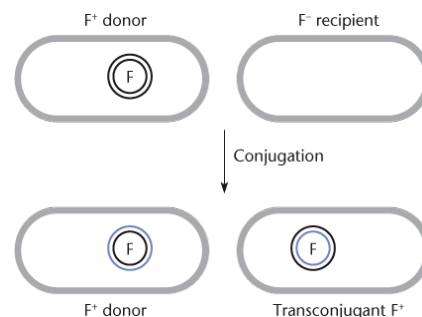


Figure 5.1

Features of conjugative plasmids

Significant amount of plasmid devoted to transfer (*tra*) genes, indicative of how complicated it is to export DNA to recipient.

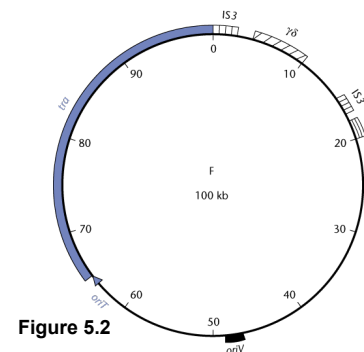


Figure 5.2

TABLE 5.1 Some F-plasmid genes and sites	
Symbol	Function
<i>ccdAB</i>	Inhibition of host cell division
<i>incBCE</i>	Incompatibility
<i>oriT</i>	Site of initiation of conjugal DNA transfer
<i>oriV</i>	Origin of bidirectional replication
<i>sopAB</i>	Partitioning
<i>traABCEFGHKLQUVWX</i>	Pilus biosynthesis, assembly
<i>traGN</i>	Mating-pair stabilization
<i>traD</i>	Coupling protein
<i>traI</i>	Relaxase
<i>traYM</i>	Accessories for relaxosome
<i>traJ, finOP</i>	Regulation of transfer
<i>traST</i>	Entry exclusion

Conjugation in Gram negative bacteria

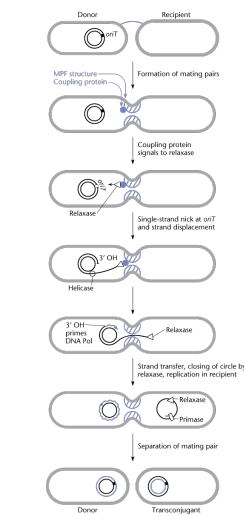
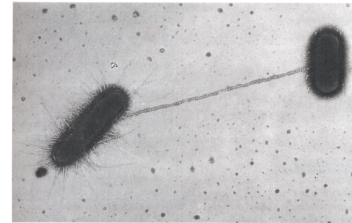


Figure 5.5

Conjugative pili



- Different plasmids express distinct pili: F pili are long, thin, and flexible, allowing efficient conjugation in liquid. Pili of IncP-type plasmids are short, rigid, and thick, restricting these plasmids to conjugation on solid surfaces.
- Pili used as specific receptors for phages: F pili bind to M13, MS2/R17 (shown here); RP4 pili bind to Pf3.
- For years, proposed that pili would “retract,” bringing the donor and recipient cells into contact

Model: F Tra apparatus

Several Tra proteins in cell envelope assemble pilus and allow “mating pair” formation (Mpf proteins). Other Tra proteins process DNA in cytoplasm (Dtr proteins). Distinct Tra protein allows contact between Mpf and Dtr functions: Coupling protein (CP). CP binds DNA/Dtr complex and mediates entry into export pathway.

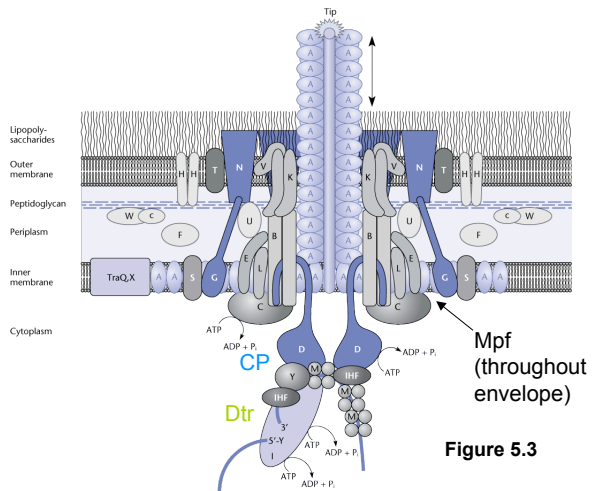
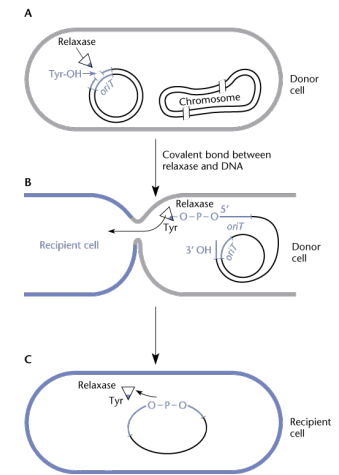


Figure 5.3

Figure 5.6



Genetic analysis of conjugative transfer

- ssDNA transfer, with complement synthesis in both donor and recipient
- Col1b-P9 (Incl) and RP4 (IncP) plasmids transfer a plasmid-specific primase for efficient recipient DNA synthesis.
- Col1b-P9 can transfer primase protein without the plasmid ssDNA

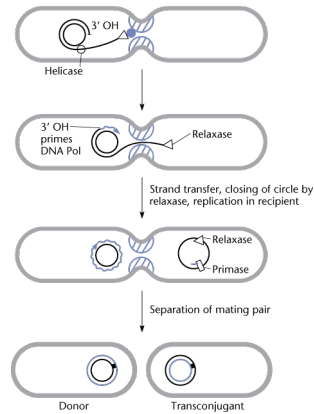


Figure 5.5 (bottom)

Conjugation in Gram negative bacteria

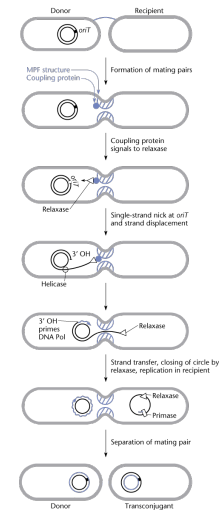
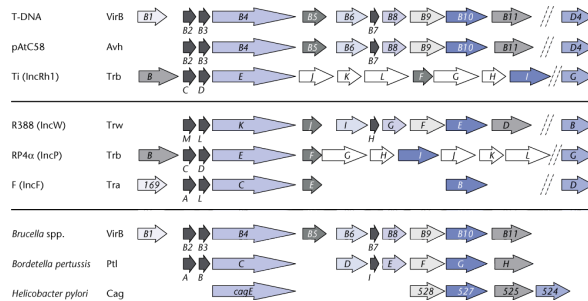


Figure 5.5

Box 5.2



Plasmid Mobilization

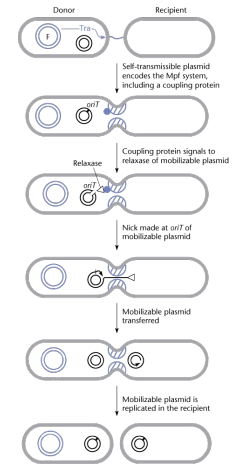


Figure 5.9

Hfr formation and resolution

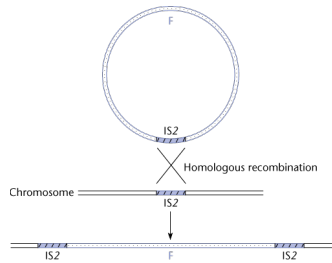


Figure 5.14

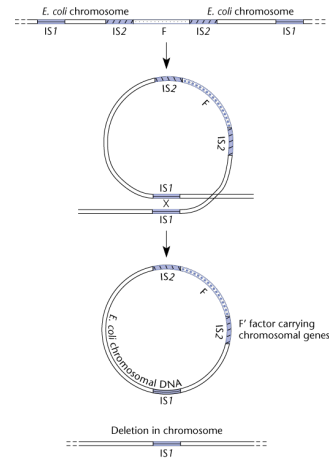
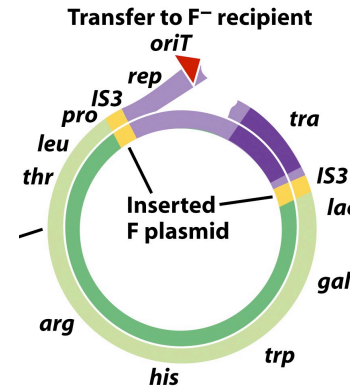


Figure 5.15

F plasmid and *E. coli* Hfrs



Brock Fig. 11.23

When plasmids recombine with chromosome, conjugation functions can lead to transfer of chromosomal DNA (next to integrated plasmid) to recipient. Efficiency of gene transfer decreases with distance from *oriT*.

Directionality of process leads to rare transfer of *tra* genes during Hfr mating.

P1 and molecular genetics

Ability of P1 to move bacterial DNA around as “generalized transducing phage” has been important for molecular genetics.

Rare P1 capsid contains fairly random segment of chromosomal dsDNA.

Transduced DNA injected into newly infected cell and must recombine with recipient chromosome in RecA/RecBCD dependent fashion.

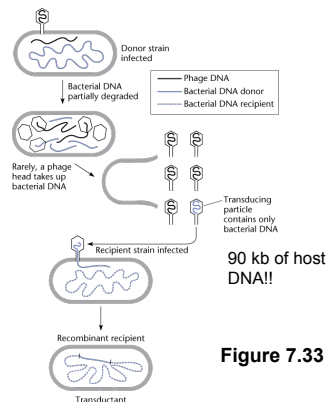


Figure 7.33