SACRAMENTO STATE
Leadership begins here.

Chapter 8:

Conjugation, Plasmids

Dr. Amy Rogers

Office Hours: Mondays & Wednesdays 9-10 AM
Sequoia 530

Lateral Gene Transfer #3:
★ **Conjugation**

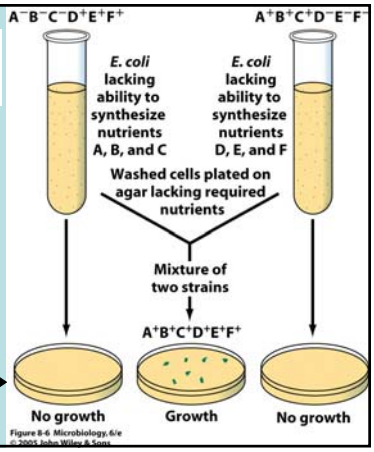
- Transfer of DNA by ***direct contact*** between two ***living*** bacteria
- Contact is made, and DNA is transferred, by way of a conjugation ***pilus*** (conjugation bridge/sex pilus/F pilus)
- Much larger amounts of DNA can be transferred by conjugation than by transformation or transduction

Discovery of conjugation: Lederberg experiment

Auxotrophic strains of *E. coli*

Only grow on nutrient-rich medium which provides nutrients they cannot make themselves

Minimal medium (does NOT provide nutrients A/B/C/D/E/F)



What happened?

Bacteria of the first strain donated the genes required to synthesize nutrients D/E/F to the second strain

and/or

Bacteria of the second strain donated the genes required to synthesize nutrients A/B/C to the first strain

★ Either way, an *auxotroph* is converted to a *prototroph*
(mutant) (wild type)

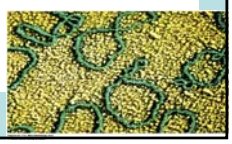
- Conversion was not efficient (about 1 in 10,000,000 cells)
- ★ Physical contact between the two auxotrophic cell types was necessary
  - Simply allowing free flow of the medium, but keeping the cells apart, did not produce prototrophs; so this was not a kind of transformation

Conjugation requires Plasmids

Plasmid: extrachromosomal genetic element

★

- Circular, double-stranded DNA molecules
- Self-replicating, often present in many copies per bacterial cell
- Genes they carry may be useful, but rarely essential, for cell growth



F (fertility) plasmids

F (fertility) plasmids:

★
- Carry genetic information for synthesis of F pili
- Direct ***their own transfer*** to a recipient cell

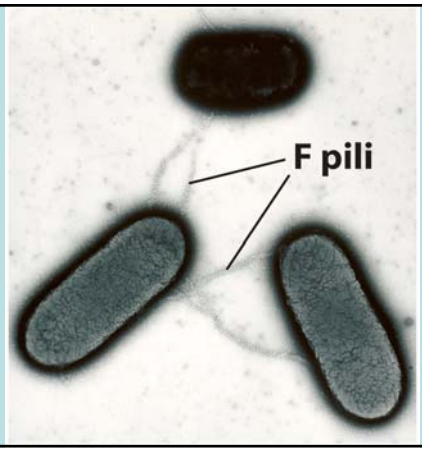
Bacteria which have F plasmids are:

F+ Donor Male

★Only F+ cells can initiate conjugation

Conjugation

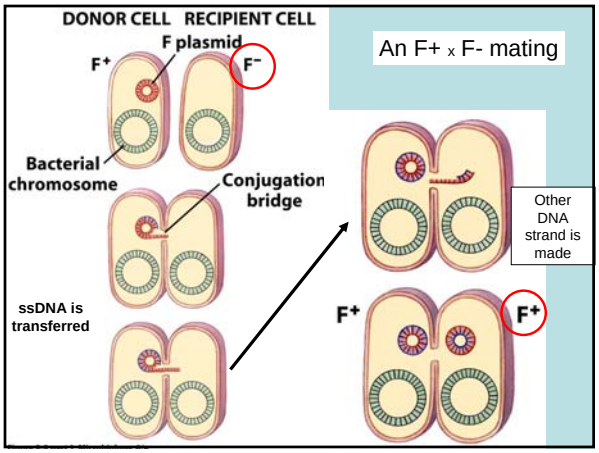
- Genes on F plasmid of F+ cell direct formation of a conjugation pilus
  - Conjugation/F/sex pilus is longer than attachment pili (fimbriae)
- Donor: F+ cell
- Recipient: F- cell
- What DNA is transferred? Primarily, the F plasmid (see later slides on Hfr and F')



★ Conjugation

- The DNA transferred from donor cell to recipient is ***single stranded***
- In both donor & recipient cells, the complementary DNA strand of the (now single-stranded) F plasmid is synthesized

⇨ ***F- recipient becomes F+***



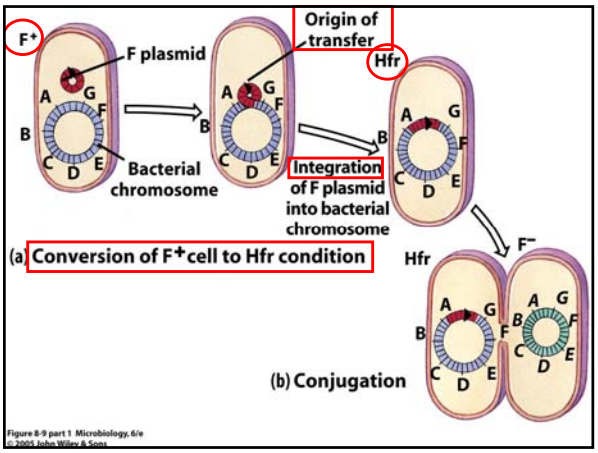
Hfr: High Frequency of Recombination

- The F plasmid is a special kind of plasmid called an ***episome***
- **Episomes** exist **either** as free ***extrachromosomal*** elements, **OR** can be ***integrated*** into the bacterial chromosome (like a prophage)
- F plasmids can integrate at any of several locations in the host chromosome

Hfr: High Frequency of Recombination

- Genes on the integrated F factor still can initiate conjugation (host is still "male")
- What DNA gets transferred? ★

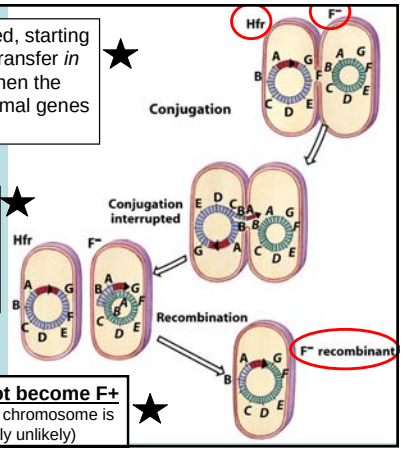
Single-stranded DNA, starting with a *bit* of the F factor, then continuing with ***adjacent chromosomal genes***



ssDNA is transferred, starting with the origin of transfer *in the F plasmid*, then the adjacent chromosomal genes ***in order*** ★

The **closer** the gene is to the F origin of transfer, the **more likely** it is **to be donated** before conjugation is interrupted ★

Recipient cell **does not become F+** unless the entire donor chromosome is passed over (highly unlikely) ★



During conjugation, because single-stranded DNA is transferred, donor bacteria ***do not*** lose any genes!

In Hfr transfers, the recipient cell ***does not*** end up with 2 copies of any genes: the original copy is recombined (spliced) out and replaced

★ Hfr strains

- In Hfr strains of bacteria, the F plasmid is ***stably*** integrated into the chromosome
  - i.e., it stays integrated over many cell generations
- Recombination of chromosomal genes into the recipient occurs 1,000x more often with Hfr than ordinary F+ donor strains
  - hence the name, High frequency of recombination

Hfr strains & mapping

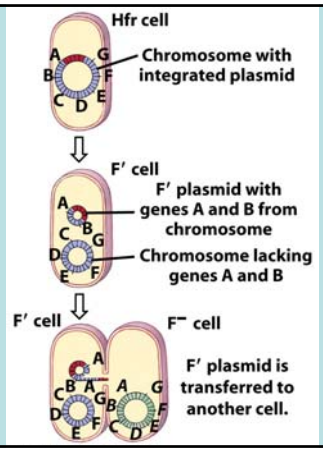
- Genes adjacent to the inserted F factor are transferred to the recipient cell first
- The longer conjugation occurs uninterrupted, the more genes get transferred (in order)
- The location of various genes on the bacterial chromosome was originally mapped using "*interrupted mating*"
  - Conjugating bacteria were put in a blender at various times, and recombinant cells analyzed for which genes they got

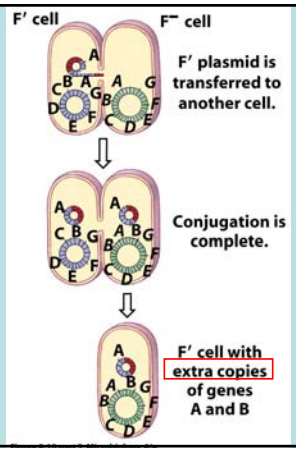
Excision of the integrated F factor: ★ F' strains

- The F plasmid, once integrated into the bacterial chromosome, can pop out again
- As with specialized transduction (prophage excision errors attach some bacterial DNA to the viral genome), the F plasmid sometimes takes a bit of adjacent DNA with it when it goes

Resulting (free) plasmid is called F' (**F prime**)

Note: F' cell has not "lost" any genes, but they *have moved* from the chromosome to the plasmid





- An F' strain (carrying a specific F' plasmid) will:
  - Convert recipient F- cells to F' (F+)
  - Always donate the same "extra" (chromosomal) genes

TABLE 8.1

Results of Selected Conjugations

Donor	Recipient	Molecule(s) Transferred	Product
F^+	F^-	F plasmid	F^+ cells
Hfr	F^-	Initiating segment of F plasmid and variable quantity of chromosomal DNA	F^- with variable quantity of chromosomal DNA
F′	F^-	F′ plasmid and some chromosomal genes it carries with it	F′ cell with some duplicate gene pairs: one on chromosome one on plasmid

Table 8-1 Microbiology, 6/e
© 2005 John Wiley & Sons

Summary of the Effects of Various Transfers of Genetic Information

Kind of Transfer	Effects
Transformation	Naked DNA from dead cell is picked up by living recipient. Recipient must be competent. Changes certain characteristics of an organism, depending on which genes are transferred.
Transduction	Transfer is effected by a bacteriophage.
Specialized	Only genes near the prophage are transferred to another bacterium.
Generalized	Fragments of host bacterial DNA of variable length and number are packed into the head of a virus.
Conjugation	Transfer is effected by a plasmid.
F^+	A single plasmid is transferred.
Hfr	An initiating segment of a plasmid and a linear sequence of bacterial DNA that follows the initiating segment are transferred.
F′	A plasmid and whatever bacterial genes adhere to it when it leaves a bacterium are transferred.

Which of the above lateral gene transfers has the potential to transfer the largest amount of DNA?

Conjugation with Hfr strain

R (resistance) plasmids

- F plasmids were the first plasmids discovered
- Other circular, double-stranded, self-replicating extrachromosomal elements (plasmids) exist e.g.

★ **R (resistance) plasmids**:
Carry genes for *antibiotic resistance*

★ R plasmids

- Like F plasmids, they carry genes needed to implement their own transfer by conjugation (*resistance transfer factor*)
- Carry one, or frequently more, resistance genes
  - Resistance genes confer insensitivity to a specific antibiotic (or class of antibiotics)

R plasmid promiscuity

- Resistance plasmids can be transferred
  - Within a species
  - Between closely related genera (genuses)
  - Between *unrelated genera*!!!
- Major problem in health care as R plasmids carrying *multiple* resistance genes can spread rapidly under ***natural selection*** within a hospital