Genetic Recombination

- If bacteria were incapable of genetic recombination, all members of a given species would be clones with differences arising only due to mutations in different lines

- If bacteria could not share genetic information as other organisms do in sexual reproduction, there would be vastly less opportunity to adapt to different environments

- As it turns out, bacteria are notorious for their ability to adapt; thus it is not surprising that elegant mechanisms exist for sharing genetic information

Ways Bacteria Exchange Genetic Material

- **Transformation -** Bacteria take up DNA from their environment and incorporate it into their genome (i.e., the Griffith experiment)
- **Transduction -** Movement of DNA between bacteria by viruses
- **Conjugation -** The direct transfer of DNA by bacteria usually via plasmids

Conjugation

1. **Discovered by Joshua Lederberg and Edward Tatum in 1946.**

2. **Unidirectional transfer of genetic material between donor and recipient bacteria cells by direct contact.**

3. **Segment (rarely all) of the donor's chromosome recombines with the homologous recipient chromosome.**

4. **Recipients containing donor DNA are called transconjugants.**

Fig. 15.2, Lederberg & Tatum (1946) Experiment demonstrating recombination in *E. coli*. Recombination of 2 complimentary auxotrophs gives rise to a strain that can synthesize all nutrients.

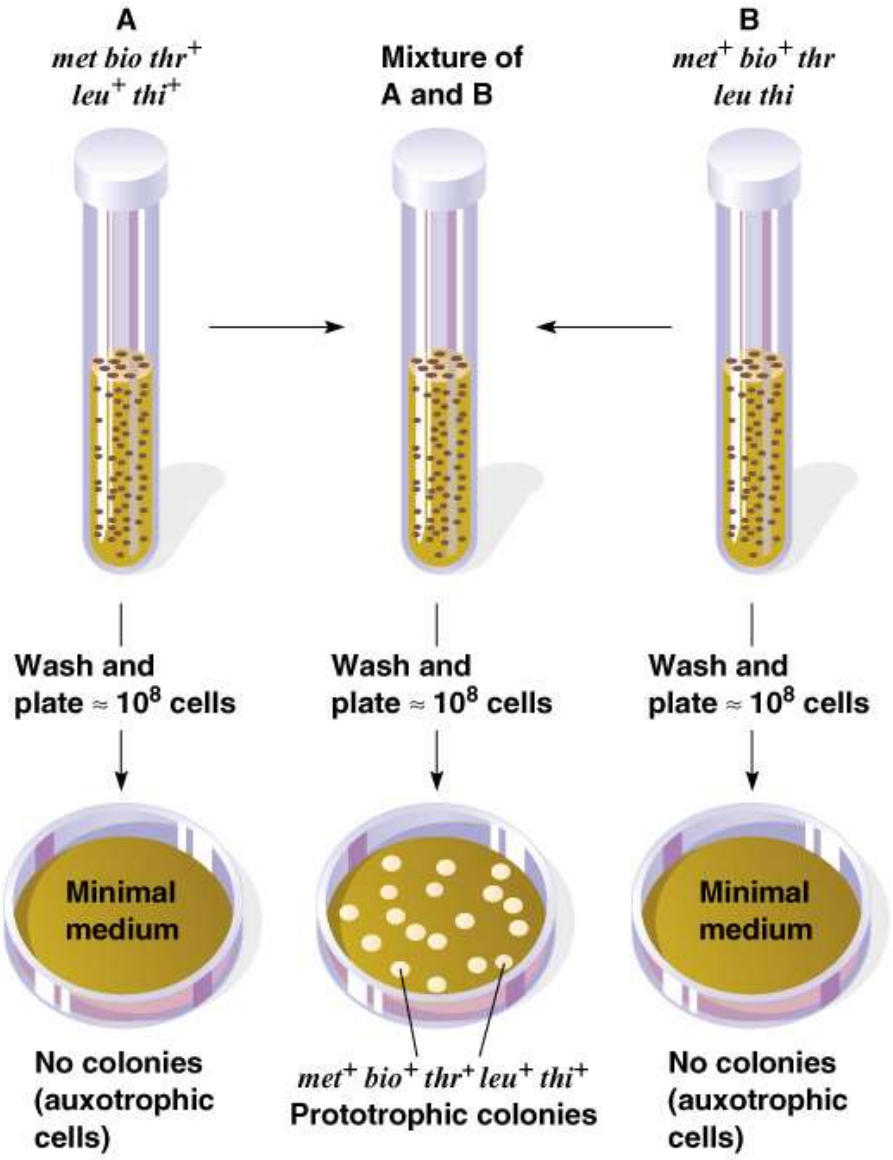
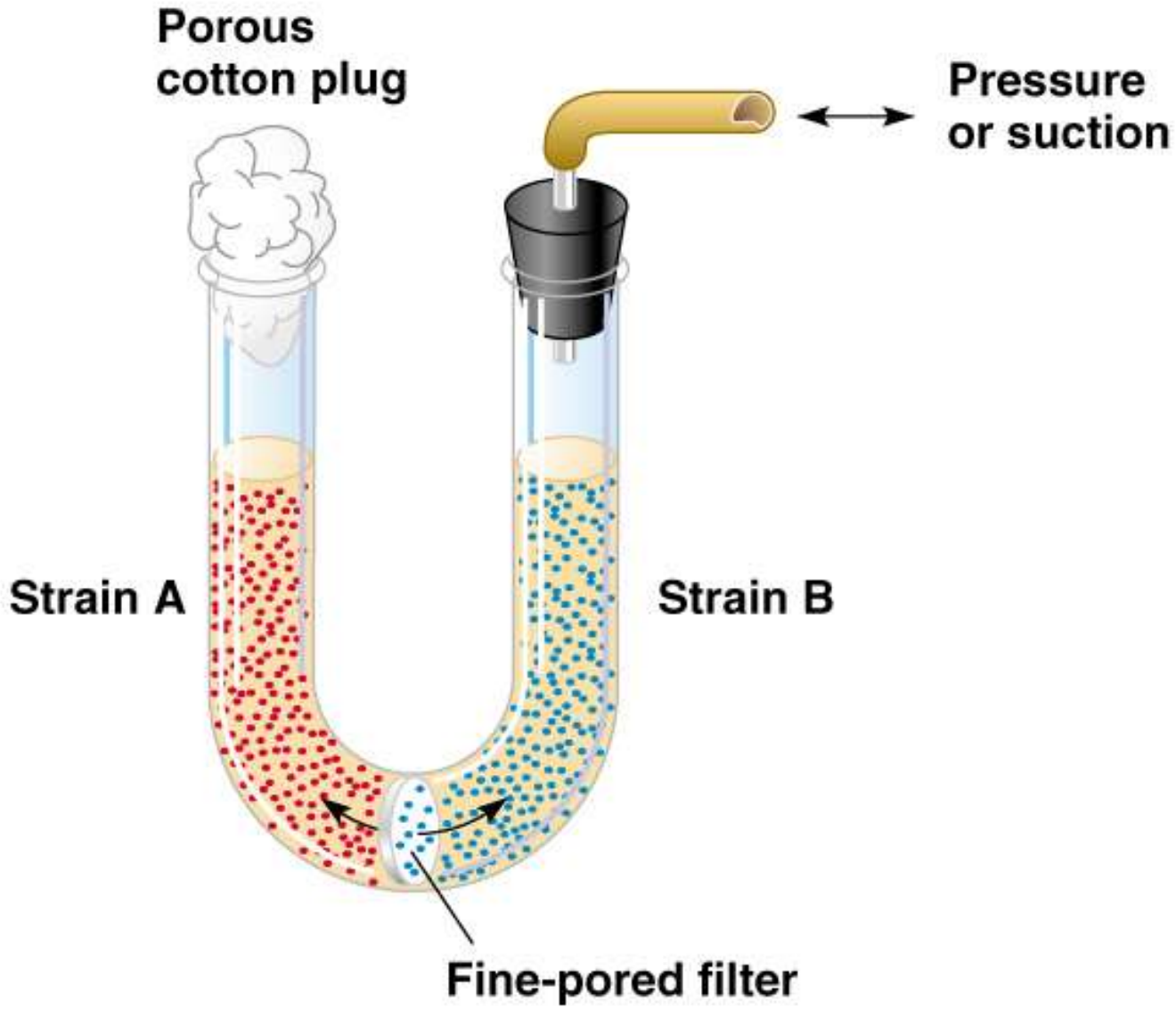


Fig. 15.3, Bernard Davis experiment demonstrated that physical contact is required for bacterial recombination.



Conjugation-transfer of the sex factor *F*:

1. **William Hayes (1953) demonstrated that genetic exchange in *E. coli* occurs in only one direction.**

2. **Genetic transfer is mediated by sex factor *F*.**

3. **Donor is F^+ and recipient is F^-.**

4. ***F* is a self-replicating, circular DNA plasmid (1/40 the size of the main chromosome).**

5. ***F* plasmid contains an origin sequence (*O*), which initiates DNA transfer. It also contains genes for hair-like cell surface (*F*-pili or sex-pili), which aid in contact between cells.**

6. **No conjugation can occur between cells of the same mating type.**

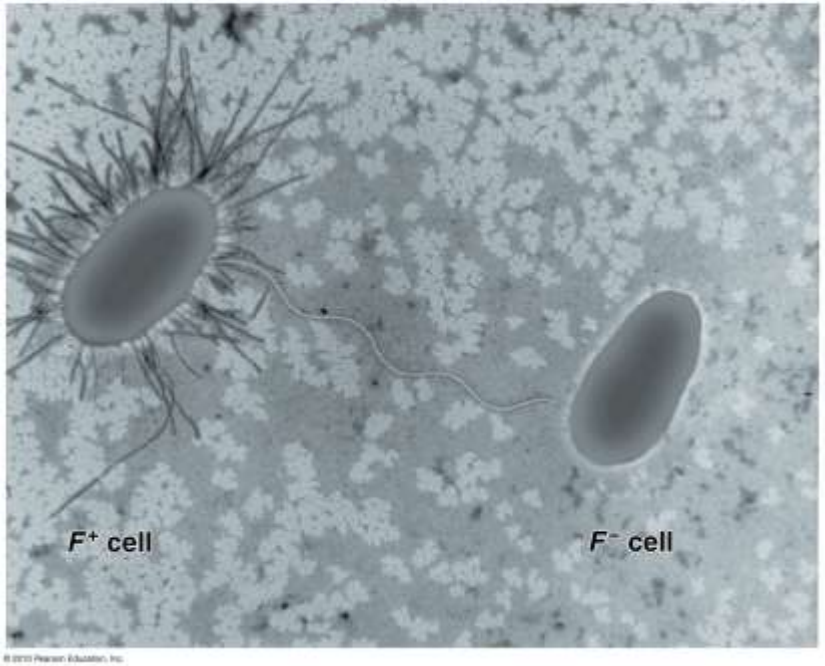
7. **Conjugation begins when the *F* plasmid is nicked at the origin, and a single strand is transferred using the rolling circle mechanism.**

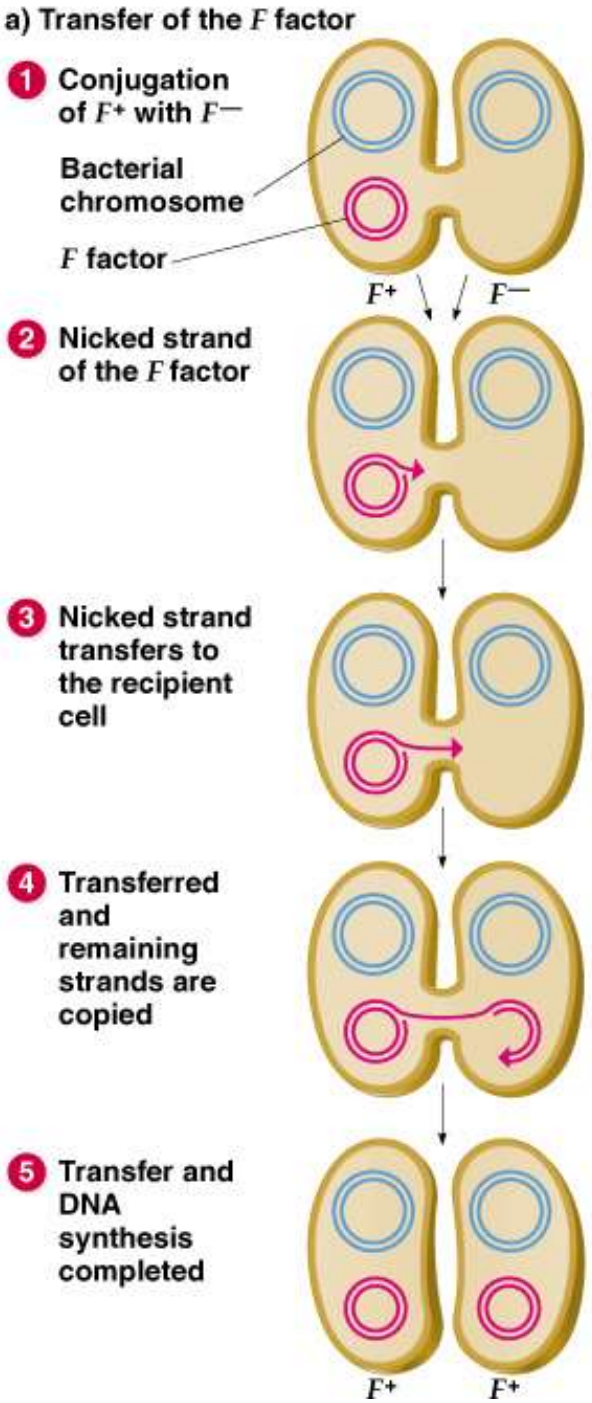
8. **When transfer is complete, both cells are F^+ double-stranded.**

Figs. 15.4 & 15.5a

Transfer of the *F* factor

a) Two bacterial cells connected by a long, tubular *F*-pilus





Conjugation of high-frequency recombinant strains:

1. **No chromosomal DNA is transferred by standard sex factor *F*.**

2. **Transfer of chromosome DNA is facilitated by special strains of F^+ integrated into the bacteria chromosome by crossing over.**

3. ***Hfr* strains = high frequency recombination strains.**

4. **Discovered by William Hayes and Luca Cavalli-Sforza.**

5. ***Hfr* strains replicate *F* factor as part of their main chromosome.**

6. **Conjugation in *Hfr* strains begins when F^+ is nicked at the origin, and F^+ and bacteria chromosomal DNA are transferred using the rolling circle mechanism.**

7. **Complete F^+ sequence (or complete chromosomal DNA) is rarely transferred (1/10,000) because bacteria separate randomly before DNA synthesis completes.**

8. **Recombinants are produced by crossover of the recipient chromosome and donor DNA containing F^+.**

Fig. 15.5b

Transfer of the *Hfr* F^+ factor

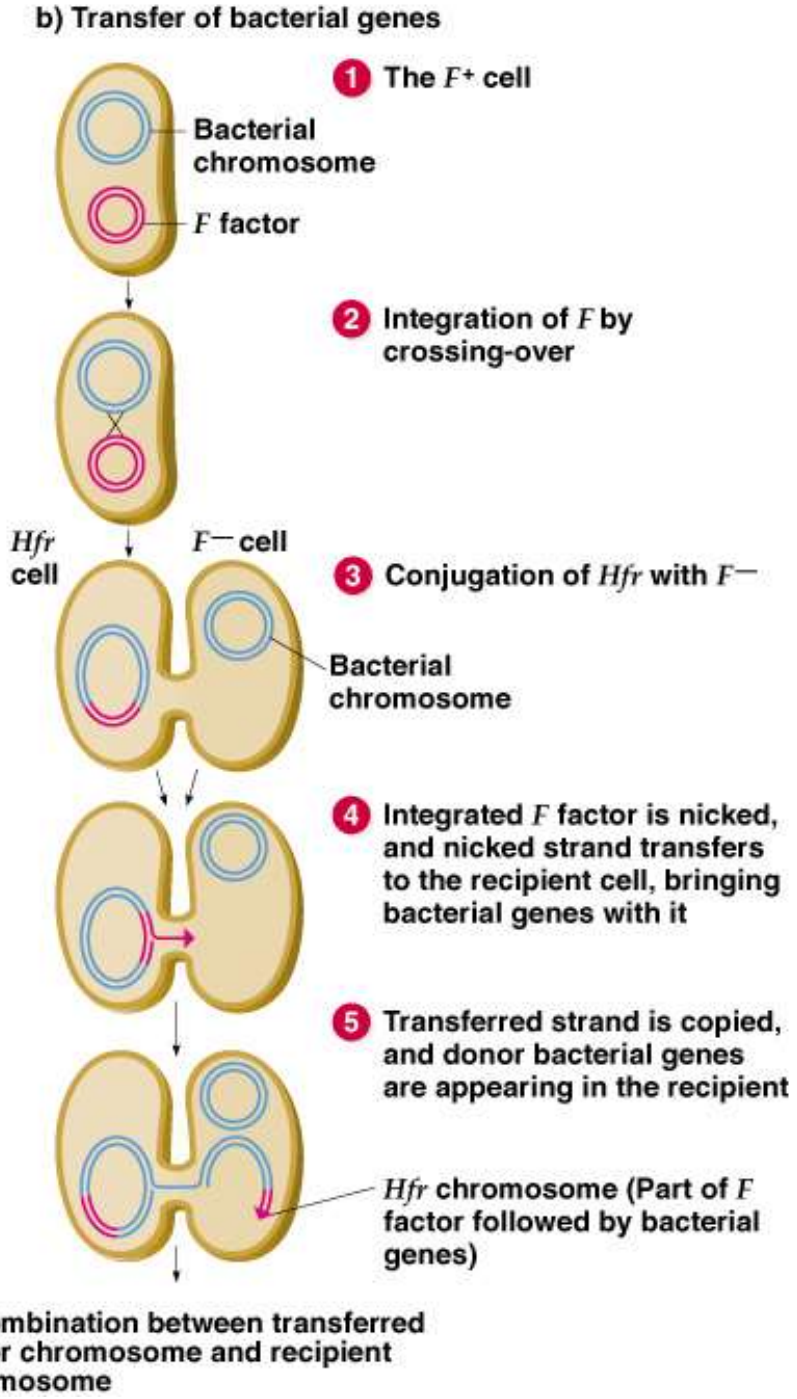


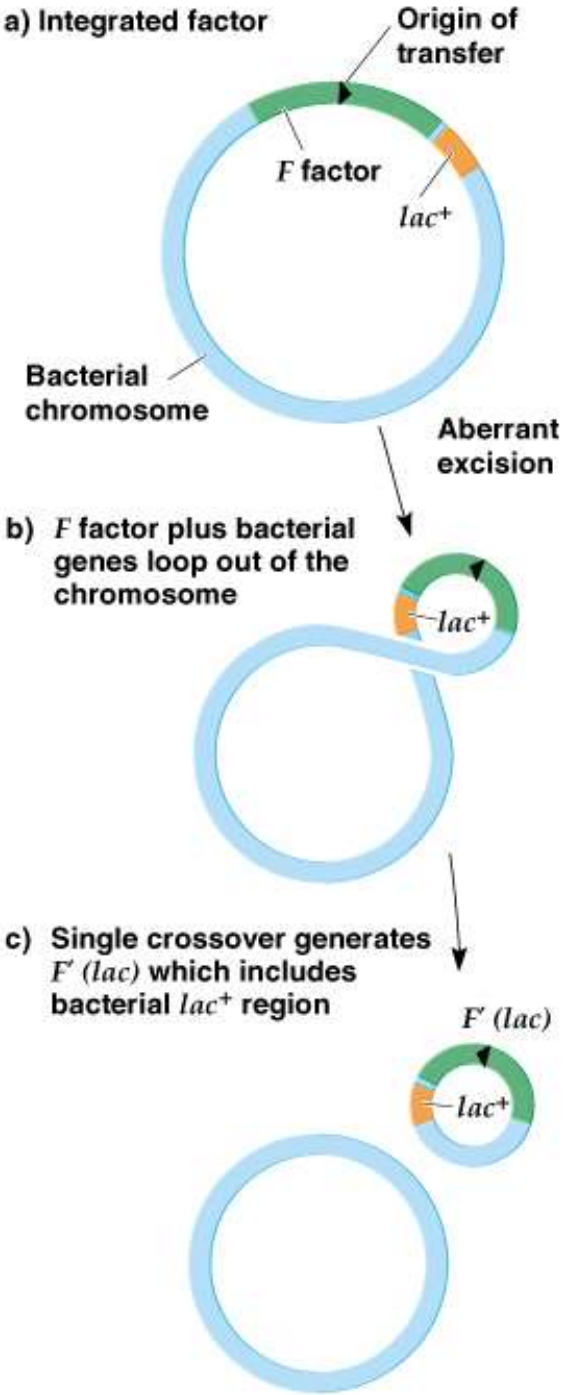
Fig. 15.6

<u>Excision of the *F*+ factor also occurs spontaneously at low frequency</u>.

1. **Begin with *Hfr* cell containing *F*+.**

2. **Small section of host chromosome also may be excised, creating an *F'* plasmid.**

3. ***F'* plasmid is named for the gene it carries, e.g., *F' (lac)***



Using conjugation to map bacterial genes:

1. **Begin with two different *Hfr* strains selected from F^+ x F^- crosses and perform an interrupted mating experiment.**

2. HfrH thr+ leu+ azi^R ton^R lac+ gal+ str^R
   F⁻ thr leu azi^S ton^s lac gal str^S

3. **Mix 2 cell types in medium at 37° C.**

4. **Remove at experimental time points and agitate to separate conjugating pairs.**

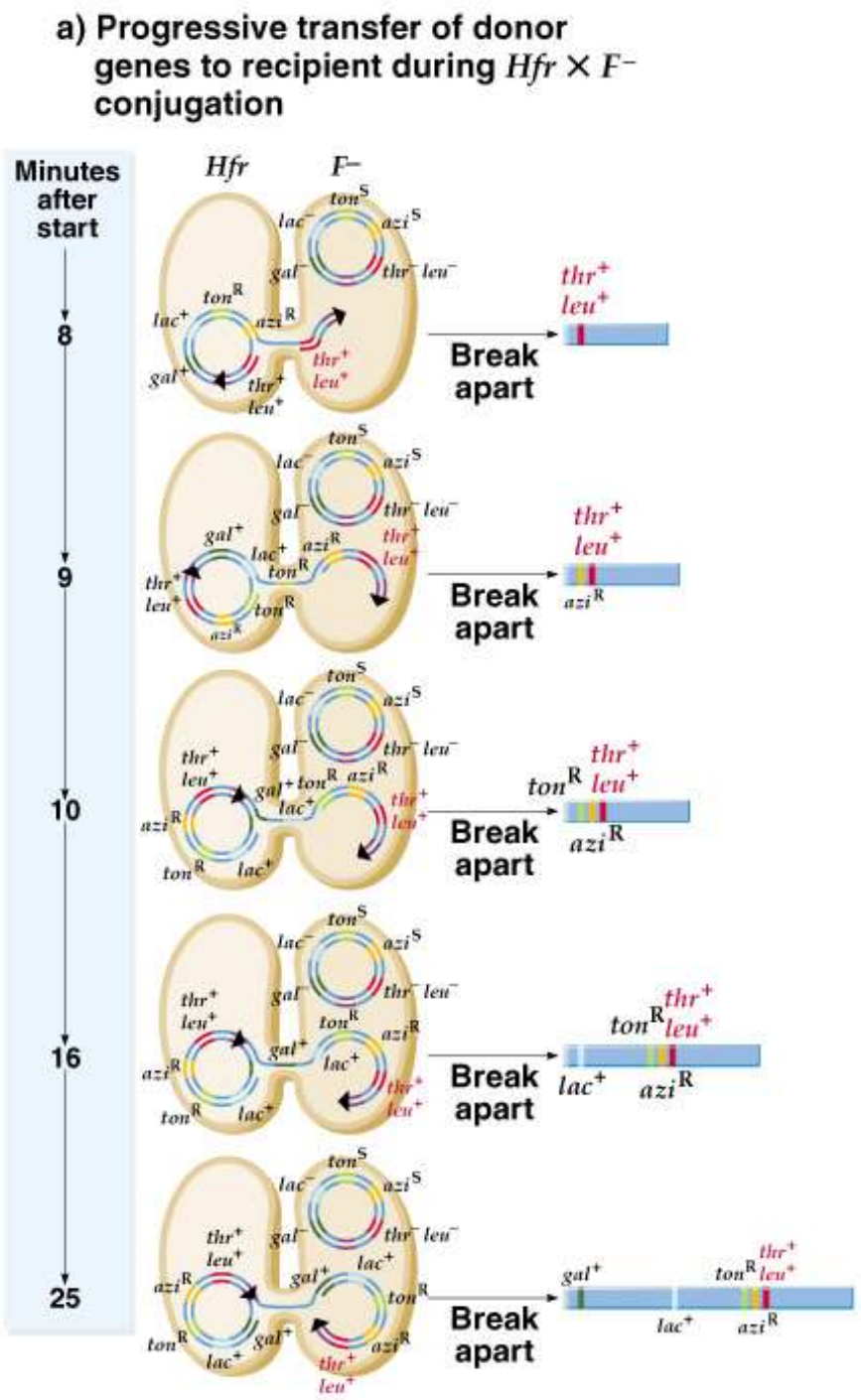
5. **Analyze recombinants with selective media.**

6. **Order in which genes are transferred reflects linear sequence on chromosomes and time in media.**

7. **Frequency of recombinants declines as donor gene enters recipient later.**

Fig. 15.7

Interrupted mating experiment



b) Appearance of donor genetic markers in recipient as a function of time

Fig. 15.7b

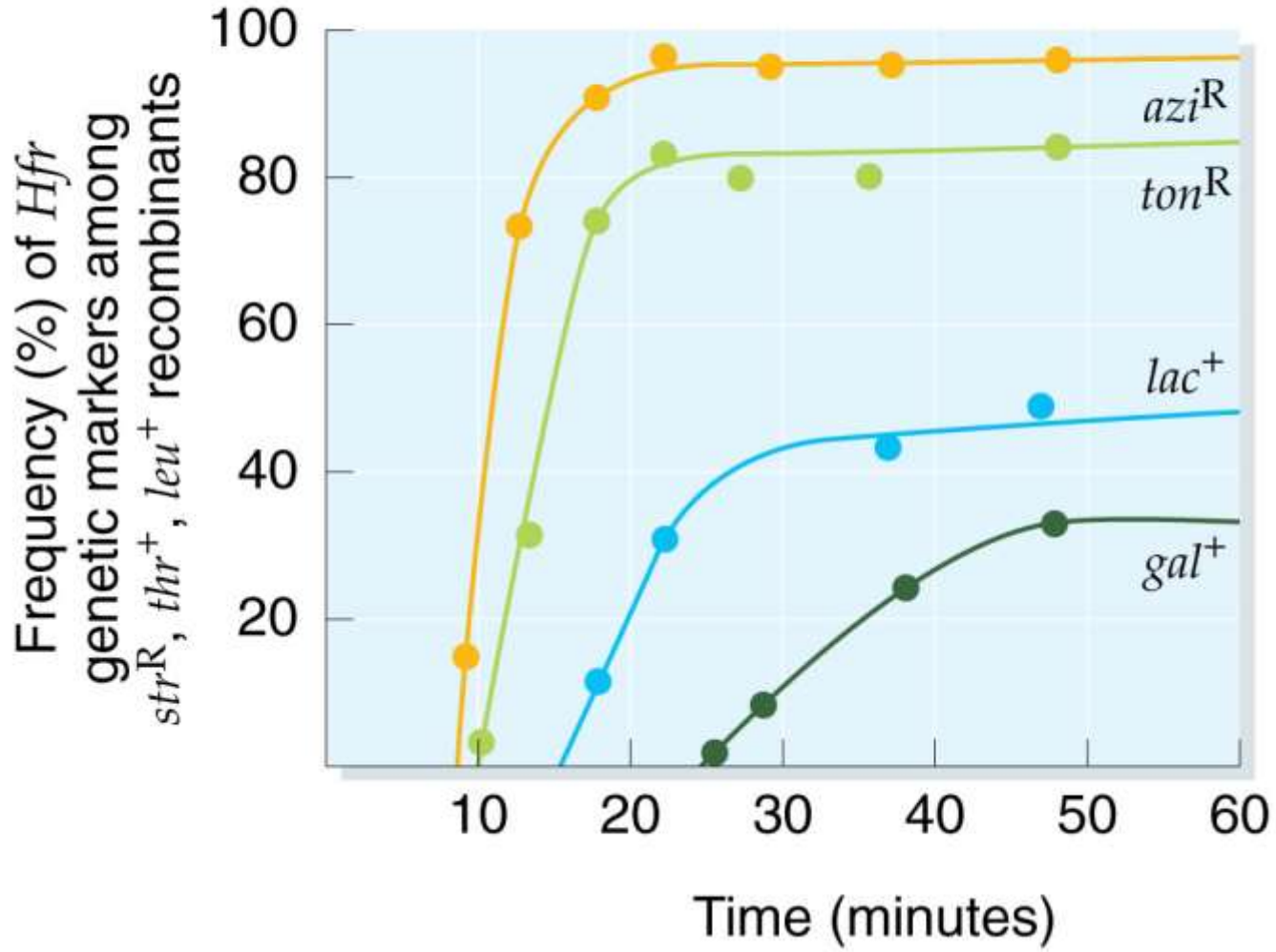
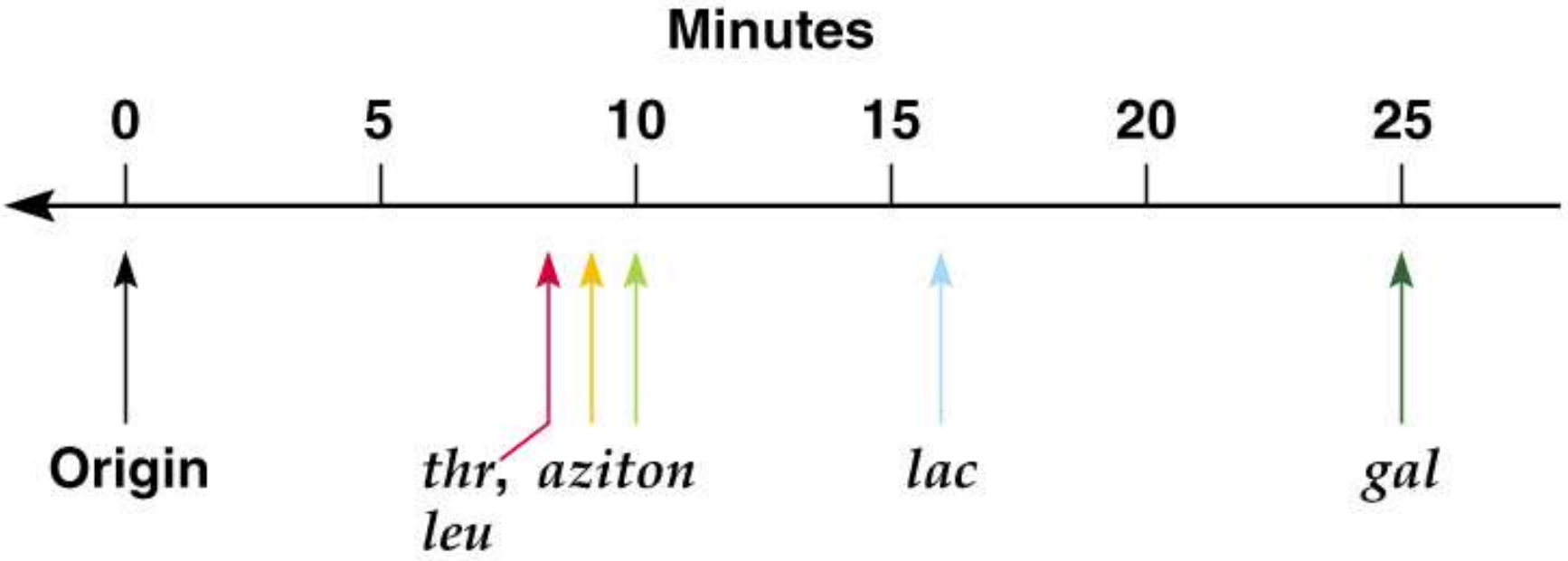


Fig. 15.7c, Genetic map-results of interrupted *E. coli* mating experiment.



Generating a map for all of *E. coli*:

1. **Location and orientation of the *Hfr F*$^+$ in the circular chromosome varies from strain to strain.**

2. **Overlap in transfer maps from different strains allow generation of a complete chromosomal map.**

a) Orders of gene transfer

***Hfr* strains:**

H origin—thr–pro–lac–pur–gal
1 origin—thr–thi–gly–his
2 origin—his–gly–thi–thr–pro–lac
3 origin—gly–his–gal–pur–lac–pro

b) Alignment of gene transfer for the *Hfr* strains

H thr–pro–lac–pur–gal
1 his–gly–thi–thr
2 his–gly–thi–thr–pro–lac
3 pro–lac–pur–gal–his–gly

c) Circular *E. coli* chromosome map derived from *Hfr* gene transfer data

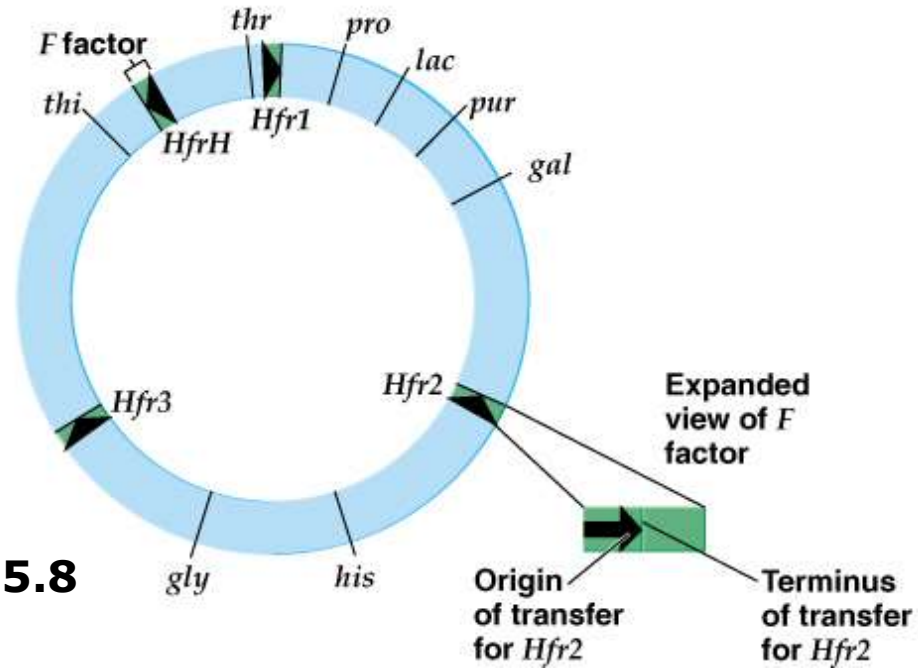


Fig. 15.8

Circular genetic map of *E. coli*

Total map units = 100 minutes

Time required for *E. coli* chromosome to replicate at 37˚ C.