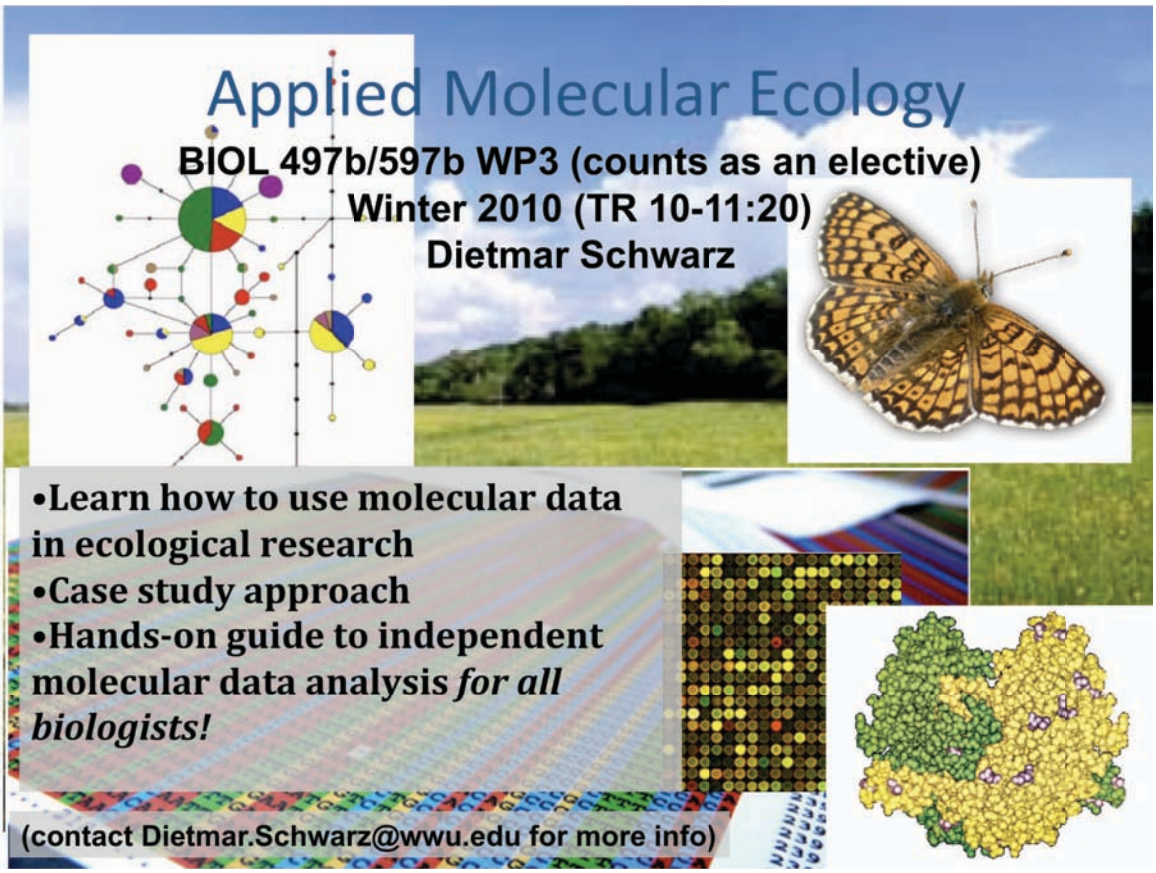
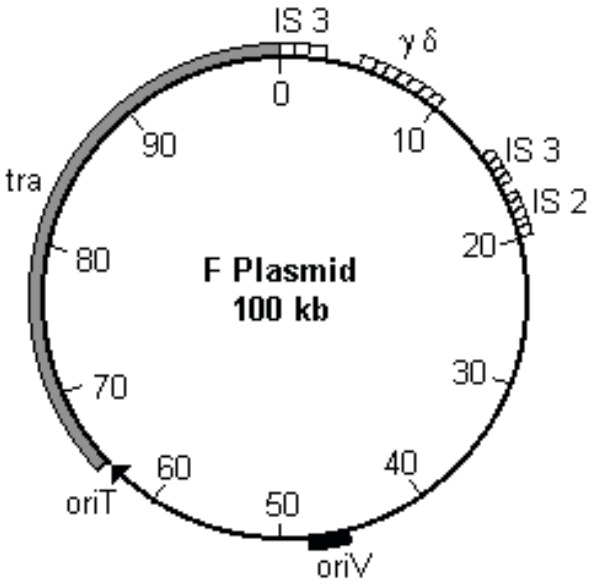

Applied Molecular Ecology
BIOL 497b/597b WP3 (counts as an elective)
Winter 2010 (TR 10-11:20)
Dietmar Schwarz
•Learn how to use molecular data in ecological research
•Case study approach
•Hands-on guide to independent molecular data analysis *for all biologists!*
(contact Dietmar.Schwarz@wwu.edu for more info)

09.11.09 Bacterial Genetics 2
Molecular mechanisms of horizontal gene transfer
F' X F-

Animation of gene transfer
http://www.blackwellpublishing.com/trun/artwork/Animations/conjugation/conjugation.html



IS 3 & IS 2 = insertion sequences
γδ = transposon Tn1000
oriV = origin of replication
oriT = origin of conjugal transfer
tra = tra functions

The tra (transfer) gene specify structural proteins that are required for pili formation and various enzymes required for "mobilization" of the DNA

Transfer of the plasmid DNA starts at ori-T and proceeds counterclockwise on the drawing shown above

Important implications of whole plasmid transfer

- ***This is a great way for a bacterial cell to acquire novel genes:*** since no requirement for cross over, resident genome does not have to have homology to incoming genes
- Also plasmid packaged AR genes come with their own transfer genes

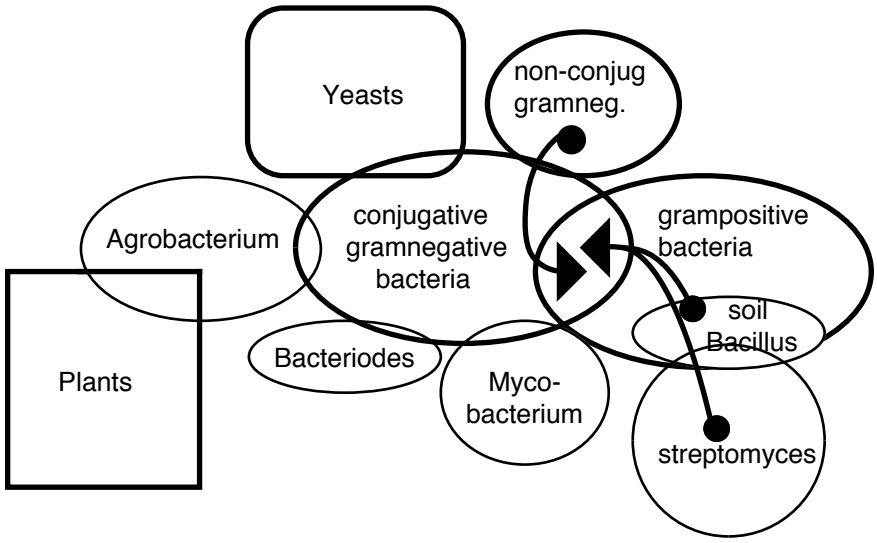
Why is the homology issue so important in the movement of antibiotic resistance genes?

In natural settings there is a tremendous amount of promiscuous plasmid exchange via conjugation (most bacterial and some archeal species encode conjugation systems) and transformation

This exchange can occur between distantly related bacterial species

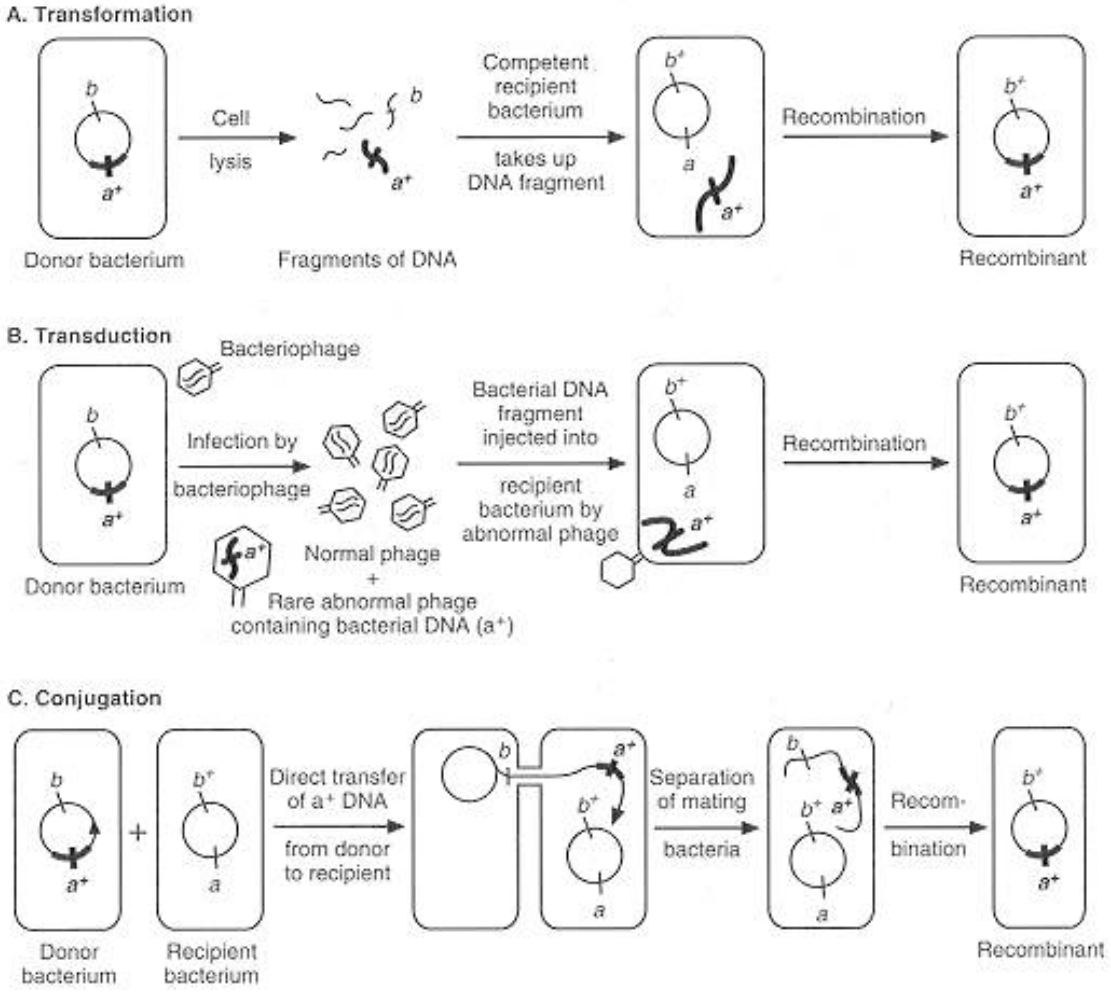
This sort of casual horizontal (lateral) transfer of genetic information (mostly on plasmids) has played a decisive role in the appearance and evolution of organisms resistant to various types of antibiotics

<u>Overlap of circles indicates documented gene exchange</u>



Range of Plasmid-Related Gene Flux .
Regions of overlap between major genetically related organisms represent gene exchange. Almost all this network is based on plasmid self-mobilization, although other means of lateral transfer maybe involved. Arrows show the proposed origin and dispersion of different antibiotic resistance determinants. Some determinants arose from antibiotic-producing organisms and soil bacteria, others from mutant organisms exposed to drugs during the antiboitic era.

In contrast to plasmid transfer, transformation, transduction and transfer of chromosomal genes via conjugation require some sequence homology between incoming DNA and resident genome to generate a stable recombinant

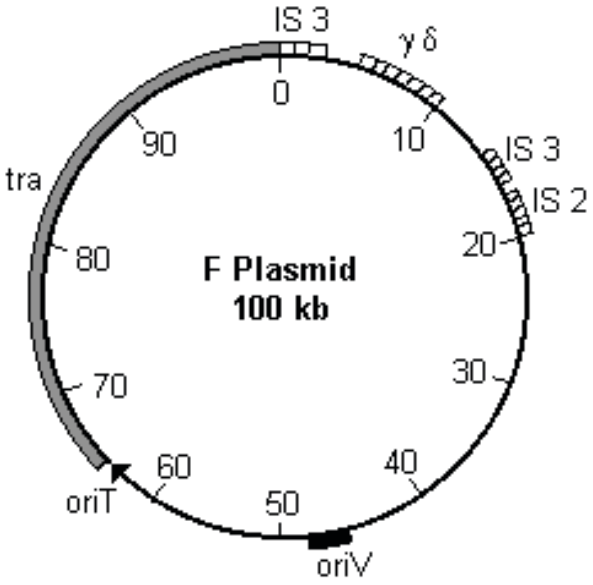


Transfer of chromosomal genes by integrated F factor

- Animation of gene transfer: Hfr X F-
- to get a stable recombinant homology must exist between some portion of the incoming DNA and the resident genome
- Since only a portion of the F factors is transferred, the recombinant does not become male

http://highered.mcgraw-hill.com/sites/0072556781/student_view0/chapter13/animation_quiz_4.html

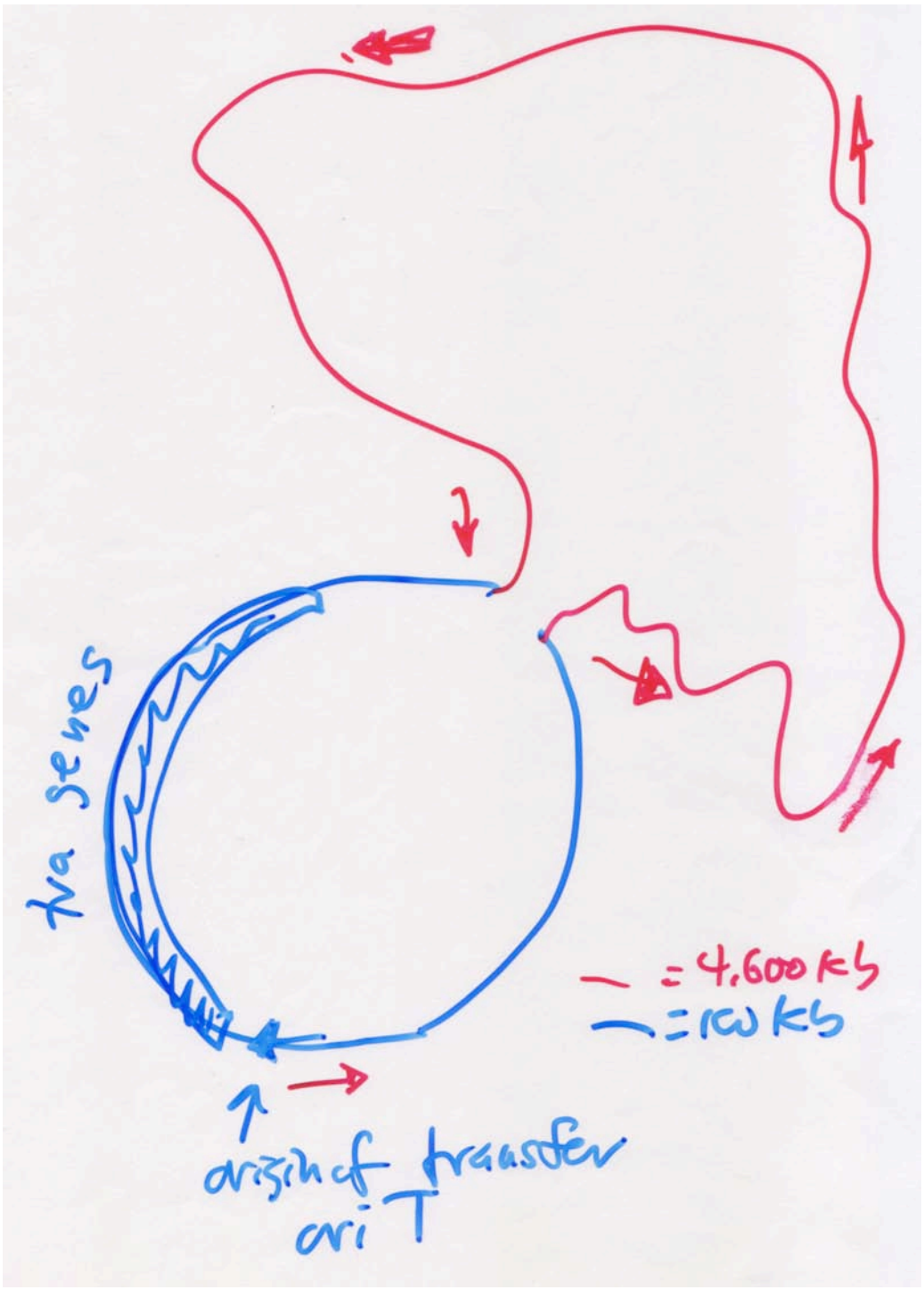
http://www.biostudio.com/d_%20Bacterial%20Conjugation.htm



IS 3 & IS 2 = insertion sequences
γδ = transposon Tn1000
oriV = origin of replication
oriT = origin of conjugal transfer
tra = tra functions

Transfer of the plasmid DNA starts at ori-T and proceeds counterclockwise on the drawing shown above

A single crossover between the the F plasmid and the bacterial chromosome will integrate the plasmid DNA into the chromosomal DNA. These crossover events take place at either the IS2 or IS3 sites. This means that during transfer of DNA by an integrated F plasmid, the entire bacterial chromosome would have to be transferred before the tra genes would enter the F- cell.



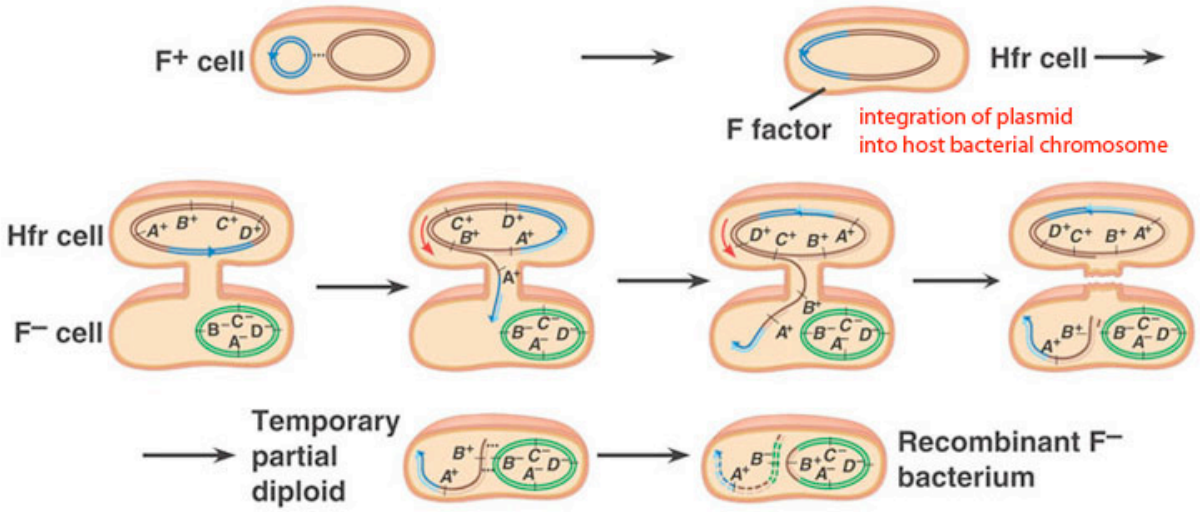
Transfer is counterclockwise in this drawing of an integrated F factor. The whole E.coli genome must is transferred before the F factor tra genes.

The molecular basis of recombination

http://www.sinauer.com/cooper/4e/animations0602.html

http://www.courses.fas.harvard.edu/~biotext/animations/holliday.html

http://engels.genetics.wisc.edu/Holliday/holliday3D.html
http://engels.genetics.wisc.edu/Holliday/index.html



(b) Conjugation and transfer of part of the bacterial chromosome from an Hfr donor to an F− recipient, resulting in recombination

Molecular basis of homologous recombination in E. coli
RecA (aka synaptase) is a central player: RecA molecules scan DNA for homology and align homologous regions forming a triplex DNA molecule

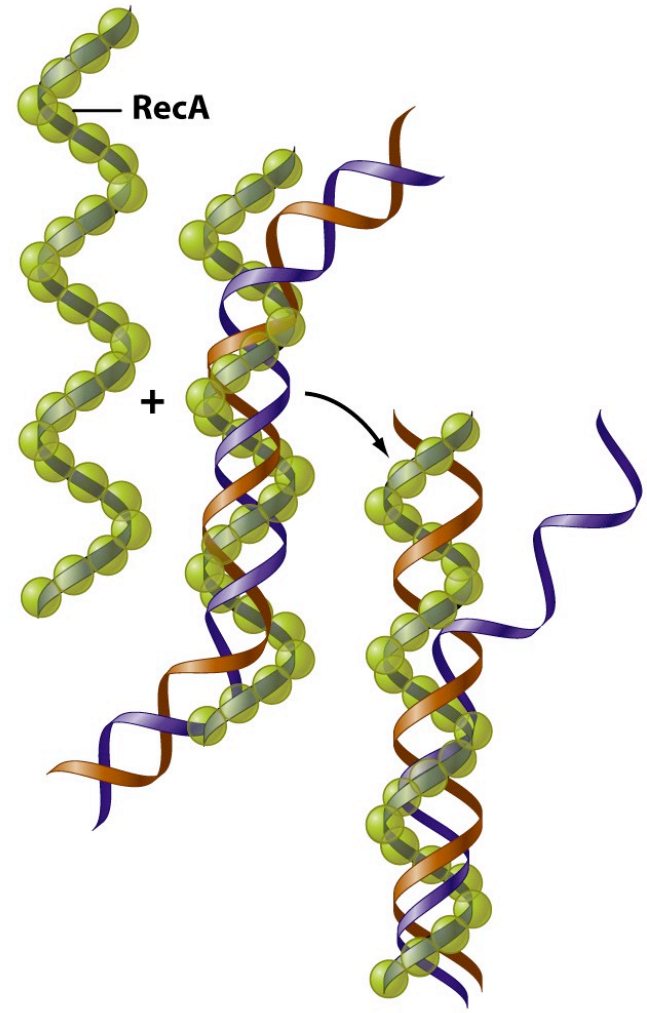


Figure 9.13a Microbiology: An Evolving Science
© 2009 W. W. Norton & Company, Inc.

RecA binds and forms a filament on a single-strand of DNA generated from DS-DNA by RecBC (see next figure)

The RecA-DNA complex can then bind a DNA duplex

When homology is found, a synapse forms and strand exchange occurs

http://www.courses.fas.harvard.edu/~biotext/animations/GeneralRecombination.html

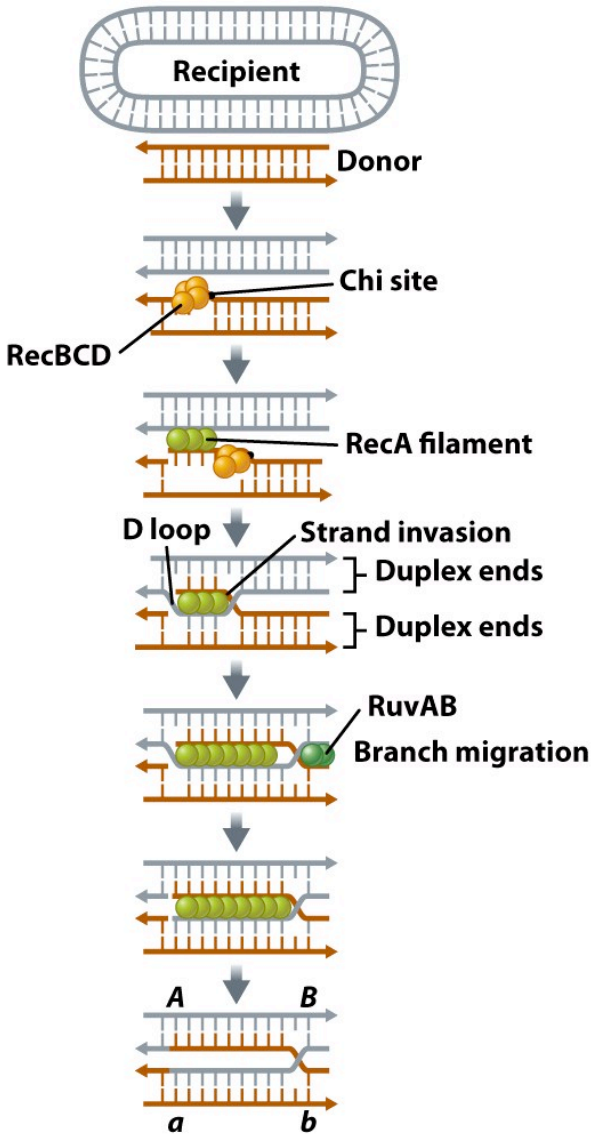


Figure 9.14 part 1 Microbiology: An Evolving Science

↓

Holliday structure

RecBCD binds to the end of donor DNA

RecBCD unwinds until a chi site is reached

At the chi site RecBCD nicks the DNA and continues unwinding

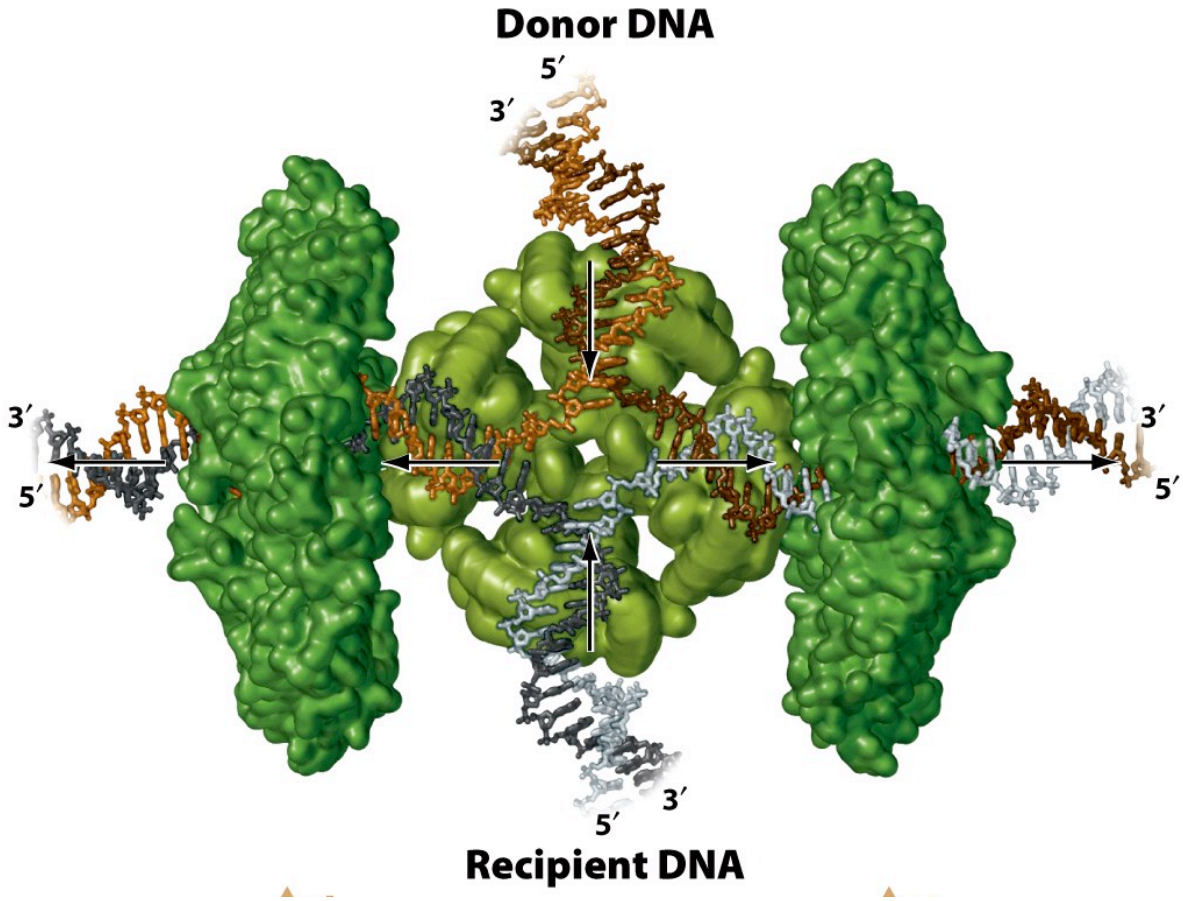
Then the RecA filament forms. RecA finds homology (at least 50 bp) and mediates strand invasion (invading strand displaces like strand in DNA duplex)

RuvAB binds at the crossover and carries out branch migration which extends base-pairing between donor and recipient strands

Endonuclease cleaves one end of D loop

Displaced ends are ligated to opposite strands

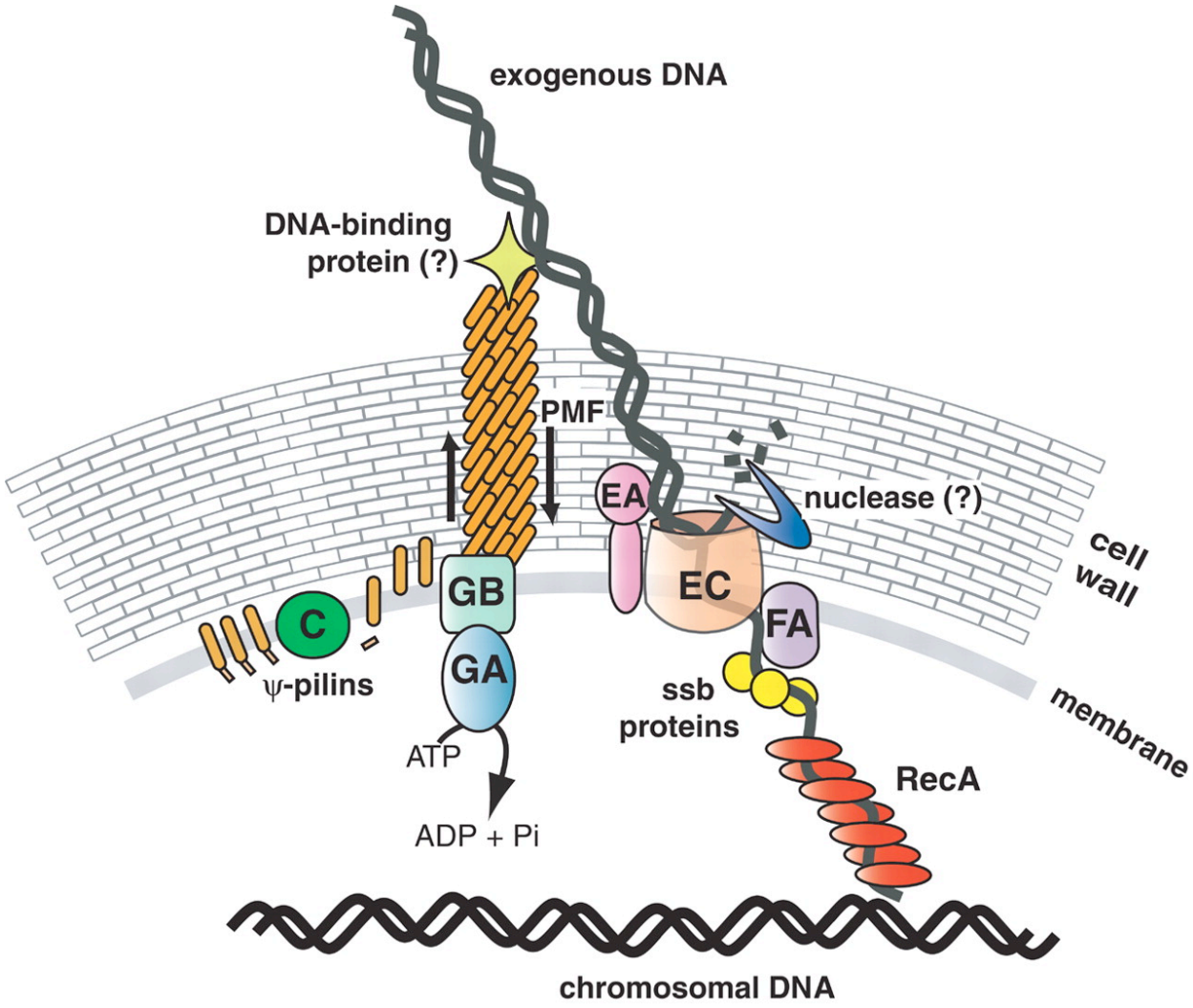
http://www.courses.fas.harvard.edu/~biotext/animations/GeneralRecombination.html



RuvAB proteins catalyze branch migration –helices are pulled in direction of arrows increasing the length of heteroduplex

http://engels.genetics.wisc.edu/Holliday/holliday3D.html
http://engels.genetics.wisc.edu/Holliday/index.html

Fig. 2. DNA uptake during transformation in *B. subtilis*. The uptake machinery is preferentially located at the cell poles. The ψ-prepilins are processed by the peptidase and translocate to the outer face of the membrane. With the aid of the other ComG proteins, the major ψ-pilin ComGC assembles into the ψ-pilus, which attaches exogenous DNA via a hypothetical DNA binding protein. Retraction of the ψ-pilus, driven by the proton motive force, and DNA binding to the receptor (ComEA) are required to transport one strand of DNA through the membrane channel (ComEC) while the other is degraded by an unidentified nuclease. The helicase/DNA translocase (ComFA) assists the process, along with ssDNA binding proteins that interact with the incoming DNA. RecA forms a filament around the ssDNA, and mediates a search for homology with chromosomal DNA. ADP, adenosine diphosphate; Pi, inorganic phosphate; PMF, proton motive force; ssb, single-stranded DNA binding protein. [View Larger Version of this Image (218K JPEG file)]



Transformatin in B. subtilis -- note RecA "delivering" ssDNA to the chromosome

		DONATES AT A	
E. COLI STRAIN	F FACTOR	HIGH FREQ.	LOW FREQ.
F^-	NONE	NOTHING	NOTHING
F^+	CYTOPLASMIC (F factor integrated in 1/1,000 cells)	F FAC TOR	CHROMOSOMAL GENES (VERY LOW FREQ)
F'	CYTOPLASMIC CARRIES A BIT OF THE BACTERIAL CHROMOSOME	F FACTOR AND BIT OF BACTERIAL CHROMOSOME	MOST CHROMOSOMAL GENES (VERY LOW FREQ)
Hfr ***➜ an Hfr strain is a laboratory artifact***	**integrated in *all cells* (site of integration varies from Hfr strain to Hfr strain)**	CHROMOSOMAL GENES (ESPECIALLY NEAR SITE OF INTEGRATION)	F FACTOR (VERY LOW FREQ)

F^+ X F^- cross: Many F^- cells converted to F^+; rarely: some bacterial genes transferred to F^- cells from rare cells with an integrated F factor

F' X F^- cross: Many F^- cells converted to F'^+; rarely: some bacterial genes transferred to F^- cells from rare cells with an integrated F factor

Hfr X F^- cross: Bacterial genes transferred (from a fixed point in the chromosome in a fixed order) to F^- cells at a high frequency. Order of gene transfer varies from strain to strain depending on where the F factor has integrated. Few F^- converted to F^+ because the F factor genes are the last to be transferred in any Hfr strain and most mating pairs don't stay tother long enough for the entire chromosometo be transferred.