Gene movement, part III and restriction-modification

F (fertility) plasmid
- Conjugative ability due to transfer (tra and F pilus genes) and adjacent oriT
- Rep/Par/Inc
- Plasmid with several IS/Tn elements: 1 IS3 disrupts tra repression system

Plasmid transfer via F conjugation

DNA transfer in F conjugation
- oriT is nicked, 5'→3' ssDNA transfer
- DNA pol III, etc replicates (leading in donor)
- Religation of oriT at end by nickase
Mobilizable plasmids

Conjugal plasmids encode proteins for an apparatus that allows DNA movement. Mobilizable plasmids (like ColEI) can exploit the apparatus encoded by a co-resident conjugal plasmid. Usually a mobilizable plasmid-specific DNA-processing function (still using ssDNA transfer process). ColE1 cannot move by itself!

Without a conjugal plasmid, ColE1 cannot mobilize from here!

F plasmid can integrate into E. coli chromosome

IS/Tns can allow plasmids to recombine with host chromosomes

F plasmid and E. coli Hfrs

When plasmids recombine with chromosome, conjugation functions can lead to transfer of chromosomal DNA (next to integrated plasmid) to recipient via conjugal apparatus.

Directionality of process leads to rare transfer of tra genes

Fig. 11.22

Fig. 11.23

Fig. 11.24
F plasmid: Hfrs and F primes

Hfr formation is reversible. Sometimes, the reversal is not precise, leading to formation of F'.

Plasmids and chromosomes

While formation of Hfrs and F prime plasmids only well described in *E. coli*, process goes on in other bacteria with other plasmids
- R prime plasmids will form (*e.g.*, IncP plasmid with *R. meliloti* cys genes)
- Hfrs have been found in *Pseudomonas*

Conjugal plasmids can mediate two distinct DNA transfer events

Conjugal or mobilizable Plasmid-containing donor cell

Conjugation: Plasmid transfer

In both cases, ssDNA transferred

Donor cell with integrated plasmid

Conjugation: Chromosome transfer

DNA must recombine with recipient; recipient rarely becomes a donor; 10-100 kb transferred

Gene movement: the bacterium fights back…

While many mechanisms to move DNA from one cell to another exist, the bacterial cell is not necessarily a “passive” recipient. Some incoming DNA can obviously have negative impact on cell (Phage infection/sensitivity). Bacteria have developed one important strategy to combat the flow of DNA into a cell: Restriction-Modification (R-M) systems
Discovery of R-M systems

Work from several phage groups (50’s-60’s):

• λ infects both B and K strains of *E. coli*, but…
  • λ preps grown on *E. coli* B strain with 10000x lower titer on K strain than on B
  • λ preps grown on *E. coli* K strain with 10000x lower titer on B strain than on K
  • Discovered that reduction in “efficiency” of infection due to strain-specific nucleases
  • Demonstrated that the R-M enzymes act indiscrimantly on dsDNA in cell; normal host DNA is protected due to its modification

Recognition sequence for *E. coli* Type II R-M enzyme

The EcoRI restriction enzyme makes staggered cuts on both strands of DNA, leaving ss “sticky ends”. The modifying enzyme adds -CH$_3$ to 1 base of each strand in recognition sequence and prevents cleavage. R-M systems widespread in Bacteria and Archaea (rare in euks).

Three types of R-M systems

<table>
<thead>
<tr>
<th></th>
<th>Type I</th>
<th>Type II</th>
<th>Type III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Example</td>
<td><em>EcoB</em></td>
<td><em>EcoRI</em></td>
<td><em>EcoPI</em></td>
</tr>
<tr>
<td>Recognition site</td>
<td>TGAN$_3$TGCT</td>
<td>GAATTC</td>
<td>AGACC</td>
</tr>
<tr>
<td>Cleavage site</td>
<td>ca 1 Kb away (distant)</td>
<td>Between G and A (in sequence)</td>
<td>24-26 bp on 3’ side (closeby)</td>
</tr>
<tr>
<td>Joint Nuclease/Methylase?</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>ATP-dependent</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>
Type II R-M enzymes

Because they have separate R/M enzymes and they cleave in recognition sequence, the restriction endonucleases of Type II systems are useful for molecular biology. Restriction enzymes with different recognition sequences have been isolated from wide variety of bacteria. Type II systems most common but Type I systems widely distributed; Type III systems rare

<table>
<thead>
<tr>
<th>Organism</th>
<th>Enzyme</th>
<th>Recognition sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. subtilis</td>
<td>BsuRI</td>
<td>GG↓C.C</td>
</tr>
<tr>
<td>H. influenzae</td>
<td>HindI</td>
<td>GTPy↓PuAC.</td>
</tr>
<tr>
<td>H. influenzae</td>
<td>HindII</td>
<td>A↓AGCTT</td>
</tr>
<tr>
<td>Nocardia otitidis</td>
<td>NofI</td>
<td>GC↓GGC.CGC</td>
</tr>
</tbody>
</table>

Generally, restriction endonucleases with larger recognition sequences (6-8 bp) are most useful for molecular biology.

Antagonizing R-M enzymes

Restriction-modification systems obviously widely distributed

Phages and plasmids have developed ways to circumvent this protection:
- Modification of T4 DNA allows it to escape the E. coli R-M system
- Several plasmids have anti-restriction systems or will reduce frequency of recognition site in the plasmid sequence.

Restriction enzymes and biotechnology

DNA can be cleaved in vitro with restriction enzymes and ligated together.

Fig. 12.5
A practical example of cloning

Steps:
1. Cut vector and insert DNA
2. Join with ligase
3. Introduce into E. coli
4. Screen on selective medium

How do we verify that our clone is good?

Cloning & recombinant DNA

- Over last 30 years a wide variety of cloning strategies have been exploited, including plasmid and viral "vectors"
- Can manipulate interesting DNA and move it into variety of bacterial or eukaryotic cells
- One strategy exploits natural ability of *Agrobacterium tumefaciens* to move plasmid DNA into plants (trans-kingdom conjugation).