Conjugative Transposons (CTns)

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What is a CTn?

• Integrated DNA segment that excises to form a circular intermediate which transfers by conjugation

• Also called ICEs (integrated conjugative elements)

• Vary widely in size (18 kbp – 500 kbp)

• Some carry genes not involved in transfer (eg, antibiotic resistance genes, nitrogen fixation genes)

• Usually able to mobilize plasmids in trans
Steps in the Transfer of a Conjugative Transposon

- **Excision**
- **Transfer**
- **Replication**
- **Integration**

**Donor**

**Recipient**
Different levels of investigation

- **Ecology:** Movement of genes in the colonic ecosystem by CTns

- Mechanisms of CTn integration and excision

- Regulation of CTn functions

- Effects of a CTn on the cell it enters
Composition of the colonic microbiota

- Numerically predominant groups
  - *Bacteroides* spp.
    - Polysaccharide fermentation
    - Opportunistic pathogen – resistance to antibiotics
  - Gram positive anaerobes (e.g. *Clostridium coccoides*, *Clostridium leptum*, *Eubacterium* spp.)
The Reservoir Hypothesis – Intestinal Bacteria As Reservoirs for Resistance Genes

- Other intestinal bacteria
- Swallowed bacteria
- Resistase intestinal bacteria
- Genes
- Fecal-oral transmission
- Bacteria
Questions

• How much transfer is actually occurring? How broad is the host range?
  – Approach: Find identical or near-identical resistance genes (>95% DNA sequence identity) in different species or genera of bacteria

• How is transfer being mediated?
  – Approach: Establish genetic linkage between resistance gene and some mobile element (CTn, plasmid) using Southern blot or PCR
Antibiotic Resistance Gene Distribution in Community and Hospital Isolates of *Bacteroides* spp.

<table>
<thead>
<tr>
<th>Source of Isolates</th>
<th>No.</th>
<th>tetQ</th>
<th>ermF</th>
<th>ermG</th>
<th>cfxA</th>
<th>CTn</th>
<th>HBU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Community (pre-1970)</td>
<td>69</td>
<td>32</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>42</td>
<td>51</td>
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<tr>
<td>Clinical (pre-1970)</td>
<td>23</td>
<td>22</td>
<td>9</td>
<td>0</td>
<td>4</td>
<td>30</td>
<td>35</td>
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<tr>
<td>Community (1996-1997)</td>
<td>102</td>
<td>81</td>
<td>15</td>
<td>8</td>
<td>3</td>
<td>84</td>
<td>74</td>
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<tr>
<td>Clinical (1980-1995)</td>
<td>87</td>
<td>86</td>
<td>30</td>
<td>18</td>
<td>14</td>
<td>85</td>
<td>84</td>
</tr>
</tbody>
</table>
### Widespread resistance genes in the human microbiota

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Resistance Gene</th>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus spp.</td>
<td>ermG</td>
<td>Bacteroides spp.</td>
</tr>
<tr>
<td>Clostridium spp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus spp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>ermB</td>
<td>Bacteroides spp.</td>
</tr>
<tr>
<td>Staphylococcus spp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clostridia spp.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>tetM</td>
<td>Campylobacter spp.</td>
</tr>
<tr>
<td>Staphylococcus spp.</td>
<td></td>
<td>Fusobacterium nucleatum</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td></td>
<td>Gardenella vaginalis</td>
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<tr>
<td>Actinomyces spp.</td>
<td></td>
<td>Haemophilus spp.</td>
</tr>
<tr>
<td>Bifidobacterium spp.</td>
<td></td>
<td>Neisseria spp.</td>
</tr>
<tr>
<td>Clostridia spp.</td>
<td></td>
<td>Veillonella spp.</td>
</tr>
<tr>
<td>Firmicute Eubacterium</td>
<td>ermF</td>
<td>Bacteroides spp.</td>
</tr>
<tr>
<td></td>
<td>tetQ</td>
<td>Prevotella spp.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Porphorymonas spp.</td>
</tr>
</tbody>
</table>
Elements responsible for transfer events

• **tetM, tetQ**
  – Conjugative transposon (Tn916, CTnDOT)
  – Tc-stimulated transfer

• **ermF**
  – Conjugative transposon (CTnDOT family)
  – Self-transmissible plasmid, mobilizable plasmid

• **ermG, ermB**
  – Conjugative transposons (CTnGERM, CTnBST)
Evidence from genome/metagenome sequences

• Sequences being seen that are associated with conjugative transposons (transfer genes, integrase genes) found in

  – Bacteroides group (Bacteroides, Porphyromonas, Prevotella)

  – Gram positives
CTnDOT – a widespread *Bacteroides* CTn

- In pre-1970s, found in about 20% of intestinal *Bacteroides* strains
- Post 1990, found in over 80% of strains
- Characteristics
  - 65 kbp
  - *tetQ, ermF*
  - Very stable in the absence of selection
  - Functions regulated by tetracycline
Different levels of investigation

• Ecology: Movement of genes in the colonic ecosystem by CTns

• Mechanisms of CTn integration and excision

• Regulation of CTn functions

• Effects of a CTn on the cell it enters
Steps in the Transfer of a Conjugative Transposon

1. **Excision**
   - The transposon is excised from the donor chromosome.

2. **Transfer**
   - The transposon is transferred to the recipient chromosome.
   - The 5' end of the transposon is labeled with mob.

3. **Replication**
   - The transposon is replicated in the recipient chromosome.

4. **Integration**
   - The transposon is integrated into the recipient chromosome.

5. **Donor**
   - The donor chromosome remains unaltered.

6. **Recipient**
   - The recipient chromosome now contains the transposon.
Excision and Integration of CTnDOT

Rajeev et al, MMBR, 2009
Characterizing the CTnDOT Excision/Integration Mechanism

- Construction of a miniature form of CTnDOT for in vivo assay of integration (suicide plasmid containing the integrase gene and the joined ends of the circular form)

- In vitro assays for integration, and steps in integration process (eg, DNA binding, cleavage, ligation)
Alignment of the Carboxyl Terminal Domain of Some Tyrosine Recombinases
Predicted structure of IntDOT
(Brian Swalla)
Mutations in the CAT Domain of IntDOT and their affect on Recombination

WT Recombination Frequency
Decrease in activity
No Detectable Recombination
15 Mutations in the CB Domain of IntDOT and their affect on Recombination
( ) = recombination frequency

H179A \( \text{WT cleavage} \)
\( \text{WT ligation} \)

K142A \( 6 \times 10^{-7} \)
\( \text{Weak ligation} \)
\( \text{WT cleavage} \)

R138A \( 1 \times 10^{-6} \)
\( \text{WT ligation} \)
\( \text{WT cleavage} \)

Y137A \( \text{no recomb.} \)
\( \text{Weak ligation} \)
\( \text{No cleavage} \)

L135A \( 5 \times 10^{-8} \)
\( \text{WT ligation} \)
\( \text{WT cleavage} \)

N183A \( \text{no recomb.} \)
\( \text{WT ligation} \)
\( \text{Weak cleavage} \)
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Steps in the Transfer of a Conjugative Transposon

donor

recipient
Regulation of excision of CTnDOT
How regulation of excision works

- Increased translation of TetQ, RteA, RteB proteins due to translational attenuation (Tc causing stalling of ribosomes on the leader region of operon)
  - Transcriptional fusion constitutive, translation regulated
  - Site directed mutations in leader region showed that mRNA structure involved

- RteA (sensor), RteB (DNA binding protein) is a two component regulatory system; activates transcription of rteC; RteC protein activates expression of excision operon (orf2c-orf2d-exc)
  - RT-PCR to detect regulated transcription
  - RteB and RteC bind DNA in vitro
  - Site-directed mutations upstream of promoter region of rteC and orf2c operon abolished transcription (activator)
Regulation of transfer genes

Transfer
- Tc-dependent excision and/or enhanced conjugal transfer
- Tc-independent repression of conjugal transfer
How regulation of transfer genes works

• When transfer genes cloned away from rest of CTnDOT, expression of tra gene was constitutive but within CTnDOT expression was regulated from nearly zero to high level expression

• Activation: Excision proteins alone are sufficient to activate tra gene expression
  – Expression of excision operon from heterologous promoter (no RteB, RteC necessary) caused activation of tra gene operon, but not repression (protein fusion to start codon of traA, RT-qPCR)

• Repression: Possible small RNA (RteR) causes repression
  – Furnishing rteR in trans with tra operon resulted in decreased expression (no effect on traA fusion, but RT-qPCR showed reduced transcription of later genes)
  – Stop codons in putative start codon had no effect on activity (probably regulatory RNA)
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Effect of CTnDOT on a recipient cell

- What is the effect on a *Bacteroides* cell of having CTnDOT enter its chromosome?
  - No evidence for disruption of genes
  - Could there be a global regulatory effect?
Approach

Microarrays to compare

No Tc, no CTnDOT
+Tc, +CTnDOT

Generate a “shopping list” of genes to be checked by qRT-PCR

(Moon and Salyers, Mol. Micro., 2007)
Results

• Expression of nearly 60 chromosomal genes were up-regulated or down-regulated by more than 7-fold

• Most up-regulated genes were genes of unknown function
  – Some were associated with cryptic CTns
  – Labeled with resistance gene to show transfer

• For one of these CTns, RteA and RteB plus Tc were sufficient. Others required intact CTnDOT

• Conjugative elements with regulatory genes may have broad effects on chromosomal genes