CLASS 2.5: 02/27/07

REPLICON STRUCTURES

A. The Replicon Structure in Chromosomes:
   - Origin =
   - Replicon =

1. Types of replicons
   a. Prokaryotic genomes
      i. Single-copy replicons
         - most of genome =
         - also can have
      ii. Multicopy replicons
         - elements present

b. Eukaryotic genomes
   i. Nuclear chromosomes =
      - have large number
      - each replicon fires
   ii. Mitochondria and chloroplasts
      - replicate

![Replication diagram](https://example.com/replication_diagram.png)
2. The Origin and bidirectional replication
   - Replication starts
   - Two strands
   - Each strand acts as
   - The point at which replication is occurring =
   - Most common form is bidirectional =

3. Prokaryotic Replicons
   - Replicon is usually
   - Includes bacterial chromosome,

   a. Stages of replication in *E. coli*
      i. Starts as distinct region =
      ii. Two replication forks move

      iii. Termination occurs at discrete sites
          - *ter* sites
          - are 2 terminator regions:
          - replication fork moves past *ter* region

      iv. At termination = 2 chromosomes are interlocked =
b. Bacterial replicons and transcription
   - Replication fork temporally
     - DNA pol moving in same direction as RNA pol can
     - Harder to resolve RNA pol and
     - Almost all active transcription units are
       - Exceptions are
c. Prokaryotic origins
   - *E. coli* has 11 copies of GATC =
     - Methylation occurs
     - Once replicated, site =
     - Hemi-methylated DNA
     - Hemi-methylation must

4. Eukaryotic replicons
   a. General characteristics
      - Eukaryotes have
        - Replication occurs
        - Each replicon is
          - Replicons located
          - May be regional controls
          - Small replicons
            - Do not
            - Replication fork moves
b. Eukaryotic origins
   - Best characterized
   - Origins identified by
     - Sequence that confers ability to replicate efficiently =
     - Structure of the ARS element (origin)
       i. AT rich sequence
       ii. Contains 4 domains
       iii. Only A well enough conserved
       iv. Target for origin recognition complex (ORC)
         - complex
         - contacts A and
         - initiation depends

B. Extrachromosomal Replicons:
   - Examples include

<table>
<thead>
<tr>
<th>Phages and plasmids live in bacteria</th>
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<tbody>
<tr>
<td><strong>Type of unit</strong></td>
</tr>
<tr>
<td>Lytic phage</td>
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<tr>
<td>Lysogenic phage</td>
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<tr>
<td>Plasmid</td>
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<tr>
<td>Episome</td>
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</table>

- Key parameter in determining
1. Replicating the ends of linear molecules
   - Recall that DNA pol synthesizes

   - DNA pol cannot initiate right at end of molecule =

   - Becomes a problem

   ![Replication of a 5' end is a problem](image)

- Strategies to overcome problem of linear end
  a. Convert linear molecule into
     - Used by
  b. DNA may form an unusual structure at end =
     - Linear
  c. Have variable ends with
     - Telomers of
  d. Covalently link
     - Adenovirus,
2. Rolling circle replication
   - Sometimes use only
   - Nick produces
   - DNA pol uses
   - Newly synthesized strand
   - Can continue to
   - Usually get cleavage of a unit length =

- Examples of rolling circle replications
  a. Amplification of
     - need lot of
     - rDNA genes are
     - cell will excise an array and
     - Can replicate
b. Phage replication
   - After rolling circle replication,

   - $\phi X174 =$
     i. DNA enters
     ii. DNA replicates
     iii. Protein A encoded from

     iv. Protein A stays associated with the 5’ end

     v. As the replication fork reaches

     vi. Protein A
     vii. Protein A nicks
4. Conjugation mechanisms
   - During conjugation can get

   a. F plasmid
      - Classic episome =
      - 100 kB element that
      - Integration occurs via recombination between

      i. Free plasmid
         - uses its own ori (oriV)
         - is maintained

      ii. When integrated into host chromosome:
         - F system
         - F-DNA replicated
         - Presence of free or integrated F episome (F⁺ cell) has

         i. F⁺ cells can
         ii. F factor contains
            - required for
         iii. If F is plasmid =
            - can convert F⁻ cell

         iv. If F is integrated =

            - Usually only
            - Mating usually
- 33 kB of the F episome contains
- The Transfer region contains
  i. $\text{traA}$ encodes pilin = 
    - several
    - each pilis =
  ii. 12 other $\text{tra}$ genes =
  iii. $\text{traS}$ and $\text{traT}$ =
    - prevents F$^+$ cell from
  iv. Other $\text{tra}$ genes stabilize

- Pilis initiates conjugation,

- Phage use pili for

\[F^+ \text{ Cell}\]

\[F^\text{- Cell}\]
b. DNA transfer of autonomous F factor
   - Transfer of F factor
   - Starts when TraM protein
     - TraY binds
     - TraI (relaxase) nicks oriT and
       5’ end of the nick
       • also unwinds
     - Freed 5’ end with TraY/I

   - Complement of transferred strand is

   - Complementary strand also must be
   - Transfer of single strand into recipient
   - Transfer ceases once single unit of F factor reaches recipient cell =

c. DNA transfer of integrated F factor
   - When F is integrated,

   - Only part of F
   - Entire bacterial chromosome must

   - Conjugation usually stops
- Most common result = 
- Single stranded transfer molecule 
- Have region of homology to host chromosome = 
- Cell with integrated F factor supports

5. Transferring bacterial genes to plants 
   - Crown gall disease occurs when

   - Bacteria causes disease 
     - Unusual infection =

   a. Properties of the Ti plasmid 
     - Contains genes for 
     - Only part of the plasmid 
       i. genes to generate 
       ii. genes for the
b. Plant cell transformation
- Requires 3 types
  i. *chvA*, *chvB*, and *pscA* = required for
    - Synthesize a poly-saccharide
  ii. *vir* region on Ti plasmid
  iii. T-DNA required

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- Requires 3 types
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c. T-DNA transfer resembles bacterial conjugation
- Are 6 *vir* genes
- When *Agrobacterium* contacts plant cell =
  - *vir* genes produce products that cause
    i. Activation of *virA* and *virG* genes
      - VirA,G = regulators that
        - causes
        - VirA-PO$_4$
        - VirG-PO$_4$ binds to
ii. \textit{virB,C,D,E} responses
- T-DNA is flanked by
- \textit{virD} has 4 open reading frames =
  - 3’ end of nick is extended =
  - Old strand becomes coated in
  - T complex (with nuclear-localization signal in VirE2)
  - \textit{virB} operon encodes
iii. Integration of T-DNA into plant genome
   - single-stranded DNA
   - sometimes find

   - dsCircle can then

   - *Agrobacterium* used as a common vector

   - remove oncogenic genes from