Lytic Phage and Transduction

I. Bacterial Virus = Phage = Bacteriophage

General life cycles
Lytic vs Lysogenic

II. Bacteriophage T7

The Lytic Life Cycle
1. Adsorption
2. Injection of Viral DNA
3. Conversion of Bacterial cell to a Phage Factory
4. Production of Phage Proteins, DNA
   early replication vs late replication
5. Assembly/Packaging into viral particles
6. Release/Lysis

The numbers game of the life cycle
MOI multiplicity of infection
Single-Burst Experiment
T7 Life Cycle
40 Kb with a 160 bp terminally redundant ends
Encodes ~50 genes.
Early Genes.
Utilize E.coli RNA polymerase promoters
Antirestriction Protein
Kinase that inactivates the E. coli RNA polymerase
RNA polymerase
Host DNA degradation genes
DNA ligase
Mid Genes
Utilize T7 RNA polymerase promoters
DNA Polymerase, Primase/Helicase
DNA metabolism
Late
Phage Particle Parts
Lysis genes
hok Disrupts cell membane
lysozyme Disrupts cell wall
Early replication occurs bidirectionally from an internal origin
Late replication occurs through recombinational initiation
Amplification and generation of concatamers
Packaging occurs processively, right o left, cutting at the redundant sequence

III. Bacteriophage T4

Linear DNA like T7 but much larger (200+genes instead of 30)
Cyclically permuted and terminally redundant
Encodes its own replication proteins, subunits, recombination proteins
Instead of its own RNA polymerase, alters the specificity of the bacterial polyerase
gp55 sigma factor (recognizes TATAAATA at -10 position)
Transcription is coupled to replication
gp45 (sliding clamp of the replication polymerase)
also acts as a transcriptional activator

Biphasic Replication
- Early origin dependant replication
- Late recombination dependant amplification
- Highly recombinagenic

Phage packaging occurs by headful mechanism (stuff the head until its full)

IV. Transduction

general transduction
- P1 phage of Escherichia coli
- P22 phage of Samonella enterica
- What makes a good transducing phage?

specialized transduction
- phage lambda

Mapping by Transduction
- Fixed distance/ short range mapping

As a tool for gene knockouts

Transduction in Evolution

V. The rII gene and its insights

Used for recombinational studies
- Recombination frequency=#recombinant progeny/total progeny
- 1 Map unit= 1% recombination frequency

Used for complementation
- Can mutant phage make wild type progeny when coinfected?

rII- rapid lysis mutants type II
- form hard clear plaques on *E.coli* instead of normal fuzzy edged plaques
- rII mutants **cannot** grow in *E.coli* carrying **phage lamda lysogens**
- rII mutants **can** grow in normal *E.coli*
- wild type T4 (r+) **can** grow in *E.coli* carrying **phage lamda lysogens**

Seymour Benzer 1950s mutants of rII

(Watson/Crick DNA structure 1953) (Messelson/Stahl Semiconservative replication 1958)
- Complementation studies of rII mutants
- Showed that all mutations fell within two genes rIIA rIIB
- Recombination frequencies: How to score for recombinants
- Intragenic supressors: Genes were not discreet units but divisable
- Mutational Spectra: Hot Spots for each mutagen

Francis Crick
- Helped ID the structure of DNA and knew it was the genetic information but...
  - How was the genetic information read?
- Trivia that he used in his experiments
  - 1.) acridine dyes induce frameshifts: (not leaky but can revert)
  - 2.) N terminus of rIIB protein is nonessential (deletion mutants showed this)
- Genetic code is unpunctuated, a triplet, and redundant***
  - Isolate rIIB insertion mutation FC0,
  - isolate rIIB supressors,
  - isolate rIIB supressors of supressors...