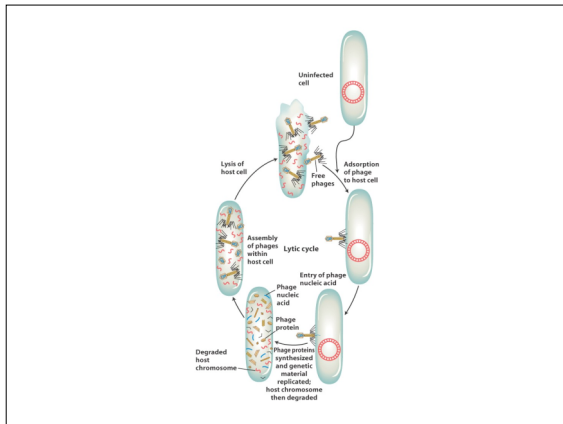
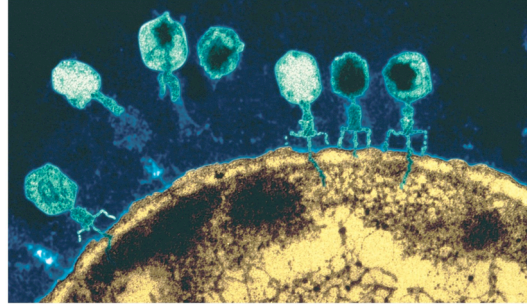


Lecture Outline 9/29

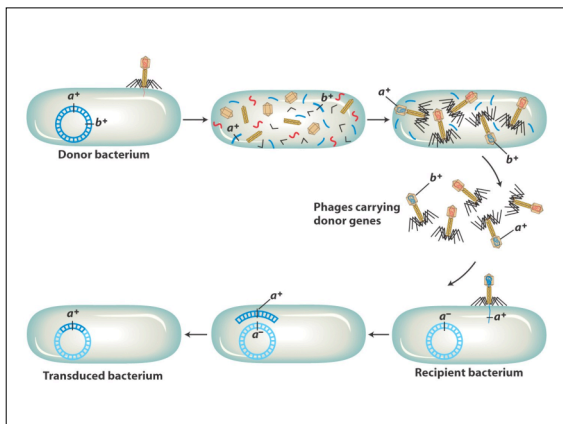
- Finish Bacterial Genetics
 - Transduction via phage
 - Fine structure mapping
- Start gene interactions
- New homework has been posted
- First gene assignment is on the website

Transduction



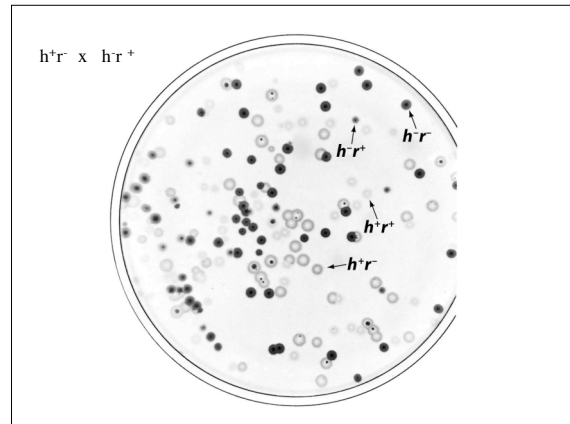
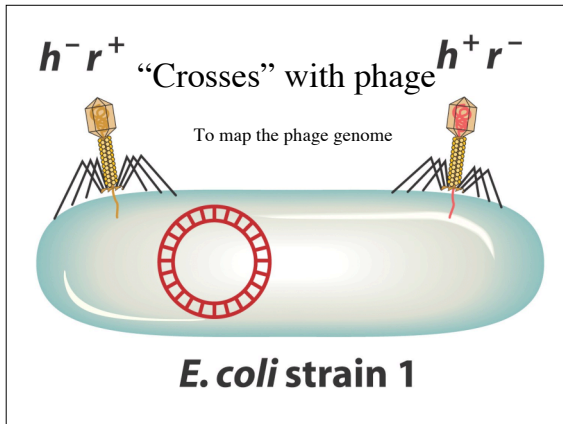
Generalized transduction

- Phage are viruses of bacteria
- Random piece of bacterial DNA incorporated into the phage
- Only small segments of chromosome can be taken up (<2 min)
- Mapping:
 - Look for co-transduction. If two genes commonly co-transduce, then they must be close together.



Example: co-transduction

- Two E. coli strains:
 - leu+ aziR x leu- aziS
 - Infect leu+ aziR with phage and collect lysate
 - Inoculate leu- aziS strain
 - Select for aziR
 - Then test if any of them also have leu+
- Results:
 - Colonies in minimal plus leucine: 50
 - Colonies in minimal 21 42% co-transduced
 - After some algebra, you can show that this corresponds to a distance of about **0.5 min**
 - *If the genes were 1 minute apart, would the number of colonies growing on minimal medium be larger or smaller than 21?*



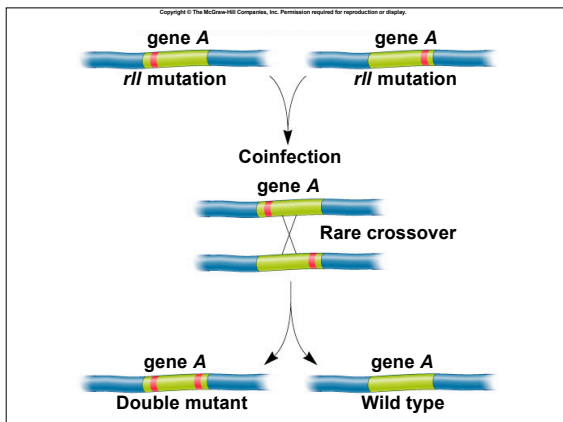
Crosses with phage

- What phage types would you predict from coinfection of bacterial cells with:

$h^+ r^+ t^+ \times h^- r^- t^-$

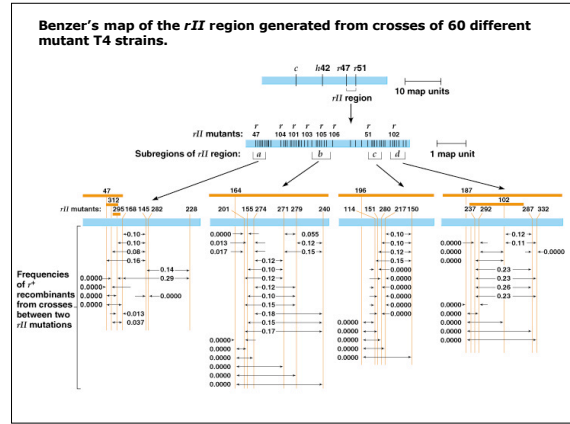
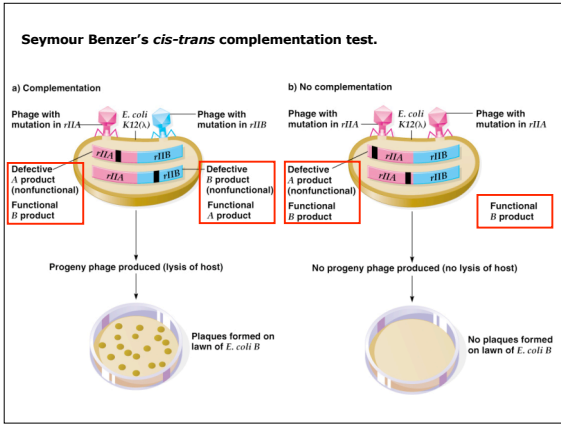
h = “host range”
r = “rapid lysis”
t = “turbid plaque”

Complementation tests



Intra-genic mapping

- Collect a huge number of independent mutants and test for complementation
- Use two non-complementing strains. Examine plaques for rare recombinants.
 - If they can grow in the selective medium, the two mutants must have recombined within the gene to produce wild type.
- Can find very rare recombination between neighboring mutations (down to a couple of bases).



Deletion mapping example

Given the following DNA sequences (a portion of the *arcA* gene from phage P22), would you expect the point mutant *arc-946* to yield wild-type recombinants if crossed against *Del(arc-191)*? What about if crossed against *Del(arc-192)*?

```

Wild-type (+)  ATGAAAGGAA TGAGCAAATGCCGAGTTCAATTTCGGTGGCC TAGAGAAGTATGGATTGGTACCGAAGT
              TACTTTCCTTACTCGTTTACGGGCTCAAGTTAAAGCCACCGGATCTTCATACCTAAACCATGGGTTCCA

arc-946 (-)   ATGAAAGGAA TGAGCAAATGCCGAGTTCAATTTCGGTGGCC TAGAGAAGTATGGATTGGTACCGAAGT
              TACTTTCCTTACTCGTTTACGGGCTCAAGTTAAAGCCACCGGATCTTCATACCTAAACCATGGGTTCCA

Here is a point mutation
              ^
              |
              v

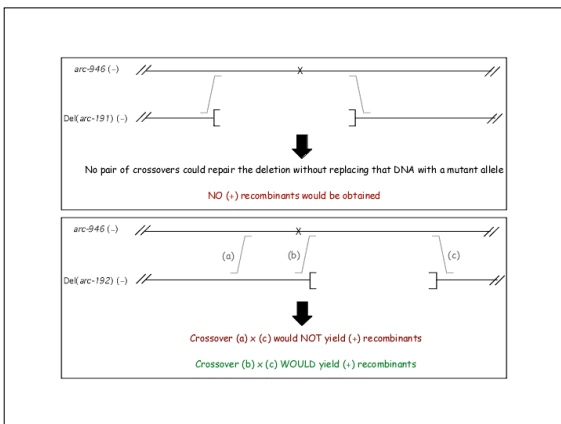
Del(arc-191) (-) ATGAAAGGAA TGAGCAAATGCCGAGTTCAATTTCGGTGGCC TAGAGAAGTATGGATTGGTACCGAAGT
                TACTTTCCTTACTCGTTTACGGGCTCAAGTTAAAGCCACCGGATCTTCATACCTAAACCATGGGTTCCA

Del(arc-192) (-) ATGAAAGGAA TGAGCAAATGCCGAGTTCAATTTCGGTGGCC TAGAGAAGTATGGATTGGTACCGAAGT
                TACTTTCCTTACTCGTTTACGGGCTCAAGTTAAAGCCACCGGATCTTCATACCTAAACCATGGGTTCCA
    
```

From <http://www.sci.sdsu.edu/~smaloy/MicrobialGenetics>

Deletion mapping

Cross point mutations with various deletions
Do you see recombinants or not?



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Deletion strain	Wild-type recombinants when coinfecting with <i>r103</i> ?
1272	No
1241	No
J3	No
PT1	No
PB242	Yes
A105	Yes
638	Yes

A1 A2 A3 A4 A5 A6 B1-B10
gene *rIIA* gene *rIIB*

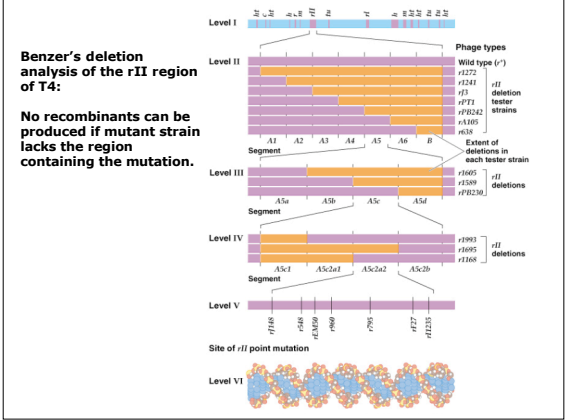


Fig. 14.22, Benzer's composite map of the rII region indicating >300 mutable sites on two different genes.

Small squares indicate point mutations mapping to a given site.

