Reading for lectures 17-19

Chp 8: from 258 (Nonoverlapping...) to 261 (…Cracking) & from 285 (8.6) to 293 (end of "essential concepts)
Chp 14: Using deletions to locate genes (496-498)
Chp 20: all except (for the time being) 724-25 (cloning) & RNA Interference (726-728).

Are **mutant a** and **mutant b** alleles (i.e. genetic alternatives)?

complementation test for function
alleles as alternative cis-acting **units** of **function**

high-resolution segregation test for recombination:
alleles as alternative **units** of segregation (**recombination**)

mutant a / **mutant b**

results may conflict

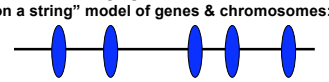
mutant a / **mutant b**

a & **b** may appear to be allelic by the functional test
(failure to complement)

…yet NOT allelic by the SEGREGATION test
(nonparental alleles recovered by meiotic recombination)

Mendel said that genes are the units of segregation, which led to the "beads on a string" model of genes & chromosomes:

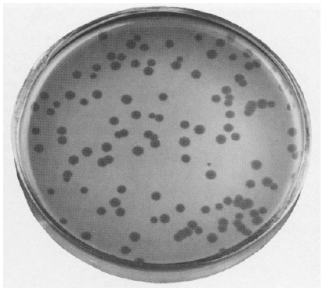
genetic map (linear like chromosome)



Benzer's question:

How is **complementation** between mutants related to **recombination** between mutants? (segregation)

Need a ***selective*** genetic system
(one with **high resolving power** for small map distances)



T4 plaques on a "lawn" of *E. coli* cells

Need a ***selective*** genetic system
(one with **high resolving power** for small **map distances**)

Rf_{a-b} = NP pfu from hybrid / total pfu from hybrid

pfu = "plaque-forming units"

hybrid = mixed infection

Phage are small, but plaques are often larger than fruit flies!
How do phage help with measuring small Rfs?

Use ***selective*** systems to easily measure **NP** pfu concentration without complication from the much larger number of **P** pfu

Benzer's system made measuring 0.0001 cM ($1x10^{-6}$) easy

…and by the way,
as an added bonus for mapping,
phage happen to have a MUCH
greater **rate of recombination per unit DNA**
than fruit flies or garden peas

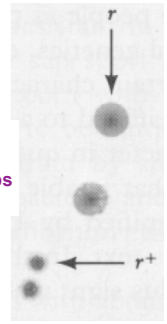
In **rII**, smallest non-0 recombination rate measured was 0.02 cM (mutants 1 bp apart)
(2 NP/10,000 total)

In my first effort at fine-structure mapping in **flies**, I measured 0.007 cM (one recombinant) for a distance (I only later found out to be) 3,100 bp
(1 NP/14,286 total)

Among Hershey's T4 collection were ***rapid lysis*** (*r*) mutants --- produced distinctively **large plaques**.

They arose spontaneously ($1:10^{-4}$) and fell into several **different complementation groups** that mapped at different places

One complementation group was ***rII*** and Benzer discovered something **special** about it that made it perfect for studying genetic fine structure.



(1) *rII* vs. *rII*$^+$ easily distinguished based on plaque morphology

(2) extremely rare ($<10^{-7}$) *rII*$^+$ recombinants easy to recover

(b.2)

T4 strain	*E. coli* strain B	*E. coli* strain K(λ)
rII$^-$	Large, distinct	No plaques
rII$^+$	Small, fuzzy	Small, fuzzy

Fig. 7.20

(1) Characterize mutants:
fell into two **complementation groups**, *rIIA* & *rIIB*
very close on a genetic map.

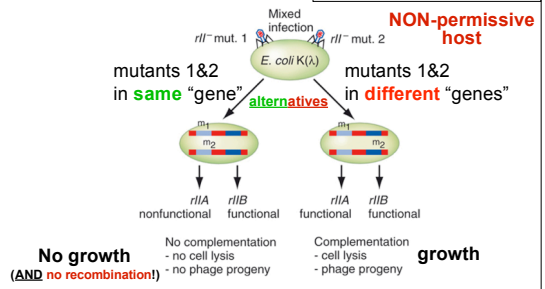
Complementation test: phenotype of the hybrid

Question: did he test by
plaque morphology
or
ability to growth on K(λ)?

p.229
Fig. 7.20 (still)

(c.1) Complementation test
(*trans* configuration)

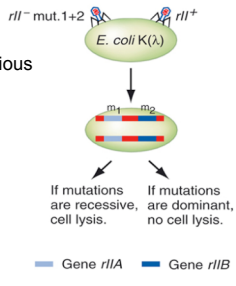
infect 1:1 mixture at "multiplicity of infection" of ~3 phage:1 bacterium



BUT WHAT did Benzer do **FIRST**?! determine that mutants are **recessive**

(for complementation test)

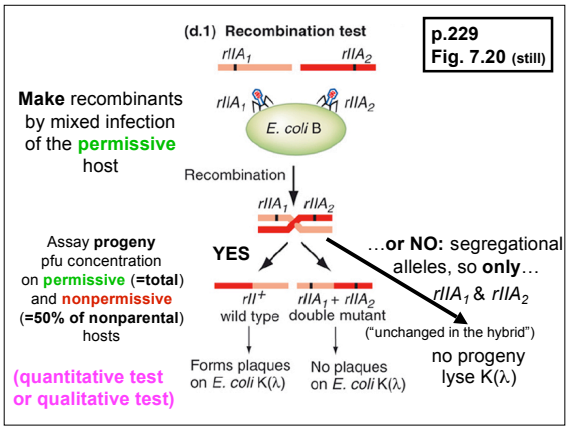
(c.2) Control
(*cis* configuration)

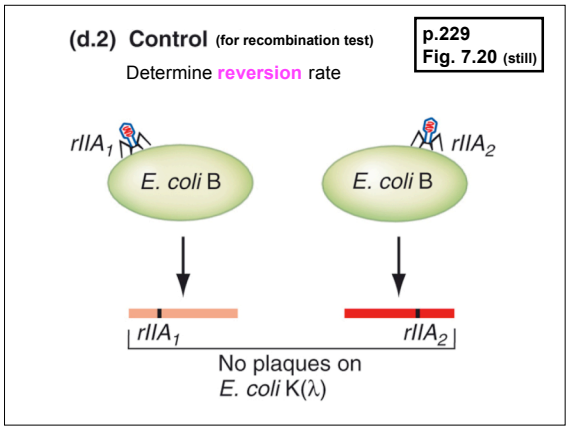


Yes, but extremely tedious (almost never do)

p.229
Fig. 7.20 (still)

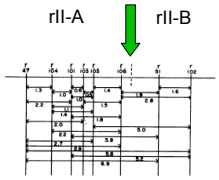
(1) Characterize mutants:
fell into two **complementation groups**, *rIIA* & *rIIB*
very close on the **genetic map**.





The first intragenic rII map

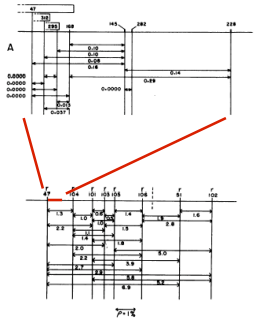
rII-A rII-B



Tests for Pseudo-allelism.—The functional relatedness of two closely linked mutations causing similar defects may be tested by constructing diploid heterozygotes containing the two mutations in different configurations.[1, 2] The *cis* form, with both mutations in one chromosome, usually behaves as wild type, since the second chromosome supplies an intact functional unit (or units). However, the *trans* form, containing one of the mutations in each chromosome, may or may not produce the wild phenotype. If it does, it is concluded that the two mutations in question are located in separate functional units.

FIG. 2.—Larger-scale map of eight rII mutants, including Doermann's r47 and r51. Newly isolated mutants are numbered starting with *101*. The recombination value (in per cent) for each cross is obtained by plating the progeny on K and on B and doubling the ratio of plaque count on K to count on B.

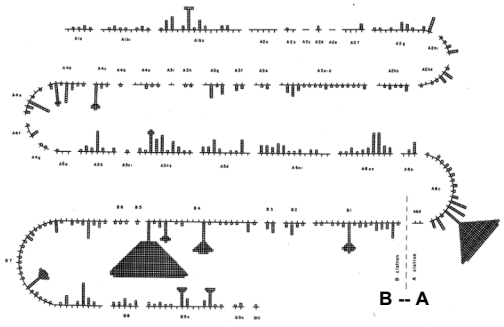
8 rII mutants mapped originally



after many more

Original 8

1,612 INDEPENDENT mutants mapped for Fig. 7.21
(and ultimately >3000)



TWO IMPORTANT QUESTIONS:

(1) How did he **accumulate** 3000+ rII mutations that he knew were **independently** generated?

(i.e. NOT progeny of the same mutant parent --- the geneticist's worst nightmare!)

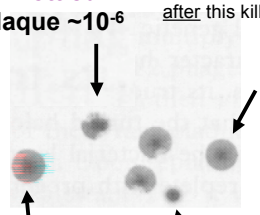
(2) How did he **map** all 3000 mutants against each other?
Certainly **not** by doing all pair-wise combinations

mixture of rII⁻ *and* rII⁺

(but remember: very low m.o.i. used for plaques …. chance of double infection <10^{-13})

rII mottled plaque ~10^{-6}

…these rII⁻ mutants had to have arisen after this killing center was founded.



rII^{mutant} ~10^{-4}

fairly rare

is this rII⁻ a relative?

rII^{wildtype}

TWO IMPORTANT QUESTIONS:

(1) How did he **accumulate** 3000+ rII mutations that he knew were **independently** generated?

(i.e. NOT progeny of the same mutant parent --- the geneticist's worst nightmare!)

(2) How did he **map** all 3000 mutants against each other?

Certainly **not** by doing all pair-wise combinations

Test each new rII mutant for **revertablility**

(one of many of Benzer's key insights)

Fell into two clear classes:

(1) revertables (single base-pair changes; "point mutants"?)

(2) nonrevertables (<<10^{-6}) (multi-base-pair deletions?)

And these two classes of mutants behaved differently in recombination tests (tests for segregational allelism)

If two mutants are **segregational alleles**, they won't be able to generate a wildtype **functional allele** by recombination

rII^{-1}/rII^{-2}--> no rII^{+} (and of course no rII$^{-1\&-2}$)

Revertables respected a segregational allelism rule that nonrevertables violate:

> Any revertable (**a**) can be a segregational allele of at most only **one** of ANY two mutants (**b** & **c**) that are **not** segregational alleles of each other

----------(b)----------
----------(c)----------
---------(rev-a)----------

…more often, rev-a was not a segregational allele of either.

----------(rev-a)----------

> Any revertable (**a**) can be a segregational allele of at most only **one** of ANY two mutants (**b** & **c**) that are **not** segregational alleles of each other

What about nonrevertables?

----------(b)----------
----------(c)----------

------[…….**nonrev**…….]---------

A nonrevertable can map to two or more different points on the genetic map at once (be completely linked to those points; 0 genetic distance)

> Any revertable (**a**) can be a segregational allele of at most only **one** of ANY two mutants (**b** & **c**) that are **not** segregational alleles of each other

What about nonrevertables?

---[…nonrev-d..]----------
----------[…nonrev-e…]--

----------[…….**nonrev**…….]---------

A nonrevertable can be a segregational allele of **both** of two other mutants, revertable or not, that are **not** segregational alleles of each other

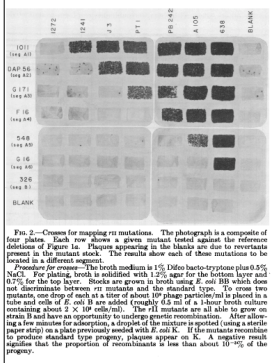


FIG. 2.—Crosses for mapping rII mutations. The photograph is a composite of four plates. Each row shows a given mutant tested against the reference deletions of Figure 1a. Plaques appearing in the blanks are due to revertants present in the mutant stock. The results show each of these mutations to be located in a different segment.

Procedure for crosses—The broth medium is 1% Difco bacto-tryptone plus 0.5% NaCl. For plating, broth is solidified with 1.2% agar for the bottom layer and 0.7% for the top layer. Stocks are grown in broth using *E. coli* BB which does not discriminate between rII mutants and the standard type. To cross two mutants, one drop of each at a titer of about 10^9 phage particles/ml is placed in a tube and cells of *E. coli* B are added (roughly 0.5 ml of a 1-hour broth culture containing about 2×10^8 cells/ml). The rII mutants are all able to grow on strain B and have an opportunity to undergo genetic recombination. After allowing a few minutes for adsorption, a droplet of the mixture is spotted (using a sterile paper strip) on a plate previously seeded with *E. coli* K. If the mutants recombine to produce standard type progeny, plaques appear on K. A negative result signifies that the proportion of recombinants is less than about 10^{-8}% of the progeny.

With rII it was easy to determine if any two mutants were or were not segregational alleles:

(i.e. would or would not produce **wildtype recombinants** from the hybrid)

Infect liquid B culture with any **two** rII mutant phage then "**spot**" a drop of that mixture on top of a **lawn** of K ask: do the progeny from lysed B "**clear**" the lawn of K?

Map of **nonrevertables** based on their **segregational allelism**

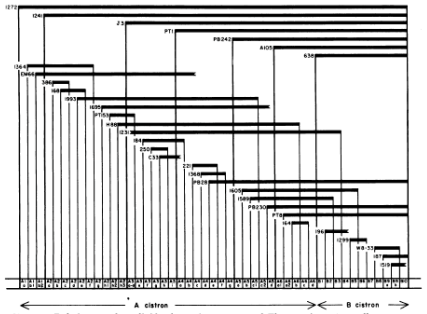
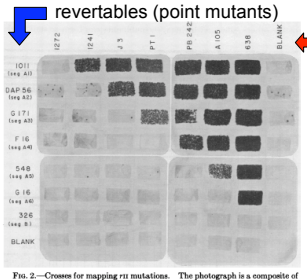
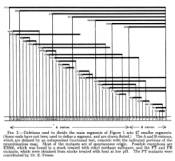


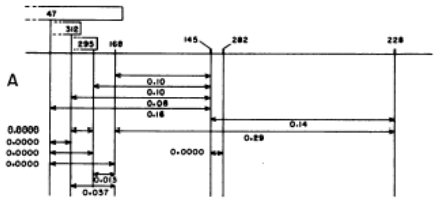
FIG. 3.—Deletions used to divide the main segments of Figure 1 into 47 smaller segments. (Some ends have not been used to define a segment, and are drawn fluted.) The A and B cistrons, which are defined by an independent functional test, coincide with the indicated portions of the recombination map. Most of the mutants are of spontaneous origin. Possible exceptions are EM66, which was found in a stock treated with ethyl methane sulfonate, and the PT and PB mutants, which were obtained from stocks treated with heat at low pH. The PT mutants were contributed by Dr. E. Freese.

revertables (point mutants)

nonrevertables (deletions)

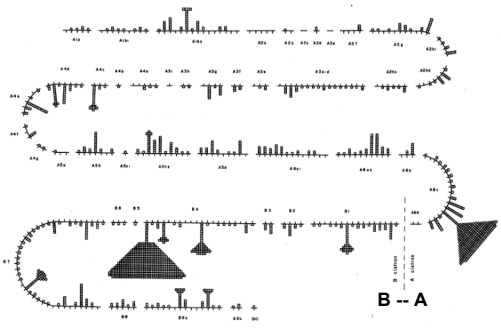






So how does the **large-scale map** generated by recombination between **mutants in different genes** (as complementation groups) compare with the **fine-structure map** generated by recombination between **mutants in the same genes** (as complementation groups)?

1,612 INDEPENDENT mutants mapped for Fig. 7.21 (and ultimately >3000)



The points on intragenic maps are:

(1) Linear (like on intergenic maps)

(2) Contiguous (like on intergenic maps)

(3) Close, but not necessarily closer within a gene than between

(4) Based on the physical size of T4, and the total map distance, adjacent points estimated to be ~2 bp apart -- hence recombination appears possible between adjacent bp.

BUT, genes are <u>not</u> infinately divisible