Mutations lecture: March 4, 2009

1. Genetic analysis of bacteria: the whys, hows, and whats
2. Luria-Delbrück and beyond: we still care!
3. Analysis of essential genes
   - ✓ RNA pol, merodiploids and amber supressors

Advantages of genetic analysis in bacteria/phage

- Numbers!
- Haploidy (most of the time; ability to make them selectively diploid)
- We don't cry when they die or don't grow (most of the time)
- Able to grow under defined conditions (temperature, nutrients, antibiotics, etc)
- Ease of gene exchange and recombination

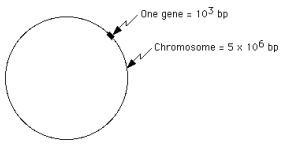
Mutations…

- Are rare, especially spontaneous ones (so working with big numbers is important).
- Will be either dominant or recessive (so working in haploid cells that can be made selectively diploid is helpful).
- That impact a process of interest are best identified via a selection or screen.
  - ✓ A selection only allows growth of a genetically distinct population of cells
  - ✓ A screen allows growth of more that one genetic population of cells, but with phenotypic differences.

Selections vs Screens

Selection	Analysis
Growth on sugar or other nutrients	Expression of gene allows growth on defined media
Antibiotics	Expression of resistance gene allows growth on antimicrobial agent; sensitive cells killed
Phage	Lytic phage will kill cells with receptor (that lack immunity)
Screen	
Fermentation of sugar	Color change on tetrazolium/MacConkey agar indicates uptake & metabolism
Cleavage of chromogenic substrate	Color on X-gal or X-phos as indicator of enzymatic activity for β-galactosidase or alkaline phosphatase

Importance of design in mutant hunts



Assuming you know what you want, what is the probability of a isolating a mutation in your favorite gene on the chromosome?

If the average gene is 10^{-3}bp and the genome is $5x10^6$, your best chance of isolating a mutation in a particular gene would be 1/5000 cells with a single mutation.

However:
↪not all mutations result in a phenotype (about 90% of base changes are silent; therefore: 1 in 50,000 candidates)
↪ some genes code for essential (or important functions), so many mutations in those won't produce viable mutants (or mutants that grow poorly)
↪ some desirable targets are much smaller than average

Genetists have to be clever (or lucky or both)

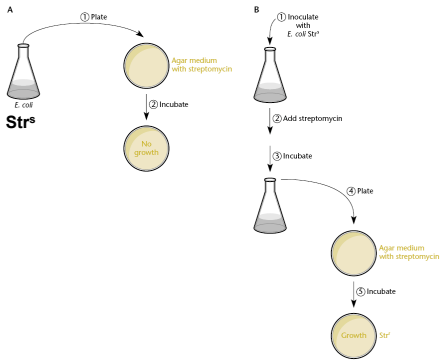
- Some problems are more easily approached than others: regulation of gene expression has been amenable to **classical genetic approaches**, using both selections and screens
- It's good to build on the success of others: **modern genetics** has tools unimagined 40 years ago, which allows analysis of complex genetic phenomena

"You get what you select for, but you don't know what you are selecting for until you get it." ***Tom Silhavy***

Isolation of bacterial mutants

Fig. 3.2

Rare to find bacteria resistant to toxic effects of selective agent. Example: one doesn't identify streptomycin-resistant *E. coli* (Str^R) unless the bacteria are plated on streptomycin containing agar.



Confluent lawn of Str^R bacteria

Important advances: Luria-Delbrück

Two competing theories about the origin of mutations in bacteria were current in 1940's:

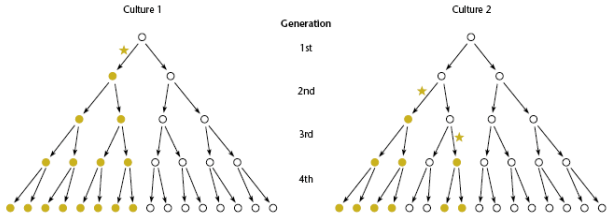
1. **Induced mutations** - mutants occur only as a specific response to selective pressure. (*e.g.,* mutants resistant to toxic agent arise in response to the application of selection to toxic agent) Luria: "Bacteriology is the last stronghold of Lamarckism."

2. **Spontaneous mutations** - mutants (wanted and unwanted) arise randomly & are present before the selection applied. Darwinian inheritance already accepted for eukaryotes.

http://www.sci.sdsu.edu/~smaloy/MicrobialGenetics/topics/mutations/fluctuation.html

Random mutations: timing is important

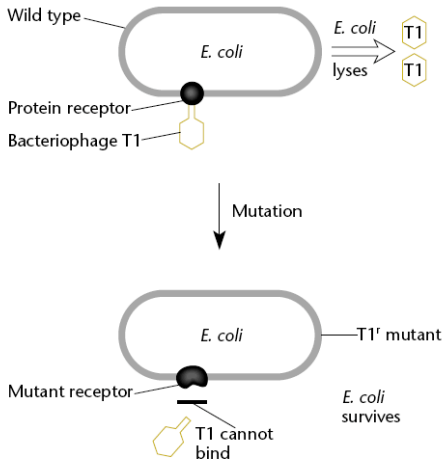
Figure 3.3



Random mutations can occur early or late in the growth cycle of the culture. In the absence of selection, the number of any particular mutant recovered from the culture will vary, depending on when the relevant mutation occurs.

Luria-Delbrück experiment: set-up

Figure 3.4



Selective pressure: resistance to lytic phage T1 from changes in OM protein FhuA (aka TonA; also binding site for 2 other phage)

Luria and Delbrück Fluctuation Test

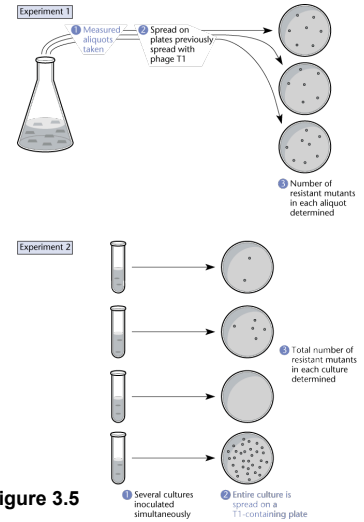


Figure 3.5

- Ton^S strain grown in culture, both as batch and as several smaller cultures. After growth, cells plated on agar with 10^{10} T1 (MOI = 1)
- If the mutation leading to Ton^R induced by exposure to T1, then there would be an equal number of mutants on all plates (within statistical variation), regardless of how cultures grown.
- If mutation to Ton^R is spontaneous and occurred before exposure to T1, then the number of Ton^R mutants isolated from each culture should vary from tube to tube, depending on when mutation occurred.

Fluctuation test data

Table 3.2

TABLE 3.2 The Luria and Delbrück experiment

Experiment 1		Experiment 2	
Aliquot no.	No. of resistant bacteria	Culture no.	No. of resistant bacteria
1	14	1	1
2	15	2	0
3	13	3	3
4	21	4	0
5	15	5	0
6	14	6	5
7	26	7	0
8	16	8	5
9	20	9	0
10	13	10	6
		11	107
		12	0
		13	0
		14	0
		15	1
		16	0
		17	0
		18	64
		19	0
		20	35

Mean: 16.7
Variance: 15
Variance/Mean: 0.9

Mean: 11.3
Variance: 694
Variance/Mean: 60.8

Importantly, the mean numbers of Ton^R mutants recovered from both of these experiments are similar.

In contrast, the large variation in the number of mutants per plate in experiment 2, (mean is much less than the variance) indicates the timing of mutation from culture to culture. One culture even contains a "jackpot" of mutants!

Beyond Luria-Delbrück

Additional studies by Newcombe (more phage) and the Lederbergs (antibiotics and replica plates) confirmed the random occurrence of mutations.

Luria & Delbrück calculated a "mutation rate" from their data, from both the number of Ton^R isolates and the number of plates with zero mutants.

Commonly accepted number:
10^{-6} to 10^{-7}/gene/generation

Analysis of essential functions by brute force

Cairns and de Lucia (1969) wanted to test whether gene coding for Kornberg's DNA polymerase was essential (and the main replicative polymerase in cell).

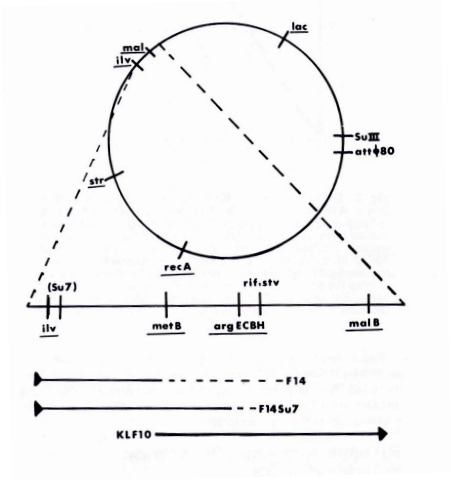
"Indscriminately" tested 1000's of colonies from heavily mutagenized culture with biochemical assay to look for incorporation of radioactive nucleotides into DNA in small cell extracts.

Found 1 mutant with less than 10% wild-type activity: PolA mutant did not have growth defect; concluded that not essential function.

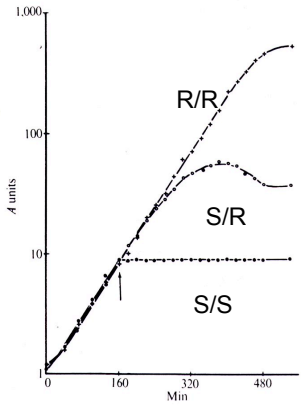
Analysis of *E. coli* RNA pol (1971)

Biochemical analysis showed that one catalytic form of RNA pol in cell. Rifampicin inhibits transcription by binding to RNA pol; Rif^R mutants make RNA pol that is Rif^R *in vitro*; mutation maps to gene for β subunit.

Scaife and colleagues: merodiploids of rif^R/rif^S made, using F' (KLF10).



Dominance test



Austin et al, Nat. New Biol. **232**: 133.

Strains tested with combinations of *rif*^{R}/*rif*^{S} alleles for growth in Rifampicin (at arrow).

Does this phenotype make sense?

RNA pol structure: http://www.pingrysmartteam.com/rifampicin/rifampicin.htm

No growth = Opportunity!

Selected 144 mutants in the *rif*^{S}/*rif*^{R} merodiploid that grow on minimal medium + Rif, calling them *rif*^{0}.

(Chromosomal marker details: Met-, RecA-, Lac- as lacZ$_{am}$)

Expected classes?

Analysis of *rif* mutants

- *rif*^{R} on chromosome: 14/144
- *rif*$^{-}$ on chromosome: 90/144
- *rif*^{S}-recessive on chromosome: 40/144