“Bacterial” genetics I

“What are the genes? What is the nature of the elements of heredity that Mendel postulated as purely theoretical units? … Frankly, these are questions with which the working geneticist has not much concern himself… If the gene is a material unit, it is a piece of a chromosome; if it is a fictitious unit, it must be referred to a definite location in a chromosome. … Therefore, it makes no difference in the actual work in genetics which point of view is taken.”

T.H. Morgan

The Relation of Genetics to Physiology and Medicine

Nobel Lecture, June 4, 1934

DNA → RNA → protein

central dogma of molecular biology
Today even the layman thinks of resistant bacteria as originating from mutation ... but when Luria and Delbrück first got together, conventional bacteriologists were by no means clear that microorganisms could be taught about genetically... Many believed that resistance was some kind of adaptation induced, in a few of the bacteria in a culture, by the exposure to the antibacterial agent.

Judson p. 55

The idea smacks of the pre-Mendelian, pre-Darwinian notion of the inheritance of acquired characteristics; Luria damned bacteriology as the “last stronghold of Lamarckism.”

Judson p. 55

Let’s all do science in Nevada

One Saturday evening ... Luria went to a faculty dance... There, watching the fluctuating returns obtained by colleagues gambling on a slot machine, he thought of the experiment that would distinguish between resistance induced in bacteria and resistance resulting from previous spontaneous mutation upon which selection acts.

Judson p. 55
What Luria perceived was that previous spontaneous mutation would pay out jackpots of resistant bacteria that would fluctuate much more widely in size than those paid out by induction. He tried the first experiment on the following morning and wrote off to Delbrueck; Delbrueck promptly replied that Luria really ought to go to church …

Judson p. 55

What Luria actually did

Sample set A:
1. Inoculate bacteria into individual cultures (1 bacterium per culture).
2. Let it grow up to a large number.

Sample set B:
1. Take an aliquot of bacteria, and start a culture (which will therefore not be clonal).
2. Let them grow up to a large number

Expose both to phage, and count, how many phage-resistant colonies per culture are found. Ask, if there is a difference between these two sample sets.

S. Luria, M. Delbrück (1943)

“If the production of resistance began only at the moment of exposure to phage, then it wouldn’t matter whether the bacteria came from many individual cultures or one bulk culture. … When Luria performed the experiment, though, the twenty separate cultures showed much wider fluctuations from the average number of resistant colonies, indicating that a few of the individual tubes contained resistant bacteria from near the beginning of the overnight growth period.”

Judson p. 56
George Beadle (left) and Edward Tatum (right) receiving their Nobel Prizes
"Conjugation" in bacteria

Take strain of *E. coli* that is auxotrophic for two distinct nutrients (thiamine and leucine).

Take different strain of *E. coli* that is also auxotrophic for two distinct nutrients, but different ones (biotine and cysteine).

Mix the two.

Ask, if ANY NOVEL PHENOTYPES APPEAR.

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J. Lederberg, E. Tatum (1946)
B.H. and A.H.

In the pre-Hayes period, mating in bacteria was envisioned as a conventional sex process, perhaps modified by aspects of “relative sexuality,” but nevertheless a standard haploid/diploid/meiosis mechanism. After Hayes, it was known that bacteria were not just small cells, but constituted a completely different kind of cell ... The terms prokaryote and eukaryote were not introduced until 1962.

Brock p. 87

William Hayes

Selman Waksman

streptomycin
Hayes expt:
Take strain A, which is streptomycin-resistant, and auxotrophic for biotin and methionine.
Take strain B, which is streptomycin-sensitive, and auxotrophic for threonine and leucine.
Mix the two on a minimal-medium plate containing streptomycin.
Wait and see.

Rich husband, poor wife is not the same as poor husband, rich wife

Cross #1:
Strain A (Str^R, B^-, M^-) × Strain B (Str^S, L^-, T^-)
Result: streptomycin completely inhibits prototroph formation (i.e., appearance of B^+, M^+, L^+, T^+ bacteria) if added before conjugation is complete.

Cross #2:
Strain A (Str^S, B^-, M^-) × Strain B (Str^R, L^-, T^-)
Result: streptomycin has no effect whatsoever. You can add it all you want, at any time, and prototrophs will still form!!

"I discussed these results with Denny Mitchison and I think it was he who first suggested that one of the parents, A, might be acting as a gene donor and the other, B, as a recipient'.
It was from this experiment that the concept of asymmetry in bacterial sexuality arose. Parent B was the recipient or 'female', the continued viability of which was essential for the whole process of recombination and segregation, while the A donor or 'male' cell was dispensable once genetic transfer had been effected."
The life cycle of a temperate phage

Three aspects of phage biology with long-term impact

1. Transduction (phage carrying additional genetic information from cell to cell) → oncoretroviruses
2. Lysogeny (phage resident in bacterial genome) → latent viruses in eukaryotic genomes
3. Recombination between phage → the fine structure of a gene

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Annual budget: $3,780.00
SUMMARY

When Salmonella typhimurium is grown in the presence of a variety of mildly distorting agents, especially weakly lytic phages, it produces a filtrable agent (PA) capable of transferring hereditary traits from one strain to another. Individual filtrates may transduce many different traits, but no more than one in a single bacterium. The activity of a filtrate parallels the characteristics of the donor cell. Nutritional, fermentative, drug resistance, and antigenic characters have been transduced. The new characters are stable after many generations of subcultures.

PA is resistant to such bacterial disinfectants as chloroform, toluene, and alcohol and to such enzymes as pronase, trypsin, ribonuclease, and deoxyribonuclease. The size of the PA particle, as determined by filtration through a 0.1 micron membrane, is about 0.1 micron. Adsorption of PA is rapid and, among various serotypes tested, is correlated with the presence of somatic antigen XIII.

The maximum frequency of transduction for any one character has been 2 x 10^-4, a limit set by saturation during adsorption. Some inter-type transfers have been observed. For example, the flagellar antigen from Salmonella typhimurium has been transduced to S. typhi to give a new serotype: IX, XII, i = -. Genetic transduction in Salmonella is common and contrasted with 'type transformation' in Hemophilus and the pneumococcus and with sexual recombination in Escherichia coli.

Indirect evidence: platings of cells and filtrates. To test the possible role of filtrable factors in this transduction, a u-tube with an 'airlock' double glass filter partition was prepared according to Davis' (1936b) design. By alternating suction between the arms of the tube, two intact populations of growing bacteria could be made to share the same medium. The integrity of the filter was verified in control experiments by leaving one compartment uninoculated. Then 10^8 cells of each parent were inoculated into twenty ml of broth and placed in either arm of the tube. Ten ml were drawn from side to side every twenty minutes for four hours while the culture grew to saturation. The two populations were washed and plated upon minimal medium. Proteus appeared in the filtrates of LA-22 but not of LA-2. Sterile filtrates of LA-2 broth cultures did not elicit proteus from LA-22. However, filtrates of mixed cultures of LA-2 and LA-22 contained one proteus per million LA-22 cells. Thus, LA-2 produced a filtrable agent (PA), under stimulation from LA-22, that could elicit proteus from LA-22. Filtrates of LA-22 cultures, containing substantial amounts of phage (PGF-22) active on LA-2, also stimulated PA production from LA-2. The role of this phage will be discussed later.

To help the further exposition of our experiments, we shall use the term transduction for genetically unilateral transfer in contrast to the union of equivalent elements in fertilization. The working hypothesis that Salmonella PA is an agent of genetic transduction provides a useful frame of reference for our discussion.
Further reading

Horace Judson
*The Eighth Day of Creation*

Thomas Brock
*The Emergence of Bacterial Genetics*