

LECTURE 8: Genetic dissection of biochemical pathways; Complementation

Reading: Ch. 3, p. 59, Fig. 3.15 and 3.18; Ch. 7, p. 206-7, 213-215

Problems: Ch. 7, solved prob II, III; 7-12, 7-16, 7-19, 7-21, 7-24 – 7-28; Ch. 3, 3-18; Ch.5, 5-31

Today, our lecture will basically be a discussion of the **Definition of a Gene**. We will concentrate on the definition of a gene as a unit of function; you will discuss the definition of a gene as a unit of structure (linear array of DNA base pairs) in other courses.

Mendel was the first to describe the unit of heredity. Although he didn't coin the term "gene" his "characters" or "constant factors" were basically defined as factors that controlled one specific phenotypic trait. So Mendel's definition of a gene might well have been "one gene, one phenotypic trait".

At about the same time that Mendel's work was rediscovered, Dr. Archibald Garrod was studying several congenital metabolic diseases. In 1902, he published his work on **alkaptonuria**, a harmless condition in which the urine of affected individuals turns black upon exposure to air. He performed biochemical analyses of affected individuals and showed that a substance called homogentisic acid, which blackens upon exposure to oxygen, accumulates in their urine. Unaffected individuals do not excrete this substance, even if they ingest it. He proposed that affected individuals were incapable of metabolizing homogentisic acid to its normal breakdown products. He noted in his paper that "the abnormality is apt to make its appearance in two or more brothers or sisters whose parents are normal and among whose forefathers there is no record of its having occurred" and that "of alkaptonuric individuals a very large proportion are children of first cousins". The horizontal pattern of inheritance should quickly lead you to the conclusion that the condition is caused by a recessive allele. Garrod, who had read Bateson's translation of Mendel's work, came to the same conclusion.

Garrod hypothesized that alkaptonuria was an "inborn error of metabolism". Although it was hypothesized at the time that homogentisic acid was a breakdown product of tyrosine, the actual biochemical pathway required to induce the change from tyrosine to homogentisic acid to final breakdown products was unknown. He proposed that affected individuals had an "alternative course of metabolism" and excreted homogentisic acid instead of the normal byproducts. Garrod studied other inborn errors of metabolism and proposed that each arose from a mutation in a gene required for a specific biochemical reaction. Garrod's definition of a gene might well have been "one mutant gene, one metabolic block".

We now know that the biochemical pathway is as follows:

Phenylalanine --> Tyrosine --> p-Hydroxyphenylpyruvate --> 2,5-Dihydroxyphenylpyruvate --> Homogentisic Acid --> Maleylacetoacetic Acid --> --> --> CO₂ + H₂O

Other mutations causing human disease in this metabolic pathway include:

Phenylketonuria (PKU): mutation in phenylalanine hydroxylase. Phenylalanine accumulates and is converted to phenylpyruvic acid which is toxic to the developing nervous system.

Tyrosinosis (very rare): mutation in tyrosine transaminase. Tyrosine levels are elevated in blood and urine. Various congenital abnormalities.

Tyrosinemia: mutation in p-hydroxyphenylpyruvic acid oxidase. Tyrosine and p-hydroxyphenylpyruvate are elevated in blood and urine. Liver failure, usually within 6 months of birth.

Albinism: mutation in tyrosinase, the first enzyme in the pathway that converts tyrosine to melanin.

In describing his work on alkaptonuria and other inborn errors of metabolism (like albinism), Garrod notes that these peculiarities are rare in the population as a whole, but that they were readily identifiable because of their overt phenotypes. Near the end of his 1902 paper, he states “May it not well be that there are other such chemical abnormalities which are attended by no obvious peculiarities and which could only be revealed by chemical analysis? If such exist and are equally rare with the above they may well have wholly eluded notice up till now. A deliberate search for such, without some guiding indications, appears as hopeless an undertaking as the proverbial search for a needle in a haystack.”

BUT that’s where genetics comes in! We can do genetic screens to find the needles in haystacks! In the 1940’s, George Beadle and Edward Tatum carried out a series of experiments to show a clear relationship between genes and enzymes that catalyze the steps of biochemical reactions. They chose the bread mold *Neurospora* for their work. Why?

- (1) Life cycle was known (can grow vegetatively as haploid or diploid cells; can mate and undergo meiosis to form haploid ascospores).
- (2) Can induce mutations!
- (3) Requires very little to grow [grows on “**minimal medium**” containing only inorganic salts, a simple sugar, and one vitamin (biotin)]

Beadle and Tatum reasoned *Neurospora* must make everything else it needs to grow (amino acids, other vitamins, nucleic acids, etc) and that the biosynthesis of these substances was under genetic control. Instead of having to rely upon existing diseases (mutations) to work out biochemical pathways (like Garrod did), they could instead select mutants in which chemical reactions were blocked.

Their experiment (genetic screen) (Fig. 7.20, p. 214):

- Mutagenize asexual spores (conidia) of *Neurospora* with Xrays or UV light
- Cross the mutagenized spores with the opposite wild-type mating type
- Collect individual ascospores and grow them on **complete medium** (containing vitamins, amino acids, etc.)
- Conidia from each culture tested on minimal media for growth. Those that fail to grow (**auxotrophs**) were tested again for growth on minimal media supplemented with amino acids or minimal media supplemented with vitamins (and of course the controls: minimal media versus complete media). Amino acid auxotrophs were then tested for growth on minimal media supplemented with individual amino acids to identify the amino acid that the mutant *Neurospora* could no longer synthesize.

They isolated four auxotrophs that could only grow on minimal media if it was supplemented with arginine. These arginine auxotrophs carried mutations that blocked arginine biosynthesis.

Each mutation mapped to a different linkage group, so they concluded that at least four genes were required for the biosynthesis of arginine. They named these genes ARG-E, ARG-F, ARG-G, and ARG-H. They then asked whether the arginine synthesis mutants could grow on minimal media supplemented with known intermediates in the biochemical pathway.

| Strain | Supplements added to minimal media | | | | |
|--------------|------------------------------------|-----------|------------|------------------|----------|
| | Nothing | Ornithine | Citrulline | Argino-succinate | Arginine |
| Wildtype | + | + | + | + | + |
| Arg-E mutant | - | + | + | + | + |
| Arg-F mutant | - | - | + | + | + |
| Arg-G mutant | - | - | - | + | + |
| Arg-H mutant | - | - | - | - | + |

Based on these results, they hypothesized a linear pathway in which each intermediate in the pathway was a product of the previous step and the substrate for the next step. Supplementing minimal media with an intermediate beyond the step blocked in an arginine auxotroph will allow it to grow. If a mutant strain is blocked at a late step in the pathway, fewer of the intermediate compounds will support the growth of the strain. If a mutant strain is blocked at an early step, more of the intermediate compounds will permit growth of the strain.

The pathway: Arg-E Arg-F Arg-G Arg-H
 N-Acetylornithine --> Ornithine --> Citrulline --> Arginosuccinate --> Arginine

They proposed that ARG-E encodes the enzyme responsible for the first step, ARG-F encodes an enzyme for the second step, ARG-G for the third, and ARG-H for the fourth. Beadle and Tatum proposed “one gene, one enzyme”. For their work, they won the Nobel Prize. Their hypothesis was later revised to “one gene, one polypeptide” to account for the fact that enzymes can be composed of two or more polypeptide chains. (In fact, these genes, one could even include “non-coding RNAs”, as researchers have demonstrated function genetically for some RNAs that don’t even code for proteins – more about this later in the course!)

Complementation Test: Operational definition of a gene (unit of function)

How can we know whether mutations that give similar phenotypes are in the same or different genes? We perform a complementation test, one of the most widely used techniques in genetics. (One can also map the mutations; if they map to different chromosomes, then they cannot be in the same gene, but if they are linked, need to perform the complementation test).

Ed Lewis first developed the complementation test for functional allelism in 1942. The test is simple: construct an individual in which one homolog of a particular chromosome carries one of the recessive mutations and the other homolog carries the other recessive mutation and see whether the mutant phenotype is expressed. If the phenotype of the transheterozygote is wildtype, then we say that the two mutations complement each other and they must be in different genes. If the transheterozygote shows the mutant phenotype, we say they do not

complement (or fail to complement) each other and that the mutations are in the same gene. A **complementation group** is a collection of mutation that do not complement each other (i.e., a group of mutations that affect the same gene).

Scenario 1: The two mutations are in the same gene

-----mut1-----
 -----mut2-----

-If mut1 and mut2 represent two different mutations in one gene, than the individual has no wild-type copy of the gene and will display the mutant phenotype. The mutations fail to complement each other.

Scenario 2: The two mutations are in different genes

-----mut1----- + -----
 ----- + -----mut2-----

-If mut1 and mut2 are mutations in different genes, than the individual will carry one wild-type copy of each gene, and thus have the wild-type phenotype. These mutations complement each other.

Note: We cannot use complementation testing with dominant alleles.

Example: *Drosophila* eye color mutations

There are several different X-linked eye color mutations: *white*, *buff*, *coral*, *apricot*, *cherry*, *ruby*, *vermillion*, *garnet*, and *carnation*. Perform complementation testing to test whether these mutations identify the same or different genes.

| Mutation | white | garnet | ruby | vermillion | cherry | coral | apricot | buff | carnation |
|------------|-------|--------|------|------------|--------|-------|---------|------|-----------|
| white | - | + | + | + | - | - | - | - | + |
| garnet | | - | + | + | + | + | + | + | + |
| ruby | | | - | + | + | + | + | + | + |
| vermillion | | | | - | + | + | + | + | + |
| cherry | | | | | - | - | - | - | + |
| coral | | | | | | - | - | - | + |
| apricot | | | | | | | - | - | + |
| buff | | | | | | | | - | + |
| carnation | | | | | | | | | - |

Results: the *white* gene has 5 different mutant alleles (w^l , w^{cherry} , w^{coral} , $w^{apricot}$, and w^{buff}); these alleles fail to complement each other. The other genes (*garnet*, *ruby*, *vermillion* and *carnation*) are separate loci. There are five complementation groups (or five different genes) for eye color on the *Drosophila* X chromosome.