Chapter 12 Lecture Notes: The Nature of the Gene

I.   How genes work or "What does a piece of deoxyribonucleic acid have to do with the color of my cat?"

Phenotype ← genotype ← gene ← DNA

The central dogmas:

protein ← mRNA ←DNA

protein ← mRNA ←DNA←RNA

II.   Preliminary thoughts on relationship between genes and phenotypes
A. 1902: William Bateson and Archibald Garrod studied alkaptonuria, a human disease characterized by urine that turns black upon exposure to air and a tendency to develop arthritis later in life.
        They made two observations (1) disease seemed to be inherited as a recessive trait and (2) people with disease secreted homogentisic acid (HA) in their urine (normal people do not secrete HA)
        They hypothesized that normal people can metabolize HA while people with alkaptonuria can not metabolize HA. Furthermore, they concluded that alkaptonuria is a genetic disease that is <u>caused</u> by the absence of an enzyme needed to metabolize HA (inborn error of metabolism).

Phenylalanine → tyrosine → p-hydroxyphenylpyruvate → HA → maleylacetoacetic acid → → → CO_2 and H_2O

B. Table 12-4: Over 40 human diseases result from inborn errors in metabolism.

C.   Another important implication of this work was that the position of a block in a metabolic pathway can be determined by the accumulation of a product that proceeds the block.

If a mutant is blocked in a metabolic pathway at some point, it can not make any of the intermidiates in the pathway that are after the block.
*   If you supply the mutant with an intermediate compound that is made before the block, there will be no effect (no final product).
*   If you supply the mutant with an intermediate compound that is made after the block, the mutant can now make the end product
*   Compound made late in the pathway will support the growth of more mutants.
*   Mutants late in the pathway can not be restored with as many compounds as mutant that are early in the pathway.
*   Analogy to Austin traffic jams

III.   Genes control the production of enzymes: The one gene – one enzyme hypothesis

1940s First convincing evidence that genes are responsible for the synthesis of enzymes.

Irradiated *Neurospora* (haploid fungus) to induce mutagenesis. They then screened for arginine auxotrophs and found three that contained mutations that mapped to different locations on the chromosome (arg-1 arg-2 arg-3).

Each of the mutants would grow when media was supplemented with arginine. Other compounds similar to arginine could also support growth:

strain	supplement		
	orthinine	Citrulline	arginine
wt	+	+	+
arg-1	+	+	+
arg-2	-	+	+
arg-3	-	-	+

Based on this data Beadle and Tatum proposed the following enzymatic pathway for the production of arginine:

Enzyme X   Enzyme Y   Enzyme Z
Precursor   →   Ornithine   →   Citrulline   →   arginine
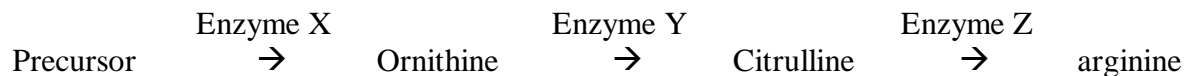
Then Beadle and Tatum made the intellectual leap that each arg mutation was in a different gene that encoded these enzymes.
        arg-1→ enzyme X
        arg-2→ enzyme Y
        arg-3→ enzyme Z

Note that this pathway was inferred entirely through genetic analysis; later biochemical work confirmed it.

Thus, examination of the one gene – one enzyme hypothesis showed that genes somehow were responsible for the function of enzymes and that each gene controlled one specific enzyme.

IV.   **How** do genes control the functioning of proteins?

A. Review of protein structure: Figures shown are using hemoglobin as an example -
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1. Proteins are macromolecules composed of a chain of amino acids linked end to end in a linear fashion (<u>primary structure</u>). Each protein has a distinct linear sequence of amino acids. This was first shown by Frederick Sanger et al. with the sequencing of the protein insulin.

(a) Primary structure
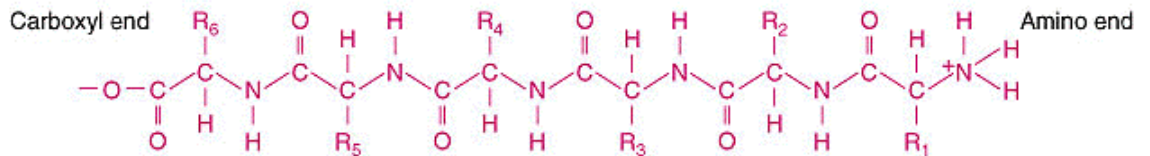
2. Polypeptides can bend into regularly repeating structures generated by hydrogen bonding between amino acids that are close together in the linear sequence (<u>secondary structure</u>). The most common secondary structures are alpha helices (Figure 12-5) and beta sheets (Figure 12-6).

(b) Secondary structure
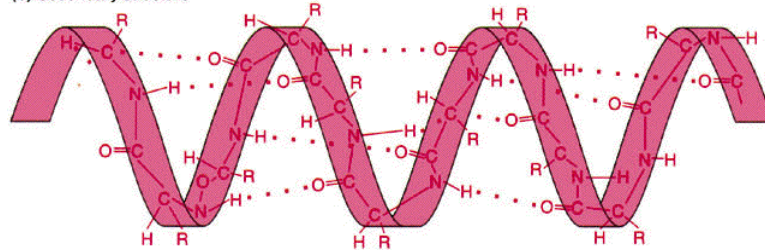
Hydrogen bonds between amino acids at different locations in polypeptide chain

3. Proteins have a 3 dimensional shape that is a result of interactions between different amino acids in the protein (<u>tertiary structure</u>). Interactions can be between amino acids that are close together or far apart in the primary structure.

(c) Tertiary structure

Heme
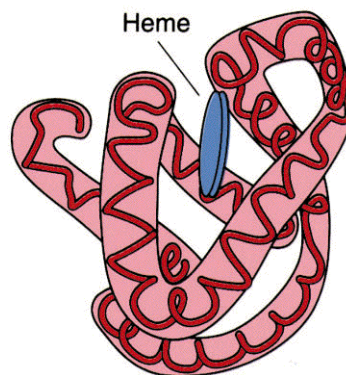
β polypeptide

4. Two or more folded proteins can bind together to form <u>quartenary structure</u>.


(d) Quarternary structure
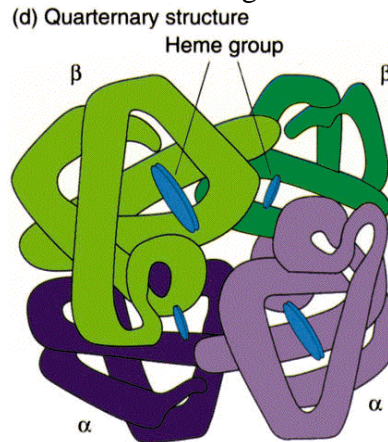
5. Proteins can be enzymatic or structural.

B. The relationship between gene mutation and altered proteins (sickle cell anemia).
1. 1910: J. Herrick first descibes sickle cell anemia. Red blood cells of affected individuals sickle at low oxygen tension causing breakage of the cell and clogging of the capillaries.
2. 1949: J. Neel and E. Beet showed that sickle cell anemia is inherited as a recessive trait. Pedegree analysis showed that there were two alleles of the Hb (hemoglobin) gene which controlled the sickle cell phenotype. Hb^A is the normal allele, and Hb^S is the sickle cell allele. The Hb^A allele showed complete dominance to Hb^S in terms of anemia, but incomplete dominance in terms of RBC shape.
3. 1950s: Linus Pauling showed that hemoglobin proteins from affected and nonaffected individuals (Hb-S and Hb-A) migrated differently on a protein gel.
4. Hemoglobin is composed of two identical alpha polypeptide chains and two identical beta polypeptide chains.
5. 1957: Vernon Ingram showed that Hb-S had an alteration in one amino acid in the hemoglobin beta subunit relative to Hb-A. Hb-S has a valine at position 6 of the beta chain, while Hb-A has a glutamate. This change was sufficient to alter the function of the hemoglobin protein.
6. It is now known that the valine substitution creates a sticky patch on the surface of Hb-S. Hb-A, of course, does not have this patch. Under conditions of low oxygen tension, a hydrophobic patch in Hb-S is uncovered which can bind to the sticky patch in another molecule of Hb-S. This causes long fibers of hemoglobin to form which give the RBC their sickle shape.
7. All of these studies together established that a single inherited gene provided the information for a single protein and that a mutation in the gene was associated with an amino acid change in the protein which was associated with a change in the activity of the protein which is associated with the anemic phenotype.

C. Colinearlity of genes and proteins

Using the *trpA* and *trpB* genes, Charles Yanofsky showed that the linear sequence of mutations was correlated with the linear sequence of amino acids in a protein.
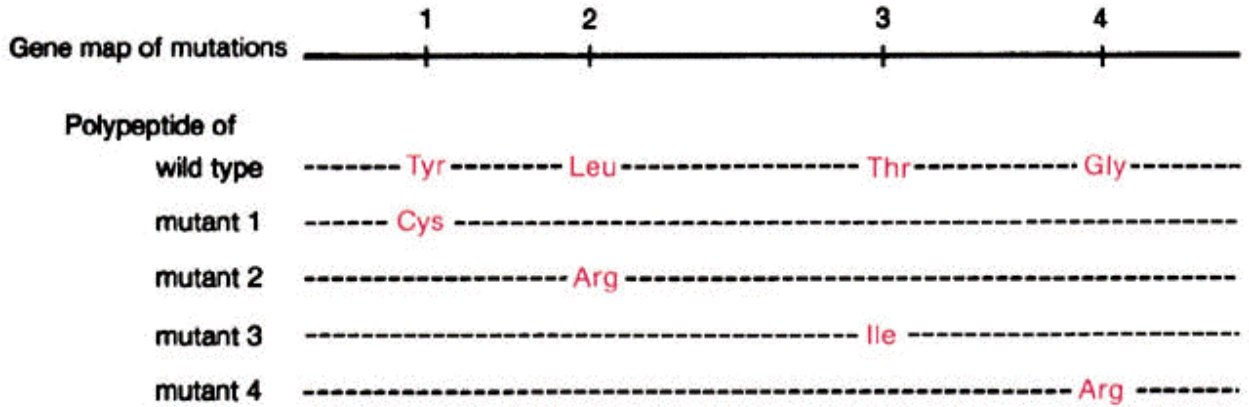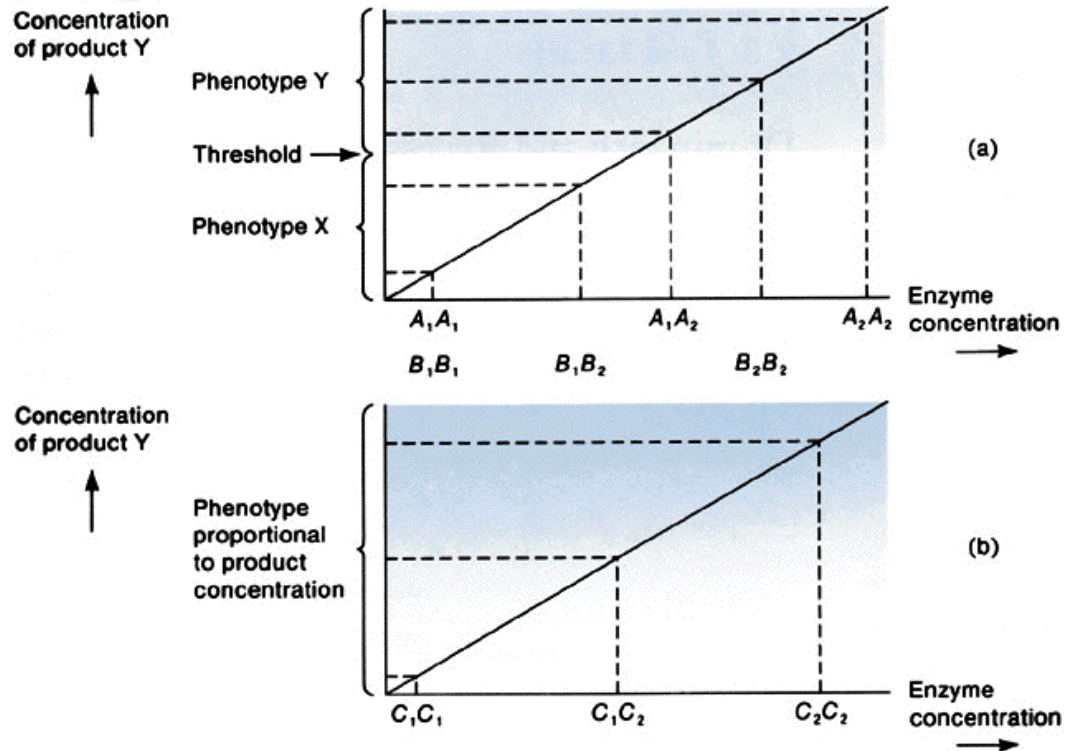

(Unchanged amino acids are not shown)

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V. Molecular explanation for previous genetic observations
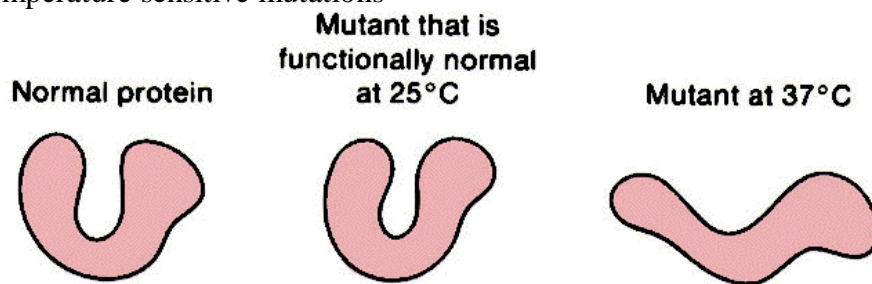A. Dominance

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Hypothetical curves relating enzyme concentration to the amount of product.

For (a), there is a threshold level of enzyme needed for the normal phenotype (Y). Assume allele 2 is the functional one. For the case of the <u>functional allele being dominant,</u> consider the three genotypes $A_2 A_2$ and $A_1 A_2$, and $A_1 A_1$. The homozygote wt ($A_2 A_2$) and heterozygote ($A_1 A_2$) produce enough functional enzyme to get the normal phenotype Y. The homozygote mutant ($A_1 A_1$) does not produce enough functional enzyme to get the normal phenotype Y and instead shows the abnormal phenotype X. For the case of the <u>mutant allele being dominant,</u> consider the three genotypes $B_2 B_2$ and $B_1 B_2$, and $B_1 B_1$. The homozygote wt ($B_2 B_2$) produces enough functional enzyme to get the normal phenotype Y. The homozygote mutant ($B_1 B_1$) and heterozygote ($B_1 B_2$) do not produce enough functional enzyme to get the normal phenotype Y and instead show the abnormal phenotype X.

For (b), there is no threshold for the normal phenotype Y; instead there is a gradient of phenotypes and the heterozygote shows incomplete dominance.

    B. Temperature sensitive mutations

Normal protein   **Mutant that is functionally normal at 25°C**   **Mutant at 37°C**

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    C. Modified Mendelian ratios – Using concepts of one gene one enzyme, you should be able to explain modified Mendelian ratios at the molecular level.
        1. Multiple alleles
        2. Lethal alleles
        3. Epistasis

VI.   Genetic fine structure
    A. Bead theory:
        1. The entire gene was viewed as the fundamental unit of inheritance, indivisible by crossing over.
        2. The entire gene was viewed as the fundamental unit of change or mutation.
        3. The gene was viewed as the fundamental unit of function.
    B. 1950s - Examination of the bead theory by Seymour Bezner: Mapping the rII locus (genetic sequencing)
        1. Used bacteriophage T4 as his system (pg. 291-294): Phage attached to bacteria → injects DNA → takes over host machinary to make progeny virus → lyses bacterial cell → repetition of infectious cycle with progeny phage → this forms a plaque or clearing on a confluent layer of bacterial cells

2. Looked at T4 mutants that had defective plaques (rII for rapid lysis)

T4 phage strain	E. coli strain	
	B	K
rII	Large	No plaques formed
rII$^+$ (wildtype)	small	Small

3. Validation of A3: The gene is the basic unit of function.
   Benzer wanted to know was the entire rII region was a single functional unit or was it made up of subunits that function independently (genes)? To answer this question, he used complementation analysis.

   Did a mixed infection of different rII mutant phage in *E. coli* K →
If mutations are in the same functional unit, them that gene product will not be made and there will be no plaques (no complementation).
If mutations are in different functional units, then each mutant will supply a wild type copy of the gene that is mutated in the other phage (complementation can occur).
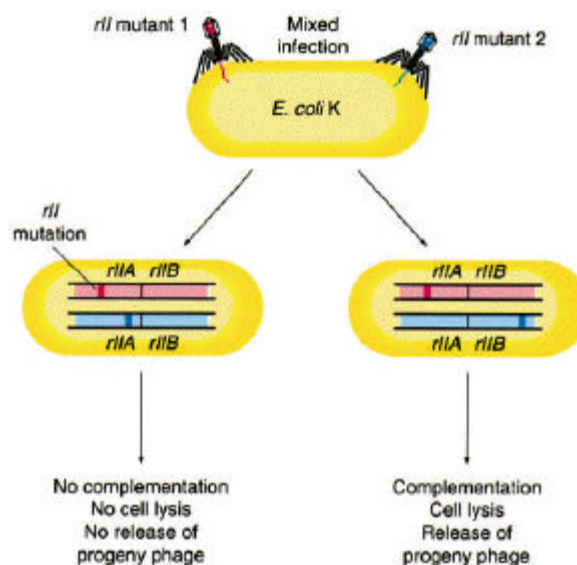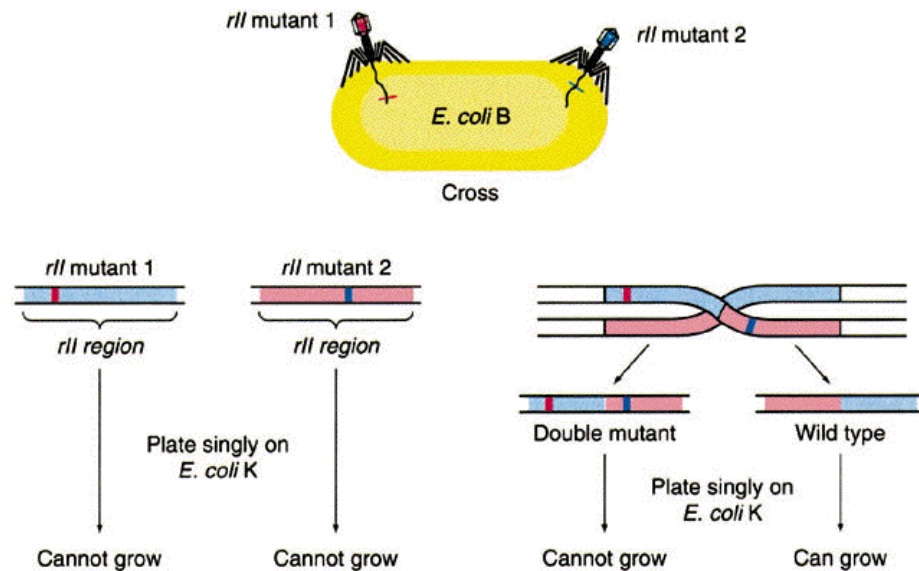

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   Showed that his phage mutants that had the rII phenotype fell into two complementation groups (rIIA and rIIB). Mutants that complement each other were in different functional units which Benzer called cistrons (genes). This type of analysis is still used today in genetics to determine the number of genes that are required for a certain phenotype.

4. Invalidation of A1 and A2 of the bead theory:

Benzer wanted to know if recombination could occur <u>within</u> genes. He used his T4 rIIA mutant phage for recombination analysis, looking for rIIA$^+$ recombinants that would form wildtype plaques on *E. coli* K. He was able to detect these <u>very rare</u> recombinants due to the incredably high sensitivity of his system (phage are produced at 10^9 phage/ml → thus, he could easily detect a recombination frequency of 1 in 10^9). Also, he had thousands of independently isolated rIIA mutants.

Benzer did a mixed infection of two rIIA mutant phage in *E. coli* B → <u>Selected</u> for rare recombinant phage (wildtype rIIA) by plating progeny phage from *E. coli* B mixed infection on *E. coli* K (which does not support rII mutant growth) → Found rare recombinant phage that could infect *E. coli* K. Thus, recombination did indeed occur <u>within</u> a gene!!!!!!

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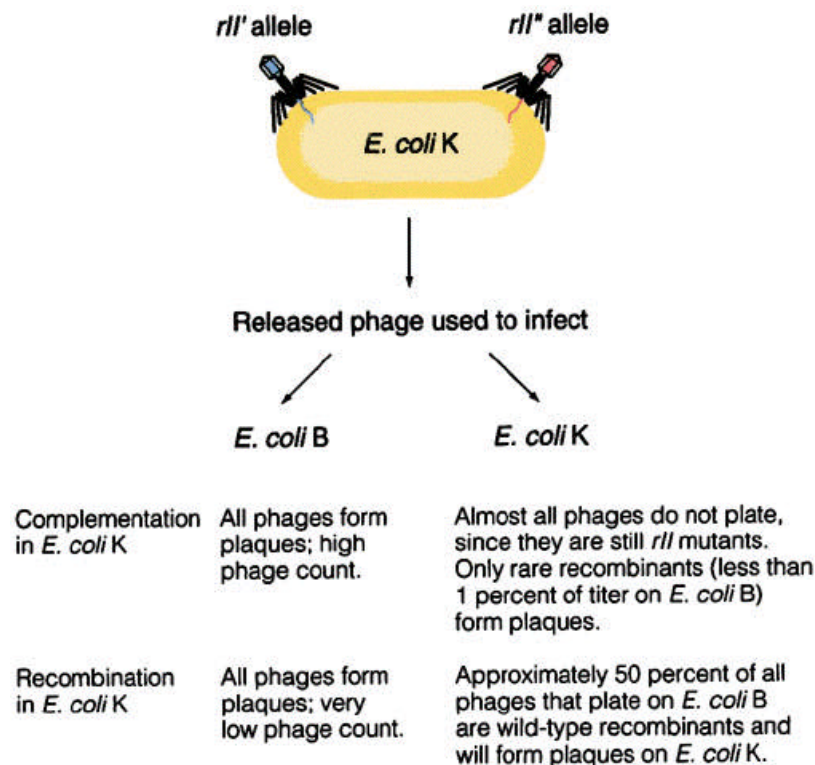

Using this recombination approach, Benzer began to construct a gene map of rIIA by calculating the recombination frequencies between the mutations in different rIIA mutants.

Benzer also used deletion mapping to localize each point mutation to a certain region of the rIIA gene. (Remember that you cannot get a recombinant when you cross a point mutation with a deletion that contains the region of the point mutation.) Then all the mutants containing point mutations in this region were crossed with each other to determine recombination frequencies. This helped speed things up as he only crossed mutants that contained point mutations that were in the same region with each other to obtain an accurate location for each mutation.

Benzer's data showed that recombination could occur between less that 5 basepairs. Subsequent work showed that recombination could occur between any two basepairs in the gene. This refuted A1 and A2 of the bead theory.

An aside on recombination analysis. Today this kind on intragenic recombination mapping would probably not be done because genes can be rapidly sequenced. However, the experiments are important to understand for historical reasons as well as a good mental exercise in genetic analysis.

5. A final note on complementation versus recombination. Please note that these are mechanistically different and thus give you different information regarding a gene or gene(s). Complementation is a mixing of gene products and does not involve a change in the genotypes of the individual chromosomes. Complementation tells you the minimum # of genes (cistrons) that control a phenotype. Recombination is the breaking and rejoining of chromosomes to produce new combinations of genes. Thus, the progeny of a cross in which recombination has occurred have new genotypes that are different from the parental genotypes. Recombination tells you the distance between genes (intergenic complementation) or mutations (intragenic complementation).

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